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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster / discussion, workshop, roundtable, and poster sessions of the 38th Annual Meeting of the Society of Toxicology, held at the Ernest N. Morial Convention Center, New Orleans, Louisiana, March 14-18, 1999.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 419.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 444.

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1 GENOMIC TECHNOLOGIES AND NEW SCREENING STRATEGIES FOR TOXICOLOGY.

M J Cunningham. *Incyte Pharmaceuticals, Inc., Palo Alto, CA, USA.*

New genomic technologies aimed at assessing molecular toxicity may now play a major role in pharmaceutical drug development. These technologies include: a.) cDNA and oligonucleotide microarrays, b.) the branched DNA (bDNA) assay, c.) the scintillation-based mRNA assay and d.) proteomics (protein gel electrophoresis/mass spectrometry). These assays may be used to screen combinatorial chemistry libraries, select drug candidates for further development or study mechanisms of toxicity. Several applications will be presented in the areas of hepatocarcinogenesis, disruption of hormonal signaling by environmental estrogens and comparing gene expression profiles of chemotherapeutic agents and peroxisome proliferators.

2 INTEGRATION OF MOLECULAR TOXICOLOGY INTO THE DRUG DISCOVERY PROCESS.

M D Todd. *Department of Toxicology, Chiron Corporation, Emeryville, CA, USA.* Sponsor: G H I Wolfgang.

Toxicity assessment of novel drug candidates is rapidly expanding into the arena of higher throughput, in vitro assays. This trend requires validated molecular endpoints, as well as sensitive and robust assays. Monitoring changes in gene expression, especially at the level of mRNA, is one area of current focus. The Quantigene Assay (bDNA) has been used to: 1) screen compounds from a combinatorial library, 2) search for novel molecular endpoints of hepatotoxicity, and 3) investigate the mechanism of cisplatin toxicity. A validation subset of combinatorial chemistry compounds was screened for changes in HSP70 and GADD45 mRNA levels. There was a significant correlation between compound structure and up-regulation of these genes and cytotoxicity. To identify new endpoints, the liver specific genes cytochrome P450 3A1 and quinone reductase as well as the oxidative stress gene, hemeoxygenase were monitored in rat hepatoma cells in response to hepatotoxins such as acetaminophen and valproic acid. Induction of CYP3A4 or QR did not correlate with hepatotoxic damage while HMO-1 was induced by most of the hepatotoxins tested. To delineate the mechanism of toxicity of cisplatin, several genes, including HSP70, c-fos, and GADD45, were monitored in target and non-target cell populations. Differences in c-fos expression and cell cycle transit were detected between target and non-target cells. These differences may help explain the greater sensitivity of the target cells to cisplatin. Overall, the studies presented here exemplify innovative ways in which molecular toxicology can be integrated into the drug discovery process.

3 MODERATE AND HIGH THROUGHPUT STRATEGIES FOR DETERMINING HEPATIC EFFECTS OF PHARMACEUTICAL LEADS.

R G Ulrich. *Strategic and Exploratory Sciences, Abbott Laboratories, Abbott Park, IL, USA.* Sponsor: M J Cunningham.

The early refinement of pharmaceutical leads can reduce drug developmental costs and enhance the potential for success. Adverse hepatic effects (toxicity and effects on drug metabolism) are commonly encountered developmental issues. While information-intensive platforms such as chip technologies and traditional investigative approaches can provide a mechanistic basis for hepatic effects for selected compounds, the evaluation of large numbers of leads for reduced adverse effects or structure-activity relationships requires the selection of precise, mechanism-linked endpoints and an assay platform that uses milligram quantities of chemical leads. Using primary cultured hepatocytes, moderate throughput, fluorescence-based assays for the detection of organelle functional changes such as phospholipidosis or mitochondrial dysfunction can be used to guide chemical synthesis and select leads with reduced toxicity. Often, changes in cell function are accompanied by changes in gene expression that can be measured quantitatively using hybridization strategies. High throughput gene expression-based assays using radiolabeled antisense riboprobes and scintillation detection or oligonucleotide, enzyme labeled branched DNA and chemiluminescence detection can be used to identify and screen for cellular responses including cell growth and death, cytochrome p450 induction and peroxisomal proliferation. By comparing results obtained from rat hepatocytes with those obtained from human hepatocytes, some prediction of potential human responses or susceptibility is also possible.

4 EVALUATING DRUG MECHANISMS AND TOXICITIES BY MICROARRAY EXPRESSION ANALYSIS.

D A Jones and A R Karpf. *Molecular Pharmacology Division, Huntsman Cancer Institute, Salt Lake City, UT, USA.* Sponsor: M J Cunningham.

Recent advances in cancer biology offer exciting new prospects for developing novel classes of chemotherapeutics that may reduce cancer mortality. These advances are coupled to rapid elucidation of the expressed human genome by means of high-throughput genomics and bioinformatics technologies. One such technology promises to advance our understanding of the pharmacology and toxicology of cancer chemotherapeutics. Microarray expression analysis allows quantitative measurement of thousands of transcripts simultaneously. This creates a comprehensive transcript profile that permits study of cellular responses in comparison to the majority of a cell's expressed genome. We have produced cDNA microarrays that encompass genes putatively involved in tumor cell chemotherapeutic responsiveness and toxicities. We are using these arrays to generate comprehensive expression profiles of cultured cells responding to current and candidate anti-tumor agents. This profiling has identified signature gene expression patterns that parallel chemotherapeutic induction of apoptosis. The presence or absence of these specific signature gene sets in cells may provide a diagnostic clue that predicts chemotherapeutic mechanisms and toxicities. This predictive ability may reveal drug toxicities early in development and could, therefore, accelerate the assessment and selection of clinical candidates.

5 THE POTENTIAL OF TRANSCRIPT AND PROTEIN PROFILING FOR THE ASSESSMENT OF CHEMICALLY-INDUCED TOXICITY.

J D Tugwood[1], I Kimber[2], G J A Oliver[1], T C Orton[1], and W D Pennie[2]. [1]*Safety of Medicines Group, Zeneca Pharmaceuticals and* [2]*Zeneca Central Toxicology Laboratory, Macclesfield, U.K.*

Many laboratories are currently exploring genomics and proteomics technologies as a means of obtaining biological information from a variety of in vitro and in vivo systems. A range of analytical methods is being developed to facilitate this, including cDNA and oligonucleotide-based microarrays, and high resolution electrophoresis coupled with mass spectrometry. While the potential for generating useful data with these technologies is great, a number of fundamental issues need to be addressed, particularly if they are to be used with the aim of evaluating chemical safety. These include whether small changes in low-abundance transcripts and proteins can be measured accurately and reproducibly, which experimental approaches are most appropriate for microarray analyses, and how best transcript and protein profiles can be deployed for assessment of adverse health effects. At Zeneca, we are tackling these and other issues through a combination of internal programmes and strategic collaborations with other companies developing the necessary technologies. Some of our initial applications of this technology are aimed at improving prediction of genotoxicity, understanding disruption of normal hormonal signalling by environmental estrogens, and exploring mechanisms of hepatocarcinogenesis. Data from both transcript and protein profiling experiments will be presented and discussed.

6 CELL CYCLE CHECK-POINTS AND CHEMICAL-INDUCED STRESS RESPONSE: SURVIVAL VERSUS DEATH.

A-N T Kong. *College of Pharmacy, University of Illinois at Chicago, Chicago, IL, USA.*

Cellular responses to toxicants, radiation, and other environmental insults can result in cell cycle arrest, proliferation, differentiation, homeostasis, apoptosis, or necrosis. Checkpoints arrest cell cycle progression before DNA replication (G1 checkpoint) or mitosis (G2 checkpoint) when the integrity of the genome is compromised either as a consequence of DNA damage, perturbed metabolism/signaling, or programmed cellular events such as senescence. Many of these checkpoints are deregulated in cancer. Recent advances in gene-targeted mice that lack these cell cycle regulators, p21(WAF1/CIP1) and p27Kip1 cyclin-dependent kinases (CDK) inhibitors showed the important functions of p21 in G1 checkpoint and p27 in replicative capacity of differentiation-associated cell divisions. Furthermore, after the hepatic toxicant carbon tetrachloride-induced liver cell death and regeneration, p21 CDK inhibitor showed two waves of expression. The first occurred in the damaged pericentral region, in conjunction with early proto-oncogene expression, the second occurred later, consistent with post-mitotic expression after regeneration was completed. The persistent effects of radi-

ation- or xenobiotic-induced DNA damage on genetic stability and fitness in yeast appeared in the progeny of survivors as many as 50 generations after exposure, implying mechanisms other than carry-over DNA damage or mutations at specific loci. Integration of signaling transduction pathways in response to cellular stress is critical for cell survival or death. Various chemicals can induce differential activation of the mitogen-activated protein kinases (MAPK) and ICE/Ced-3 proteases (caspases). Studies with phenolic compounds showed that low concentrations activated MAPK leading to gene expression and cell survival, whereas higher concentrations activated caspases leading to apoptosis. In summary, this symposium will focus on the cell cycle checkpoints, genetic stability, cell death and cell survival. The information from this symposium may be of importance in assessing the potential risks of environmental toxicants leading to cellular injury.

7 ROLES OF CYCLIN-DEPENDENT KINASE INHIBITORS IN CELL CYCLE CHECKPOINTS: LESSONS FROM KNOCKOUT MICE.

H Kiyokawa. *Cancer Center, University of Illinois, Chicago, IL, USA.* Sponsor: A-N T Kong.

Cyclin-dependent kinases (CDKs) form the central machinery of the cell cycle. The serine/threonine protein kinase activity of each CDK depends on the association of the regulatory cyclin subunit, and is subject to negative regulation by inhibitory subunits, known as CDK inhibitors. In mammalian cells, cyclin D/CDK4 (or CDK6) and cyclin E/CDK2 regulate the G1 to S transition, cyclin A/CDK2 controls S phase progression, and cyclin B/CDK1 is essential for the G2 to M transition. When genomic stability is compromised by DNA damage, perturbation of the nucleic acid metabolism, or shortened telomere, checkpoint mechanisms suppress the CDK activity to cause cell cycle arrest in either G1 or G2. The CDK inhibitors, including $p21^{WAF/Cip1}$ and $p27^{Kip1}$, are involved especially in the G1 checkpoint. DNA damage or perturbation of nucleotide pool induces the transcription of p21 in a p53-dependent manner. It has been shown that p21-knockout mice develop normally, but p21-deficient cells in culture are impaired in their ability to undergo G1 arrest following γ-irradiation or treatment with PALA, an inhibitor of uridine synthesis. These data are consistent with the G1 checkpoint function of p21. p27 has a unique function programmed in a cell-intrinsic fashion. p27-knockout mice, we previously created, exhibit a gigantism-like phenotype. Our studies suggest that p27 determines the replicative capacity of differentiation-associated cell divisions. p27-deficient cells undergo additional cell divisions during differentiation before they finally arrest in G0, ending up with increased numbers of differentiated cells. In contrast to p21-deficient cells, p27-deficient cells have normal sensitivity to γ-irradiation. However, p27-deficient cells are partially resistant to rapamycin- or TGF-β-induced growth arrest, suggesting that p21 and p27 regulate distinct types of G1 checkpoints. Our comparative studies on proliferation and apoptosis of p21- and p27-deficient cells will be further discussed.

8 $p21^{(WAF1/CIP1)}$ EXPRESSION IN THE LIVER FOLLOWING CARBON TETRACHLORIDE ADMINISTRATION.

A L Tyner, A L Gartel, E Goufman, M Feuerman, and M S Serfas. *Dept. of Molecular Genetics, University of Illinois College of Medicine, Chicago, IL, USA.* Sponsor: A-N T Kong.

The cyclin dependent kinase inhibitor (CKI) $P21^{(WAF1/CIP1)}$ can induce G1 arrest, and its expression coincides with the cessation of replication in many systems. To address the role of p21 during liver regeneration, we examined p21 expression using in-situ hybridization and RT-PCR, following administration of carbon tetrachloride, which induces liver cell necrosis. We found that carbon tetrachloride-induced liver damage leads to two waves of p21 expression. The first occurs in the damaged pericentral region, in conjunction with early proto-oncogene expression. The second wave of p21 expression occurs later, and is consistent with post-mitotic expression after regeneration is complete. In the healthy adult liver we also detected p21 expression in a few cells in a pattern that was suggestive of rare post-mitotic expression. Since the tumor suppresser protein p53 has been shown to activate p21 gene expression following DNA damage, we examined p21 expression in p53 deficient mice following administration of carbon tetrachloride. In these animals the early wave of pericentral specific p21 induction was still detected, indicating that activation of p21 transcription is not p53 dependent. These data suggest a role for p21 expression subsequent to normal cell division in normal and regenerating liver. As an inhibitor of cell replication and a component of a cell cycle checkpoint, p21 expression may generally precede necrosis in cells subjected to damage.

9 THE EFFECT OF RADIATION AND XENOBIOTIC-INDUCED DNA DAMAGE ON GENETIC STABILITY IN YEAST.

R J Brennan and R H Schiestl. *Dept. Cancer Cell Biology, Harvard School of Public Health, Boston, MA, USA.*

The development of a "hypermutable" phenotype is thought to be a critical component of the multistage development of an invasive tumor from a normal somatic cell. Cultured mammalian cells which survive exposure to ionizing radiation and other DNA damaging agents often subsequently develop a genomic instability reminiscent of that observed in tumor cells. Such delayed effects can include a reduced plating efficiency, an increased frequency of chromosome abnormalities, increased levels of genetic recombination and an increased mutation frequency. We have studied the development of similar phenotypes in the yeast Saccharomyces cerevisiae. Following exposure to gamma radiation, a high proportion of colonies derived from surviving cells displayed significantly elevated frequencies of genetic deletions up to 50 generations post-irradiation. Other phenotypes observed included elevated frequencies of forward mutation and low plating efficiency. The appearance of High Frequency of Deletion (HFD) clones was dose-dependent and the elevated deletion frequency was dominant when HFD cells were mated with unexposed cells. Deletion frequencies and forward mutation frequencies appeared to be correlated. These delayed effects cannot be explained by the carry-over of DNA damage, due to the large number of generations between exposure and observation; nor can they be due to mutations at specific loci, as they appear in a significant proportion of survivors. Similar phenotypes were also observed following exposure to other DNA damaging agents (UV, alkylating agents, oxidants). Elucidating the mechanism by which these effects arise may shed light on the development of genomic instability in human cancers.

10 CHEMICAL-INDUCED DIFFERENTIAL ACTIVATION OF MAPK AND CASPASES IN CELL SURVIVAL AND DEATH.

A-N T Kong, R Yu, S Mandlekar and L Wei. *College of Pharmacy, University of Illinois at Chicago, Chicago, IL, USA.*

Cellular responses to chemical-induced stress can signal proliferation, differentiation, hemeostasis, apoptosis or necrosis. To better understand the underlying molecular mechanisms after exposure to chemicals, we investigated the signal transduction pathways, the mitogen-activated protein kinase (MAPK), and the ICE/Ced-3 protease (caspase) pathways, activated by different agents. Butylated hydroxyanisole (BHA) and its metabolite, t-butylhydroquinone (tBHQ), both are well known phenolic antioxidants (PAs) used as food preservatives, strongly activated c-Jun N-terminal kinase 1 (JNK1) and/or extracellular signal-regulated protein kinase 2 (ERK2) in a time- and dose-dependent fashion. Free radical scavengers N-acetyl-L-cysteine, or glutathione, inhibited ERK2 activation and, to a much lesser extent, JNK1 activation by PAs, implicating the role of oxidative stress. Under conditions where MAPKs were activated, PAs also activated transcription factors NF-(B, AP-1, or ARE (antioxidant response element), with the induction of early genes c-jun, c-fos, and Phase 2 genes GST, NQO. Increasing the concentrations of PAs, the activities of caspases were stimulated and resulted in apoptotic cell death. Further increase in concentrations led to rapid cell necrosis. Other phytochemicals including phenylethyl isothiocyanate (PEITC), sulforaphane, and green tea polyphenols, also differentially regulated the activities of MAPKs or caspases. A model is proposed, that at low concentrations, these chemicals (BHA, PEITC) activate MAPK pathway leading to induction of gene expression which may protect the cells against toxic insults and enhance cell survival. At relatively high concentrations, these agents activate both MAPK, and the caspase pathways, leading to apoptosis. The balance between these signaling pathways may dictate the fate of cells upon exposure to chemicals. (Supported by NIH grants ES06887 and GM49172.)

11 COGNITIVE TESTS: INTERPRETATION FOR NEUROTOXICITY?

W Slikker, Jr. and B D Beck. *Division of Neurotoxicity, National Center for Toxicological Research, Jefferson, AK, USA and Gradient Corporation, Cambridge, MA, USA.*

The aim of this workshop is to help provide a scientific basis for the appropriate interpretation of cognitive tests with respect to neurotoxicity and human health risk. Cognitive tests should measure the total functional output of the nervous system, yet cognitive function cannot be directly

observed. The lack of direct measures makes interpretation of cognitive tests problematic. The Intelligence Quotient (IQ), for example, reflects factors in addition to intelligence, such as socioeconomic status; the difficulty in interpreting cognitive function tests in humans exposed to neurotoxicants is thus complicated by a range of factors, such as the adequacy of control of confounders. Difficulty in test interpretation is an issue with species other than humans. To overcome these problems, sophisticated assessment tools have been developed to assess selected aspects of complex brain function (cognition) and their alteration by toxicant exposure. Improvement of the interpretation of animal cognitive function tests has resulted from data generated from carefully designed operant and non-operant problem solving tasks, especially those that can be modeled in both animals and humans. These include delayed matching to sample (short-term memory); repeated acquisition (learning); temporal discrimination (timing ability); and progressive ratio (motivation). Interpretation of cognitive test results in exposed humans with respect to causation is enhanced by consistency with results from animal species. Challenges still remain regarding interpretation of results with respect to adverseness of effect. This workshop will discuss: the range of cognitive tests available; their use and reliability in different species for predicting neurotoxicity; and the appropriate interpretation of such tests with respect to overall function, including whether an effect is adverse or not.

12 ASSESSMENT OF COMPLEX COGNITIVE FUNCTION IN RODENTS AND EXTRAPOLATION ACROSS SPECIES.

D A Cory-Slechta. *Department of Environmental Medicine, University of Rochester Medical School, Rochester, NY, USA.*

Cognitive function subsumes three overlapping and interactive behavioral processes: learning, memory and attention. The elaboration of a learning impairment in the rodent and its differentiation from other interpretations is most easily achieved using repeated learning paradigms in conjunction with a performance-only assessment. Potential behavioral and neurobiological mechanisms of a learning impairment can be further determined using carefully designed probe procedures in such paradigms. Delayed match-to-sample procedures offer generally the same precision and interpretative facility within a memory context. Both approaches exhibit substantial cross-species generality of the associated performance. Attention deficit remains a global behavioral construct with no unifying theoretical basis. However, reaction time and vigilance paradigms are useful for evaluating sustained attention across species. Aversion to delays has recently been suggested as the basis for attention deficits and can be evaluated across species using delay of gratification and self-control approaches. Interpretation of such data can be difficult if important experimental variables are not controlled. The use of these approaches to cognitive function across species offers not only the soundest basis for precise risk characterization and for elaboration of both behavioral and biological mechanisms of neurotoxicant effects: precise characterization of behavioral deficits in experimental animals can then to be compared to effects observed in human populations with known exposures, and experimental animal experiments can attempt to mimic the precise behavioral deficits identified in populations with defined neuropsychiatric or neurodegenerative conditions.

13 ASSESSMENT OF COMPLEX BRAIN FUNCTION IN BOTH NON-HUMAN PRIMATES AND HUMANS USING IDENTICAL BEHAVIORAL TASKS.

M G Paule. *National Center for Toxicological Research, Jefferson, AR, USA.*

The use of animal models for predicting the potential effects of neuroactive agents on complex brain functions in humans is critical. This need has led to the development of automated systems for administering identical behavioral tasks to both animals and humans. Maintaining task continuity across species allows for direct observations of interspecies similarities and differences in a variety of functional domains and should assist in the extrapolation of data from lab animals to humans. Data from identical operant behavioral tasks, designed to require responses thought to depend upon specific functions including motivation, color and position discrimination, time estimation, and short-term memory, and learning, have been obtained from both rhesus monkeys and children. Remarkable interspecies comparabilities portend the utility of such an approach in neurotoxic risk assessment procedures.

14 ASSESSING THE EFFECTS OF NEUROTOXICANTS ON CHILDREN'S COGNITION.

D Bellinger. *Children's Hospital, Boston, MA, USA.* Sponsor: W Slikker, Jr.

Most studies evaluating the cognitive effects of environmental exposures on children employ test batteries consisting of tests of global (or apical) function (e.g., IQ, general development), supplemented by tests of specific domains (e.g., language, attention, visual-motor integration). This presentation will address several issues that need to be considered in the analysis and interpretation of such data: (1) apical test scores represent final common pathways, so they may be sensitive to neurotoxicant exposure but lack specificity; (2) apical test scores generally reflect the product of a child's past learning rather than the process of learning, providing relatively little insight into behavior mechanisms; (3) apical test scores are influenced by many biological, sociological, and psychological factors, creating the potential for misattribution of effect (e.g., confounding bias) and/or effect modification; (4) the supplementary tests used are often not well matched in terms of psychometric characteristics, complicating the effort to draw interferences about the relative magnitude of a neurotoxicant's impact on different aspects of cognitive function; (5) traditional neuropsychological tests are administered in the context of an interaction between examiner and child, requiring close attention to issues of quality control in test administration.

15 HUMAN NEUROBEHAVIORAL TEST METHODS FOR STUDYING NEUROTOXICITY IN WORKING POPULATIONS.

W K Anger, D S Rohlman, and D Storzbach. *Oregon Health Science University, Portland, OR , USA.*

Tests of cognitive performance have been widely applied in human behavioral neurotoxicology research since clinicians initiated workplace or field studies in the 1960s. Reports of statistically significant differences between workers and controls reveal consistent but unique patterns of cognitive deficits associated with chronic exposures to metals (e.g., lead, mercury), organophosphate pesticides, and solvent mixtures, the agents/categories for which there is a sufficient body of evidence to establish replication. Those deficits are consistent with reports of human poisonings and animal research on the same chemicals. Evidence of the impact of human subject variables (e.g., age, education, gender, motivation) on standard neurobehavioral tests used in this field establishes the importance of these variables in selecting appropriate comparisons (controls or eferencts) just as do parallel factors in animal research. The impact of parameters of human tests (e.g., match-to-sample, selective attention task, progressive ratio) that are comparable to tests employed in animals when studying neurotoxic chemicals will be described. The sensitivity of human cognitive tests to low-concentration chemical exposures will be discussed, citing evidence from recent research on occupational groups (e.g., orchard workers) and veterans returning from the Persian Gulf.

16 RELATIONSHIPS BETWEEN BIOPERSISTENCE, IN VITRO DISSOLUTION RATE, AND FIBER TOXICITY.

D M Bernstein[1] and T W Hesterberg[2]. [1]*Consultant in Toxicology, Geneva, Switzerland;* [2]*Johns Manville Corporation, Littleton, CO, USA.*

For many years, inorganic fibers have been viewed with suspicion as potential pulmonary toxins. However, extensive new research reveals that inorganic fibers encompass a broad range of biological reactivity, from innocuous to pathogenic. The fibrous shape is problematic in that it permits penetration into the lower lung of very long particles that cannot be fully phagocytized and cleared by macrophages. If the fiber contains a high proportion of soluble components, it quickly dissolves in the lung environment and thus has little time to induce toxicity. Research suggests that only those fibers that persist in the lung can lead to disease. In contrast to asbestos, synthetic vitreous fibers chemically and physically degrade in the lung relatively quickly, with the new generation of vitreous fibers clearing from the lung within weeks or days. This workshop focuses on fiber biopersistence as a determinant and predictor of fiber toxicity and examines the possible mechanisms of biopersistence, including dissolution rate. Fiber biopersistence research is also discussed as the scientific basis for fiber classification schemes adopted by the Europe Union and Germany and those that are currently being developed in the US.

17 SCIENTIFIC BASIS OF BIOPERSISTENCE METHODS TO CHARACTERIZE THE CARCINOGENIC POTENTIAL OF FIBERS.

G Oberdörster. *University of Rochester, Department of Environmental Medicine, Rochester, NY, USA.*

The assumption that biopersistence of a fiber in the lung correlates with its long-term toxicity and carcinogenicity is well supported by experimental data. Methods to determine pulmonary biopersistence include fiber administration in rats by intratracheal instillation or by inhalation over a five-day period. The subsequent disappearance of fibers in the lung is due to physical (cellular removal; breakage) and chemical (dissolution; leaching) processes. Since there are significant species differences (rat vs. human) of these different processes, the extrapolation of results from rat biopersistence studies to humans may not be as straightforward. The focus is on fibers > 20 μm in length. The problem for inhalation of these fibers is that respirability in rats is very poor compared to humans. Intratracheal instillation results in other problems, such as clumping of the fibers, especially if long ones are present, and induction of significant inflammatory reactions when high doses (>1 mg) are delivered. Although the inhalation method is not without problems when it comes to administering long human respirable fibers, it is still superior to intratracheal instillation which is prone to influences by artifacts. However, a promising method combining the advantages of inhalation and instillation and avoiding their disadvantages is the use of intratracheal inhalation in rats. This method has successfully been used to deliver particles to the alveolar region for determining long retention halftimes, it circumvents the rat's nasal filter and can deposit human respirable fibrous and non-fibrous particles into the pulmonary region of the rat. The method is easy to establish and should be tested for its suitability for biopersistence testing.

18 THE BIOPERSISTENCE OF SYNTHETIC VITREOUS FIBERS: A CRITICAL DETERMINANT OF THE BIOLOGICAL ACITIVITY OF FIBERS.

J G Hadley and W Eastes. *Owens Corning Science and Technology Center, Granville, OH, USA.*

The lung has highly efficient defense systems to protect the gas exchange surfaces from inhaled particles. Airway geometry precludes large particles from entering the deep lung and those that do penetrate can be readily phagocytized by alveolar macrophages. The fibrous shape is unique in being able to penetrate the deep lung with a dimension (length) which precludes effective macrophage ingestion. Absent a removal mechanism, long fibers, if durable and in adequate concentrations will accumulate and lead to disease. Identifying the relationships between fiber composition, in vitro and in vivo durability, and chronic effects has led to the development of a simple predictive model of the biological effects of inhaled fibers based on their composition. This understanding of the critical nature of fiber durability provides guidance for fiber manufacturers to assure the safety of their products and has important implications for the regulation of synthetic vitreous fibers.

19 IMPORTANCE OF THE BIOPERSISTENCE OF MINERAL FIBERS TO FIBER TOXICITY.

H Muhle and B Bellmann, and O Creutzenberg. *Fraunhofer Institute of Toxicology and Aerosol Research, Hannover, Germany.*

The biopersistence of various synthetic mineral fibers (glass, stone, ceramic) was investigated after 5day inhalation exposure or intratracheal instillation of rats. The test protocols proposed for regulation in the European Community were used (ECB/TM/26 and ECB/TM/27). Only a limited database is available to correlate the biopersistence to chronic toxicity data. Some information exists for chronic inhalation studies and after intraperitoneal instillation. Studies after inhalation exposure for up to 90 days and a post-treatment observation period are useful to investigate the inflammatory potential, the proliferation rate of the bronchial and alveolar epithelia, and the impact on the macrophage mediated clearance. Such studies were performed with MMVF 21 and RCF 1. Further studies are currently performed. For soluble fibers only transient effects are seen whereas for persistent fibers the effects are more pronounced.

20 COMPARISONS OF LUNG CLEARANCE MECHANISMS OF TWO ORGANIC FIBER-TYPES: IN VITRO AND IN VIVO MECHANISTIC STUDIES.

D B Warheit. *DuPont Haskell Lab, Newark, DE, USA.*

Studies were undertaken to investigate the mechanisms through which inhaled p-aramid fibrils (RFP) are biodegraded in the lungs of exposed rats and hamsters; in contrast cellulose fibers are biopersistent. In earlier 2-week inhalation studies, rats were exposed to RFP (419 or 772 f/ml) or to Thermocell mechanical wood pulp cellulose fibers (300 and 575 fibers/ml). Hamsters were exposed to RFP concs. of 358 and 659 f/ml. The lungs of fiber-exposed animals were assessed over time for clearance and biopersistence. p-Aramid was biodegradable, as evidenced by progressive shortening of inhaled RFP (mean RFP lengths decreased from 12.5 um to 7 um with increased residence time in the lung). In contrast, lung clearance of cellulose was slow with mean lengths and no shortening of retained fibers. To investigate the mechanisms of RFP biodegradation, we have postulated that lung fluids activate RFP following deposition and are then vulnerable to enzymatic attack in the lungs. To test the hypothesis, RFP or cellulose were instilled into the lungs of rats and the lungs digested 24 hrs postexposure using two different digestion techniques, 1) a conventional ethanolic KOH method, and 2) an enzyme method (which simulates lung enzymes). The enzyme but not KOH method artificially cleaved the RFP recovered from rat lungs. Next, RFP or cellulose fibers were incubated with saline or lung fluids and then processed by the 2 digestion techniques. Mean lengths of RFP processed with KOH and evaluated by SEM were 13.4 um; in contrast, mean lengths of RFP samples, incubated in lung fluids and treated with the enzyme method were 8.8 um. The enzyme digestion method had no discernible effect on shortening of cellulose fibers, indicating that the results with p-aramid were specific. Our results suggest that components of lung fluids coat and catalyze the p-aramid, rendering the fibrils vulnerable to enzymatic attack, and this could result in the mechanism of transverse cleavage of inhaled p-aramid fibrils in the lungs of exposed rats and hamsters.

21 EFFECTS OF LEAD ON CELLULAR AND HUMORAL IMMUNE RESPONSE OF ENVIRONMENTALLY EXPOSED CHILDREN.

A P Pineda-Zavaleta, G García-Vargas, A Gómez-Muñoz, V H Borja-Aburto, E Vera, M E Cebrián and E S Calderón-Aranda. *Sección de Toxicología Ambiental, CINVESTAV IPN, México DF.*

Lead has different toxic effects on immune system as suppressed cell mediated immunity or/and enhanced humoral response has been reported in humans occupationally exposed and animals. Thus, we investigated the immune response in 65 children having blood lead concentrations (PbB) ranging from 3 to 50 μ g/dl. Children were divided in three groups according to their PbB: low (PbB ≤ 10 μ g/dl), medium (10 < PbB < 25 μ g/dl) and high exposure (PbB ≥ 25μ g/dl). The cell mediated response was evaluated as lymphoproliferation to tetanic toxoid (Txt) or to phytohaemagglutinine (PHA). Humoral response was evaluated by quantitating Txt specific immunoglobulines G (IgG) after programmed immunization. Finally, we evaluated unspecific responses, such as nitric oxide (NO) and superoxide (O_2) production by macrophages, activated either directly with interferon-γ and lipopolysaccharide or indirectly with lymphocytes and PHA. Production of $O_2^-$was directly related to PbB. The IgG titer was also directly related to PbB up to 22 μ g/dl, suggesting an enhanced Th_2 activity, and then decreased without reaching control group values. This course of action suggests that at high PbB, cells are downregulated or too damaged to activate or produce antibodies. The NO production, by indirect activation, was inversely related to PbB, suggesting a decreased Th_1 activation, whereas no significant impairment was observed upon direct activation. There were no significant associations among PbB and the other parameters evaluated. Thus, lead exposure could result in two different immunotoxic effects on children: 1) The altered cell mediated NO production could prevent an efficient response to intracellular parasites and 2) The increase in specific IgG titers, suggest that lead may induce autoimmune responses by enhancing autoantibodies production by a mercury-like mechanism.

22 EMBRYONIC EXPOSURE TO LEAD MODULATES TH1/TH2 ASSOCIATED IMMUNE FUNCTIONS AND IS INFLUENCED BY DEVELOPMENTAL STATUS.

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Following a prior developmental immunotoxicology study in rats, the current study utilized a second species, the chicken, to examine potential direct effects of lead on the developing immune system. Lead acetate at sublethal doses or control solution (sodium acetate) was administered to fertilized Cornell K strain White Leghorn eggs via the air sac at day 5 or day 12 of embryonic development. Immunological assessment was performed in female chickens at 5-6 weeks of age. The day 5 lead exposed group (10 ug Pb/egg dose) showed significantly elevated anti-BSA antibody production with no change in the delayed-type hypersensitivity (DTH) response to BSA when compared with the control. In contrast, day 12 lead exposed groups (100, 200, 400 ug pb/egg doses) demonstrated both increased anti-BSA antibody production and depressed DTH response to BSA compared with that of the control. Nitric oxide production from macrophages was significantly decreased in day 5 (10 ug pb/egg dose) and day 12 (50 ug pb/egg dose) lead treated groups. This present study suggests that lead has direct persistent immunotoxic effects on the developing immune system in the absence of the maternal system; the range of effects is influenced by the developmental stage of exposure. The results support the hypothesis that embryonic exposure to lead disrupts the Th1/Th2 balance by inducing a strong Th2 response (antibody production) and/or a suppressed Th1 response (DTH response). (Supported by NIEHS grant #ES05950.)

23 MERCURY: EFFECTS ON IMMUNE RESPONSE IN MICE MAY INVOLVE GLUTATHIONE DEPENDENT MECHANISMS.

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Immunotoxic effects of mercury (Hg) are well described, although most research has dealt with Hg-induced autoimmunity. As reported at SOT 98, we have found that Hg impairs murine resistance to *Plasmodium yoelii*, a murine malaria. In these studies, we examined the effects of Hg exposure on acquisition of immunity to malaria. Male and female BalbC and CD1 mice were exposed to $HgCl_2$ (100 µg/kg, s.c.) every other day for 11 days. For immunization, they were administered (i.v.) irradiated sporozoites of *P. yoelii* on days 12 and 26 On day 40, they were challenged with live sporozoites. Exposure to Hg, over 5 weeks earlier, blocked acquisition of immunity to *P. yoelii*. Mechanisms of these effects are unknown. In general Hg inhibits Th1 and upregulates Th2 responses; consistent with this, we found that exposure of mouse splenocytes or human monocytes to 10^{-9} to $^{-6}$ M $HgCl_2$ in vitro inhibits production of interferon γ and increases production of IL-4. Hg also blocks LPS-induced nitric oxide synthase. These are important elements of host response to malaria and may explain at least in part decreased resistance. Depletion of cellular glutathione by DEM in vitro also inhibits interferon and nitric oxide production. This suggests that immunotoxic effects of Hg may involve interactions with thiol proteins. The mechanisms by which Hg interferes with the acquisition of immunity are not known. (Supported by grants from the W Alton Jones and Heinz Family Foundations.)

24 EFFECTS OF *IN VIVO* EXPOSURE TO HEXACHLOROBENZENE AND *TRICHINELLA SPIRALIS* INFECTION ON CYTOKINE EXPRESSION AND PRODUCTION BY CULTURED SPLENOCYTES FROM LEWIS AND BROWN NORWAY RATS.

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In a previous study, exposure of Wistar rats to the immunotoxic compounds bis(tri-*n*-butyltin)oxide, hexachlorobenzene (HCB), and benzo(a)pyrene was found to affect IL-2, IFN-γ, and IL-2R α -chain (IL-2Rα) mRNA expression. No exposure effects on IL-4 expression were found. To study whether this lack of effect was due to the nature of the compound, the rat strain used, or the parameter tested, Lewis (TH1-prone) and Brown Norway (BN; TH2-prone) rats were exposed to various doses of HCB for 6 weeks. HCB exposure was previously found to increase serum IgE levels, an IL-4 dependent response, in BN but not Lewis rats. As a positive control for expression and production of TH1 and TH2 cytokines, BN rats were infected with *Trichinella spiralis*. Spleen cells were cultured with Con A and analyzed for IL-2, IFN-γ, IL-2Rα , IL-4, and IL-10 expression, and IFN-γ, IL-4, and IL-10 production. HCB exposure resulted in Lewis rats in reduced IFN-γ and IL-10 expression and reduced IFN-γ , IL-4, and IL-10 production, with no or small effects in BN rats. *T. spiralis* infection resulted in increased IFN-γ , IL-4, and IL-10 expression and increased production of IL-10 and IFN-γ . Remarkably, IL-4 production was strongly reduced. In both studies, however, IL-4 production was below or around the detection limit. In sum: (1) A good correlation exists between cytokine expression and production for IFN-γ and IL-10. (2) IL-10 seems more useful than IL-4 as a parameter for TH2 cytokines. (3) A poor correlation exists between effects on IL-4 and effects on IgE. (4) Lewis, BN, and Wistar rats (data for Wistar are obtained from earlier observations) show different response profiles after HCB exposure, with Lewis and BN being more sensitive than Wistar. (5) *T. spiralis* infection induces both the TH1 cytokine IFN-γ and the TH2 cytokine IL-10.

25 AFLATOXIN B_1 EXPOSURE IMPAIRS HOST RESISTANCE THROUGH DEFECTS IN MACROPHAGE DERIVED CYTOKINE PRODUCTION.

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Aflatoxin B_1 (AFB_1), is a well characterized environmental carcinogen. In addition to its known carcinogenic endpoint, AFB_1 has been demonstrated to be immunosuppressive. We have previously shown that male Fisher 344 rats intratracheally exposed to AFB_1 (750 µg/kg) and subsequently challenged with *Listeria monocytogenes*, are unable to clear the systemic infection as compared to controls. These data demonstrate that there is a functional impairment in cell populations intrinsic to infectious immunity resulting from an acute dose of AFB_1. Resolution of infection with *L. monocytogenes* is dependent upon activation of the macrophage system through cytokine interactions, namely TNFα and IFNγ. To investigate the mechanism whereby AFB_1 affects cellular immunity, splenic macrophages were harvested from AFB_1-treated rats, cultured *in vitro* with LPS 2 days following treatment, and TNFα production was subsequently quantitated by ELISA. There was a striking suppression (54% of controls) of macrophage TNFα production from AFB_1-treated animals, whereas splenic lymphocytes from AFB_1-treated animals showed no difference in Con A-stimulated IFNγ production. These data demonstrate that there is a defect in the macrophage mediated immune response rather than an impairment in T cell function. Similarly, there was an equal degree of TNFα suppression (50%) when splenic macrophages were isolated from untreated rats and exposed *in vitro* to 30µM AFB_1 for 24 hr, as compared to cells treated with media alone. These data suggest that AFB_1 is metabolized by the macrophage itself to a reactive intermediate which then results in functional impairment. We conclude that the mechanism of AFB_1-induced immunosuppression involves a defect in macrophage secretion of the proinflammatory cytokine TNFα.

26 IMMUNOTOXICITY TESTING: A COMBINED LISTERIA IMMUNIZATION/INFECTION MODEL IN WISTAR RATS.

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The Plaque Forming Cell (PFC) assay with sheep red blood cells or an alternative T cell dependent B cell response is generally recommended for testing immunotoxicity. Investigation of other aspects/functions of the immune system require additional studies. A more comprehensive and possibly more sensitive model, reducing the number of studies required, is under development. In this model an immunization procedure using dead, irradiated *Listeria monocytogenes* bacteria with a subsequent infection with live virulent *L monocytogenes* is combined. Female Wistar rats were immunized with dead *L. monocytogenes* bacteria at days 0, 9 and 18. The immunization doses ranged from 10^5 to 10^9 bacteria per animal. All applied doses resulted in clear T cell responses as measured by proliferation assays with spleen cells at day 28. Moreover, administration of especially the higher doses (>10^6 bacteria per animal) resulted also in a marked IgM and IgG (specifically IgG2a) production on days 18 and 28. There was no clear distinction between immunization protocols wich used either two (days 0 and 18) or three (days 0, 9 and 18) applications of the antigen. These findings indicate that the immunization procedure at least may be used as an alternative to the PFC assay.

However, infecting the animals subsequently with live *L. monocytogenes* and determining the survival of the bacteria two days thereafter in liver and spleen not only enables to investigate effects on the innate immunity but also on the adaptive immunity. Currently a validation study is underway in which effects of an immunosuppressive drug (cyclophosphamide) and an immune enhancing compound (the adjuvant alumiumhydroxide) are investigated with this new model. It is expected that the model being developed will be a good and more comprehensive alternative to the PFC in which, besides effects on the T cell dependent B cell response, also effects on the innate immunity and the interaction between the adaptive and the innate immunity can be tested.

27 A MOUSE MODEL OF INFLUENZA EXHIBITS INCREASED MORBIDITY, MORTALITYAND THYMIC ATROPHY ASSOCIATED WITH ALTERED LUNG CYTOKINE PATTERNS FOLLOWING EXPOSURE TO MILD ULTRAVIOLET RADIATION (UVR).

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Low level exposure to dioxin, UVR and ozone increases mortality in mice subsequently infected with influenza virus without increasing virus titers in the lung; little toxicity is induced in uninfected animals. Protective immunity to influenza is not compromised by UVR, even though UVR is known to suppress contact and delayed-type hypersensitivity. In this study, the nature of the increased mortality was investigated using a BALB/c female mouse model exposed to 8.2 kJ/m^2 UVR 3 days prior to infection with mouse-adapted Hong Kong influenza A/68 virus. We hypothesized that UVR exposure, which induces interleukin-10 (IL-10), tumor necrosis factor (TNF) and other mediators, alters cytokine responses to influenza virus. After UVR or control light pre-exposure and subsequent viral or sham infection, groups were sacrificed daily from 0-6 days. The UVR-virus group exhibited increased body weight loss, thymic atrophy and pulmonary erythrocyte congestion compared with the light-virus group. The virus-induced lymphocytic lung infiltrate on day 5 was also dampened by UVR. Lung virus titers and numbers of opportunistic bacteria and elevated serum cortisol levels were similar in both infected groups. No elevation in serum IL-10 or TNF was found. In both lung lavage (BAL) fluid and homogenates (LH), influenza virus caused an increase in TNF, IL-1α , IL-6, IL-10, total IL-12 (p40), and interferon (IFN)-γ , but not IL-12 p70, GM-CSF, IL-4, IL-5, or IL-1β . In LH samples, virus-induced total IL-12 (mostly p40) and IFN-γ were dampened by UVR, whereas IL-10 induction was not altered. In BAL, virus-induced IL-10 and IFN-γ release in the lung on day 5 was decreased by UVR. TNF or IL-1α levels were similar in both BAL and LH from the UVR-virus group and the light-virus groups. IL-6 levels were elevated in the UVR-virus group. The data suggest that alteration of protective cytokines by environmental agents may be a mechanism for increasing the morbidity and mortality to influenza virus in the nonimmune host following primary infection. (This abstract does not reflect EPA policy.)

28 SUPPRESSION OF ALLERGIC IMMUNE RESPONSES TO HOUSE DUST MITE (HDM) IN RATS EXPOSED TO 2,3,7,8-TCDD.

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Exposure to various xenobiotics, including NO_2, diesel exhaust and certain pesticides, has been reported to exacerbate pulmonary allergic hypersensitivity responses. To determine whether TCDD exposure affects allergic immune responses and subsequent clinical disease, Brown Norway rats were injected i.p. with 0, 1, 10 or 30 μ g TCDD/kg 7 d before intratracheal (i.t.) sensitization to semipurified house dust mite allergen (DMA). After 14 d, rats were challenged with DMA and immediate bronchospasm was measured. At this time point and 2 and 7 d later, inflammatory cells in bronchoalveolar lavage fluid (BALF), DMA-specific IgE levels in serum and BALF, and DMA-driven cell proliferation in bronchial lymph nodes and spleen were evaluated. TCDD exposure decreased immediate bronchoconstriction and IgE levels after DMA challenge; 7 d later, DMA-specific IgE responses remained suppressed, while total IgE levels increased in rats exposed to the highest dose of TCDD. DMA challenge significantly increased cellular and biochemical indicators of lung injury, all of which were suppressed by TCDD exposure. The proliferative response of lymph node cells, but not spleen cells, to DMA was suppressed at the highest TCDD dose and the splenic response to Con A was elevated. At present, we have no explanation for elevated total IgE levels. Nevertheless, these data show that TCDD exposure suppresses, rather than increases, local allergic sensitization to HDM and subsequent expression of clinical disease on re-exposure to antigen. (This abstract does not reflect EPA policy.)

29 EXPOSURE TO TCDD SUPPRESSES THE RECRUITMENT OF T CELLS TO THE LUNGS OF C57Bl/6 MICE IINFECTED WITH INFLUENZA VIRUS.

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The immune response to infection with influenza virus is exquisitely sensitive to suppression by halogenated aromatic hydrocarbons such as 2,3,7,8–tetrachlorodibenzo–Dioxin r (TCDD). Exposure of C57Bl/6 mice to TCDD (1–10 μg/kg bodyweight) caused a dose–responsive increase in mortality following infection with a nonlethal strain of influenza virus (A/HKx31). The association between enhanced susceptibility to the pathological effects of influenza virus and TCDD exposure has been reported by other laboratories; however, the mechanism is unknown. It is generally accepted that a positive outcome from a primary infection with influenza virus is dependent upon the generation of an effective cell–mediated response, which includes the activation and differentiation of naive CD8 T cells in the regional lymphoid organs and the subsequent migration of effector cytotoxic T lymphocytes 9 (eCTL) to the lung. We have tested the hypothesis that exposure to TCDD suppresses the activation and differentiation of CD8 T cells, leading to decrease CTL in the lungs. Initial experiments have revealed that exposure to TCDD dose–dependently decreased the recruitment of cells to the lungs in response to influenza virus. Moreover, flow cytometric analyses of bronchoalveolar lavage (BAL) cells showed that, when compared with BAL cells from infected controls, exposure to TCDD decreased the number of CD8 lymphocytes and there was a decrease in the percent of CD8 BAL cells bearing an eCTL phenotype (CD44). However, when compared on a per cell basis, the CTL activity 9 of BAL cells against HKx31–infected targets was equal to that of virus–infected controls, which suggests that exposure to TCDD affects the development of eCTL rather than the eCTL lytic machinery. In conclusion, reduced CTL in the lung are one mechanism which may underlie the enhanced mortality observed following influenza infection and exposure to TCDD.

30 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD) DIFFERENTIALLY ALTERS THE COURSE OF LEISHMANIASIS IN RESISTANT AND SUSCEPTIBLE MICE.

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T helper type (Th)-1-like cellular immune responses can be suppressed by TCDD in mice, although the exact mechanism(s) involved are unclear. In contrast, little is known of the effects of TCDD on Th-2 driven cellular immunity. We used a model of experimental cutaneous leishmaniasis to compare the effects of TCDD on both Th-1 and Th-2 responses. Most mouse strains successfully resist infection with *Leishmania major* via a Th-1 response [interferon (IFN)-γ^{high}, interleukin (IL)-4^{low}], whereas BALB/c mice are lethally susceptible to infection due to an ineffective Th-2 response (IFN-γ^{low}, IL-4^{high}). TCDD was given orally (0, 0.6, 2.5, 10 or 40 μg/Kg) one day prior to infection of each mouse in one rear foot pad with 10^6 *Leishmania major*. Foot lesion size was followed as an index of disease status. Lesions of vehicle-treated C57Bl/6 (B6) mice reached a maximum of ~1.5 mm on day 26 and had completely resolved by day 75. TCDD did not alter maximum lesion size; however, a dose-dependent delay in lesion resolution was observed (a 59 day delay in the 10 μg/Kg dose group). Although the number of *L. major* per lesion varied significantly between dose groups, the variation was not dose-dependent suggesting a lack of a direct TCDD effect on *L. major*. Ex vivo cytokine production by lesion-draining lymph node cells indicated no dose-dependent change in IFN-γ production, whereas IL-4 production decreased in a dose-dependent manner — from 8 pg/mL of supernatant for vehicle-treated mice to undetectable for mice given TCDD at 10 μg/Kg. Other standard dose-dependent effects of TCDD in B6 mice (decreased thymus weights and specific antibody levels) were observed. In contrast to B6 mice, TCDD significantly slowed the progression of lesion development in BALB/c mice. Whereas euthanization of vehicle-treated BALB/c mice was necessary 49 days after infection because of the severity of foot lesions, euthanization of BALB/c mice treated with TCDD at 10 μg/Kg was not necessary until 96 days after infection. We spec-

ulate that TCDD-induced suppression of IL-4 production plays a role in immune modulation in BALB/c mice infected with *L. major*.

31 ABILITY OF SPLENIC DENDRITIC CELLS (DC) FROM 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD)-TREATED MICE TO INDUCE T CELL ACTIVATION EX VIVO.

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Dendritic cells are a population of antigen presenting cells whose primary function is to activate T cells. Previously we reported that *in vivo* exposure of mice to TCDD altered the expression of costimulatory/adhesion molecules on the DC. Specifically, the expression of B7-2, CD40, ICAM-1 and CD24 molecules was enhanced and LFA-1 was suppressed on the DC taken from TCDD-treated mice. In order to evaluate the impact of these phenotypic alterations on DC function, we tested the ability of DC to activate T cells *in vitro*. DC were enriched from spleens of C57BL/6 mice which had been treated with vehicle or TCDD (15μg/kg) 3 days prior to sacrifice. DC were then incubated with either allogeneic T cells or KLH-specific cloned T cells. Proliferation was measured as ^{3}H-TdR uptake. Cytokine production, including IL-2, IFN-γ, and IL-12 in the allogeneic cultures, or IL-4 in the T clone cultures, was measured by ELISA. *In vivo* exposure to TCDD did not suppress the ability of the DC to activate T cells. On the contrary, all responses that were statistically different indicated enhanced activation. T cell proliferation tended to be enhanced and, in one experiment, IL-4, IFN-γ, and IL-12 production was increased. Thus, under the conditions of these experiments, TCDD exposure *in vivo* does not suppress, but may change, the way DC stimulate basic T cell activation. The impact, if any, on immune function remains unknown. (Supported by NIEHS grant #s ES07060 and ES003966.)

32 LIVER PROTEIN ADDUCTS OF QUINONES AND SEMIQUINONES FROM METABOLIC ACTIVATION OF PENTACHLOROPHENOL.

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Metabolic activation of pentachlorophenol (PCP) to chlorinated quinones and semiquinones was investigated *in vitro* in a microsomal or peroxidase mediated activation system and *in vivo* in rodents. Mono-S- and multi-S-substituted cysteinyl adducts derived from tetrachloro-1,2-benzoquinone (Cl_4-1,2-BQ) were identified in liver cytosol treated with PCP *in vitro* and in B6C3F1 mice administered with PCP *in vivo*. Adduct levels increased with increasing dosage of PCP in B6C3F1 mice (0-40 mg/kg body weight). No Cl_4-1,2-BQ-derived liver protein adducts were detected in PCP-treated Sprague-Dawley (0-40 mg/kg body weight) and Fischer 344 rats (60 mg/kg body weight/day in the diet for 6 months). These results confirmed our previous observation that Cl_4-1,2-BQ is a mouse-specific quinone derived from bioactivation of PCP *in vivo*. In parallel, we confirmed that tetrachloro-1,4-benzoquinone (Cl_4-1,4-BQ)-derived adducts in 3-methylcholanthrene and phenobarbital-induced rat and mouse microsomes were 3-7 fold higher than in non-induced microsomes *in vitro* where levels of Cl_4-1,2-BQ, tetrachloro-1,4-semiquinone, and tetrachloro-1,2-semiquinone derived adducts remained unchanged. The K_m for the formation of all PCP-derived adducts in 3-methylcholanthrene-induced microsomes was to 52 μ M for both rats and mice. When cumene hydroperoxide was used as a cofactor, with or without microsomes, the levels of quinone and semiquinone adducts increased by 8-130 fold in rats and 1.5-40 fold in mice compared to using NADPH as a cofactor. Taken together, these results suggest that species differences exist in the metabolism of PCP to quinones and semiquinones, and that cumene hydroperoxide supported bioactivation is the dominant pathway for the conversion of PCP to quinoid derivatives *in vitro* in both rats and mice. (This work was supported by the National Institute of Environmental Health Sciences through grant P42ES05948.)

33 PROTEIN ARYLATION IS NOT BY ITSELF SUFFICIENT FOR HEPATIC NUCLEAR TRANSLOCATION OF THE 58-KDa ACETAMINOPHEN BINDING PROTEIN (58-ABP).

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APAP overdose can result in covalent binding (CVB) of APAP to cellular proteins with subsequent hepatotoxicity. In particular, APAP targets the 58-ABP in the cytosol with subsequent translocation from the cytosol to the nucleus before there are detectable perturbations of the nuclear membrane. Earlier studies demonstrated translocation after a toxic dose but not after a nontoxic dose of APAP. However, the non-toxic dose was also associated with less covalent binding. To determine this translocation occurs with binding in the absence of toxicity male CD-1 mice were administered either multiple non-toxic doses of APAP (400 mg/kg, ip, 2 times/day) for either 2, 4, or 10 days and then killed the following day, or they were administered a single toxic dose of APAP (600 mg/kg, ip) and killed 1, 2, or, 4 hours after challenge. APAP CVB and 58-ABP in cytosolic and nuclear fractions were detected by western blot analysis using anti-APAP antibody or anti-58-ABP antisera, respectively. Bound APAP was detected in cytosolic and nuclear membrane fractions from treated mice, but not controls. By contrast, in mice dosed repeatedly with the nontoxic dose of APAP, by 10 days the level of APAP adduct was similar to that seen after toxic doses yet there was no toxicity and no detectable 58-ABP in the nucleus. Thus when approximately equivalent APAP CVB to cytosolic and nuclear membrane proteins was present in each treatment group, 58-ABP translocation was only observed after a toxic APAP dose. These data suggest that APAP CVB alone is not sufficient for translocation and that translocation may reflect a cellular response to toxic insult. (Boehringer Ingelheim Pharmaceuticals Predoctoral Fellowship in Toxicology, NIEHS E507163.)

34 IDENTIFICATION OF A NOVEL 210 KDA PLASMA MEMBRANE PROTEIN ARYLATED BY ACETAMINOPHEN IN MOUSE LIVER.

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Selective covalent binding of acetaminophen (APAP) to cytosolic, microsomal, mitochondrial and nucleic, but not to plasma membrane proteins has been reported *in vivo*. In these studies, male CD-1 mice were injected *i.p.* with 600 mg APAP/kg. Controls received vehicle only. A mixed canalicular (cLPM) and basolateral (bLPM) plasma membrane fraction was generated by percoll density gradient, and probed for APAP covalent binding by western blotting using anti-APAP antibodies. Alkaline phosphatase activity and western blot analysis of P-glycoprotein served as markers of cLPM enrichment, while western blot analysis of Na+/K+ ATPase was used as a marker of bLPM enrichment. A single protein adduct of approximately 210 kDa was detected on this mixed membrane fraction, with maximal binding detected at 2-4 hrs after APAP challenge. In an attempt to better understand the identity and/or function of this APAP binding protein, mice were injected *i.v.* with 12 μmol/kg indocyanine green (ICG), a non-biotransformable anionic dye mostly excreted into bile, immediately followed by APAP challenge. Interestingly, ICG pretreatment of mice increased APAP binding to this protein. Further studies are needed to determine the function and role of this protein in the development of APAP toxicity. (Supported by Zeneca Pharmaceuticals and UCONN Research Foundation.)

35 A COMPARISON OF THE COVALENT BINDING OF VESNARINONE AND CLOZAPINE TO HUMAN NEUTROPHIL POLYPEPTIDES *IN VITRO*.

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The use of vesnarinone, a novel positive inotropic agent, has been associated with an incidence of agranulocytosis of 0.3 %. We have previously shown that activated neutrophils metabolize vesnarinone to a reactive iminium ion. In this abstract we describe the use of an anti-vesnarinone antiserum to characterize the covalent binding of the vesnarinone iminium ion to human neutrophils *in vitro*. Immunochemical studies demonstrated that vesnarinone reactive metabolite (0-100 μM) (generated directly by addition of HOCl to drug) covalently modified neutrophil polypeptides with apparent molecular masses of 126, 98, 90, 57 and 41 KDa. Further studies revealed that the 57 KDa vesnarinone adduct was formed by temperature-dependent degradation of the 98 KDa adduct. When vesnarinone was incubated with neutrophils in the presence of the cell activator phorbolmyristate acetate (PMA), a large number of vesnarinone modified-polypeptides (including major 126, 98 and 90 KDa adducts) were observed. When the covalent binding of clozapine, another drug that causes agranulocytosis, and vesnarinone were compared in PMA-activated neutrophils, the pattern of polypeptides modified was different (major drug-polypeptide adducts were 58 and 49 KDa with clozapine and 126, 98 and 90 KDa with vesnarinone). However, with both drugs polypep-

tide adducts of 126, 45, 41 and 31 KDa were formed suggesting that a subset of neutrophil proteins are covalently modified by both drugs. In addition, incubation of either drug with myeloperoxidase in the presence of H_2O_2 led to covalent modification of the enzyme. It may be that covalent modification of these common cellular targets plays a role in the agranulocytosis caused by the two drugs. (This research was supported by PMAC and the Medical Research Council of Canada (Grant number MT-13478).)

36 METABOLISM OF TRIMETHOPRIM BY ACTIVATED HUMAN NEUTROPHILS AND RAT LIVER MICROSOMES: IMPLICATIONS FOR TRIMETHOPRIM-INDUCED AGRANULOCYTOSIS AND LIVER TOXICITY.

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An antibacterial agent, trimethoprim (TMP), is normally used synergistically with sulfonamides. Its use is associated with agranulocytosis and liver toxicity. In this study, we demonstrated that trimethoprim was oxidized by activated human neutrophils, as well as a combination of myeloperoxidase/hydrogen peroxide/chloride, or hypochlorous acid to a reactive pyrimidine iminoquinone methide intermediate with a MH^+ ion of m/z 289 as detected by electrospray mass spectrometry (ESMS). In the presence of N-acetyl-L-cysteine (NAC), the pyrimidine iminoquinone methide could be trapped as three NAC adducts. The three NAC adducts were separable on HPLC but showed the same protonated molecular ion of m/z 452. The proton nuclear magnetic resonance spectrum of the major NAC adduct showed that the NAC group was at the 6 position of the pyrimidine ring. Incubation of trimethoprim with isolated rat-liver microsomes in presence of NAC gave the same set of TMP-NAC adducts. LC/MS/MS of the two minor metabolites of TMP generated by neutrophils and rat-liver microsomes indicated that they were the two diastereomers in which NAC was attached to the *exo*-cyclic prechiral carbon of the pyrimidine iminoquinone methide. We propose that the formation of this pyrimidine iminoquinone methide by both neutrophils and hepatic microsomes may be responsible for trimethoprim-induced idiosyncratic agranulocytosis and hepatotoxicity. (This work was supported by the PMAC and MRC of Canada.)

37 COVALENT BINDING SITES OF CLOZAPINE IN HUMAN NEUTROPHILS *IN VITRO* DETECTED BY CONFOCAL AND ELECTRON MICROSCOPY.

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Clozapine is often effective in the treatment of refractory schizophrenia but its use is limited by a high incidence of agranulocytosis. We recently demonstrated by immunoblotting that a reactive metabolite of clozapine covalently binds to several neutrophil proteins, both *in vitro* and *in vivo* in patients taking the drug. In this study, we used antibodies against clozapine and myeloperoxidase combined with confocal microscopy and electron microscopy to determine the localization of the covalent binding in neutrophils that occurs *in vitro*. When the reactive metabolite was generated externally with HOCl, confocal microscopy demonstrated that the reactive metabolite penetrated the cell and the pattern of binding appeared homogeneous except that no binding was detected in the nucleous. In contrast, when only clozapine and H2O2 were added to neutrophils, the pattern of covalent binding (visualized with a second layer fluoresceine antibody) appeared to match the pattern observed with an antibody against myeloperoxidase labeled with rhodamine. This suggests that the reactive metabolite was formed by myeloperoxidase/ H2O2. The results with electron microscopy using a second layer antibody tagged with gold, supported this hypothesis because most of the staining occured in the granules which is the location of myeloperoxidase. It was also observed in earlier *in vivo* studies that covalent binding occured to granulocytes but not to lymphocytes. These results suggest that most of the *in vivo* covalent binding of clozapine is due to a myeloperoxidase-dependent reactive metabolite. (This work was supported by a grant from the Medical Research Council of Canada (13478).)

38 INHIBITION OF GLUTATHIONE S-TRANSFERASES BY REACTIVE METABOLITES OF BUTYLATED HYDROXYTOLUENE.

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Butylated hydroxytoluene (BHT) is a phenolic antioxidant that causes a number of adverse biological effects in animals due to the formation of reactive metabolites including a quinone methide (BHT-QM) and a hydroperoxide (BHTOOH). Both of these metabolites are highly cytotoxic in vitro but little is known about their specific cellular targets responsible for toxicity. The electrophile BHT-QM is active in stress gene assays employing recombinant HepG2 cell lines by stimulating the xenobiotic response element and glutathione S-transferase (GST) and P450 1A1 promoters. The catalytic activity of P450 1A1 was enhanced in HepG2 cells treated with BHT-QM while GST activity was substantially decreased in these cells. Studies with purified GST demonstrated that the quinone methide is an irreversible inhibitor of dinitrochlorobenzene conjugation, presumably through alkylation of a critical nucleophile. With BHTOOH, GST exhibited peroxidase activity but the ability to conjugate dinitrochlorobenzene was greatly compromised. Further studies demonstrated that BHTOOH oxidizes methionine residues of GST to methionine sulfoxide (MetSO) and that this process occurs much more readily than with H_2O_2. Although it is possible that cysteine oxidation also occurs with BHTOOH, methionine residues appear to be more susceptible to this hydroperoxide. Investigation of BHTOOH-treated proteins by electrospray mass spectrometry confirmed the formation of MetSO and facilitated identification of the residues affected. These data indicate that both alkylating and oxidizing metabolites of BHT inhibit an important detoxification pathway. (Supported by NIH grant CA41248.)

39 GLUTATHIONE CONJUGATION OF ELECTROPHILIC METABOLITES OF 1-NITRONAPHTHALENE IN RAT TRACHEOBRONCHIAL AIRWAYS AND LIVER, IDENTIFICATION BY MASS SPECTROMETRY AND PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY.

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1-Nitronaphthalene (1-NN) is a mutagenic nitroaromatic which has been detected in emissions from both heavy- and light-duty diesel engines, as well as in urban airborne particles. 1-NN is a cytochrome P450 (P450)-bioactivated, non-ciliated bronchiolar epithelial (Clara) cell cytotoxicant. The current studies examined the metabolism of 1-NN to electrophilic metabolites which were trapped as glutathione conjugates in highly susceptible (lung) and less susceptible (liver) tissues in rat. Significant depletion of reduced glutathione was observed in all levels of tracheobronchial airways of rats treated with 200 mg/kg 1-NN, ip. This was consistent with the HPLC radioactivity profiles demonstrating five putative glutathione conjugates isolated from liver microsomal incubations with 1-NN, glutathione S-transferase and tritiated [^{3}H] glutathione. Mass spectrometry in positive ion mode yielded a *m/z* of 497 (M+H), and daughter ions of *m/z* 479 (loss of water) and *m/z* 306 (glutathione), which were consistent with the formation of hydroxy-dihydro-glutathionyl derivative of 1-NN. Proton nuclear magnetic resonance (NMR) spectroscopy analysis suggested the formation of two major conjugates from liver microsomal incubations, namely 1-nitro-5-glutathionyl-6-hydroxy-5,6-dihydronaphthalene and 1-nitro-5-hydroxy-6-glutathionyl-5,6-dihydronaphthalene. These data show that P450 metabolizes 1-NN in the liver to an intermediate 5,6-epoxide, which is further metabolized by glutathione S-transferase to form 1-NN glutathione conjugates. HPLC radioactivity profiles demonstrate that major conjugate(s) generated in the lung differ from those generated in the liver, thus suggesting that either the intermediate epoxide is formed at different carbon positions, or the stereoisomer of the liver conjugate is formed in the lung.

40 FORMATION OF 1,1-DICHLOROETHYLENE EPOXIDE IN CENTRILOBULAR HEPATOCYTES OF MURINE LIVER.

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Hepatocellular injury ensues after treatment of mice with 1,1-dichloroethylene (DCE), a chemical used in the plastics industry. Necrosis of centrilobular hepatocytes is ascribed to P450-catalyzed formation of a reactive metabolite believed to be the DCE epoxide. *In vivo* studies were undertaken to test the hypothesis that *in situ* formation of the epoxide mediates the DCE-induced centrilobular damage. Epoxide levels were estimated by trapping the metabolite with glutathione (GSH), and identifying the conjugated products as *S*-(2,2-dichloro-1-hydroxy)ethyl glutathione [B] and 2-(*S*-glutathionyl) acetyl glutathione [C]. HPLC analyses showed that [C] was the major product found in liver cytosol isolated from mice treated *in vivo* with [^{14}C]DCE; the amounts of [B] formed were negligible. Epoxide levels were

dose-dependent at DCE doses ranging from 25 to 225 mg/kg (1.9 to 15.4 nmol/mg protein). Pretreatment of mice with buthionine sulfoximine (BSO) significantly decreased GSH content and inhibited formation of the epoxide (83%) from DCE (125 mg/kg). Pretreatment of mice with diallyl sulfone, an inhibitor of CYP2E1, also significantly decreased epoxide formation (82%). Immunohistochemical studies with a polyclonal antibody specific for conjugate [C] demonstrated staining in the centrilobular region of the liver lobule. The level of staining was dose-dependent and was highest at 225 mg/kg DCE. Staining of adjacent liver sections with a CYP2E1 antibody revealed labeling that was also located in the centrilobular region, and coincided with the areas in which [C] was found. These data demonstrated that the DCE epoxide is formed *in vivo*, and is localized in the target centrilobular hepatocyes. (Supported by MRC of Canada (Grant MT-11706) and NCI/NIH (CA 73220-01).)

41 CYP2F1 KINETIC STUDIES WITH DEUTERATED ANALOGS OF 3-METHYLINDOLE REVEAL MECHANISMS OF DEHYDROGENATION TO 3-METHYLENEINDOLENINE.

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The reactive intermediate 3-methyleneindolenine (3MEI) is a major contributor to the systemic pulmonary toxicity of 3-methylindole (3MI). Dehydrogenation of 3MI by specific cytochrome P450s leads to the formation of 3MEI. To study this dehydrogenation mechanism, inter- and intramolecular isotope effect studies were conducted using CYP2F1—a human P450 enzyme that is selectively expressed in lung tissue and known for its ability to catalyze dehydrogenation reactions. For the intermolecular studies, four deuterated analogs at the 3-methyl position of 3MI were used (3MI-d0 through 3MI-d3). As suspected, increased deuterium substitution at the 3-methyl position caused sequential decreases in V_{max}. However, enzyme inhibition was observed at high substrate concentrations for all four isotopologs. Interestingly, the inhibition increased with increases in deuterium substitution. A possible mechanism for the inhibition may be isotopically sensitive formation of a mechanism-based inhibitor of 2F1. Intramolecular isotope effect studies with 3MI-d1 and 3MI-d2 isotopologs revealed an isotope effect (^{D}k) of 3.9 ± 0.53. A low isotope effect such as this may be explained by metabolic switching to form oxygenated rather than dehydrogenated products of 3MI. However, oxygenated products were not observed, and an alternate mechanism involving an aminium cation radical that inactivates the enzyme is proposed as an explanation for the relatively low intramolecular isotope effect and isotopically sensitive inhibition. Thus, deuterium-substituted isotopologs of 3MI were useful tools in elucidating the catalytic mechanisms of the human lung P450 enzyme CYP2F1. (Supported by USPHS grant HL13645.)

42 DOSE-DEPENDENT PROTEIN ADDUCT FORMATION IN RATS AND HUMANS AFTER PERCHLOROETHENE INHALATION.

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Perchloroethene (PER) is an environmental contaminant. In bioassays for carcinogenicity PER was found to increase renal tumors in male rats. Toxic effects of PER after repeated administration are likely caused by bioactivation. PER bioactivation occurs by two pathways. Oxidation by cytochrome P450 results in trichloroacetyl chloride, which binds to lipids and proteins. Glutathione *S*-conjugate formation from PER and further processing of the formed *S*-(trichlorovinyl)glutathione to *S*-(trichlorovinyl)-*L*-cysteine followed by cysteine conjugate ß-lyase catalyzed cleavage results in the reactive dichlorothioketene which also binds to proteins. The objectives of this study were to quantify the dose-dependent formation of protein adducts from PER in rats and humans using antibodies specific for N^{ε}-(trichloroacetyl)-*L*-lysine (TCL) or N^{ε}-(dichloroacetyl)-*L*-lysine (DCL) in proteins. Male and female rats were exposed to 400, 40 and 10 ppm PER for 6 h and sacrificed at various time points. Formation of DCL and TCL in proteins was quantified in subcellular fractions from liver and kidney and in blood. In addition, male and female human volunteers were exposed to 10 and 40 ppm PER and formation of protein adducts in blood was analyzed using antibodies and GC/MS after immunoaffinity enrichment of modified proteins. In liver and kidney subcellular fractions and blood of PER-exposed rats, dose-dependent formation of DCL and TCL in proteins was observed. Highest concentrations of DCL were present in kidney. Blood concentrations of DCL were 5 to 10 fold lower than in kidney. Highest concentrations of TCL were found in the liver. In human blood samples taken 0 and 24 h after PER inhalation, TCL containing proteins were present in low concentrations. DCL containing proteins were not detected either by Western blotting or by GC/MS after immunoaffinity chromatography. The obtained results indicate a dose-dependent covalent binding of PER metabolites to proteins in rat liver, kidney and blood and suggest that covalent binding is much lower in blood of humans exposed under identical conditions.

43 A MECHANISM FOR THE ADDUCTION AND CLEAVAGE OF PEPTIDES BY NITROGEN DIOXIDE.

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Nitrogen dioxide (NO_2), is an environmental pollutant and a nitroxide species is hypothesized to mediate reactions which result in the adduction and/or cleavage of peptides by NO_2. A mechanism for the adduction and cleavage reaction of peptides by NO_2 is presented. Phosphate buffered solutions of the model peptides (G4KG5), (K10) and alpha-1-proteinase inhibitor were exposed to varying molar ratios of NO_2 to target peptide. Analysis of the post exposure peptide solutions by HPLC and Matrix Assisted Laser Desorption Ionization (MALDI) Mass Spectroscopy demonstrated significant preferential fragmentation of the K10 polypeptide as compared to the G4KG5 control peptide. Exposure of alpha-1-proteinase inhibitor to NO_2 also revealed significant fragmentation. The fragmentation pattern of alpha-1-proteinase inhibitor is consistent with cleavage at sequences which are rich in positively charged amino acids. With proteins, the reaction may be conformational-dependent, in that the polypeptide backbone of positively charged amino acids tends to be acessible to solvent which may allow for the reactive uptake of NO_2. We conclude that NO_2 reactive uptake occurs at aqueous-surface interfaces, and as such, amino acid residues which are accessible to solvent appear to serve as excellent substrates for derivatization by NO_2. (Supported by NIH ES00287.)

44 N-ACETYLBENZIDINE PEROXYGENASE METABOLISM BY PROSTAGLANDIN H SYNTHASE.

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Synthesis of prostaglandin H_2 by prostaglandin H synthase (PHS) results in a two-electron oxidation of the enzyme. An active reduced enzyme is regenerated by reducing cofactors, which become oxidized. This report examines the mechanism by which PHS, from ram seminal vesicle microsomes, catalyzes the oxidation of the reducing cofactor N-acetylbenzidine (ABZ). During the conversion of 0.06 mM ABZ to its final end product 4'-nitro-4-acetylamino-biphenyl, a new metabolite was observed when 1 mM ascorbic acid was present with either 0.2 mM arachidonic acid or 0.5 mM H_2O_2 as substrate. This metabolite co-eluted with synthetic N'-hydroxy-N-acetylbenzidine (N'HA) and not N-hydroxy-N-acetylbenzidine. The new metabolite was identified as N'HA by ESI/MS/MS. N'HA represented as much as 10% of the total radioactivity recovered by HPLC. When N'HA was substituted for ABZ, PHS metabolized N'HA to 4'-nitro-4-acetylaminobiphenyl. Inhibitor studies demonstrated that metabolism was due to PHS not cytochrome P-450. DMPO, mannitol, and superoxide dismutase did not alter metabolism. Oxygen uptake studies did not demonstrate a requirement for molecular oxygen. When $[^{18}O]H_2O_2$ was used as substrate, ^{18}O enrichment was observed for 4'-nitro-4-acetylamino-biphenyl, but not N'HA. A 97% enrichment was observed for one atom of ^{18}O and 17 ± 7% for two ^{18}O atoms. The rapid exchange of ^{18}O-N'HA with water was suggested to explain the lack of enrichment of N'HA and the low enrichment of two ^{18}O atoms into 4'-nitro-4-acetylaminobiphenyl. Results demonstrate a peroxygenase oxidation of ABZ and N'HA by PHS and suggest stepwise oxidation of ABZ to N'-hydroxy, 4'-nitroso, and 4'-nitro products.

45 AN NMR ANALYSIS OF THE REACTION OF UBIQUITIN WITH [ACETYL-1-13C] ASPIRIN.

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The acetylation of ubiquitin by [acetyl-1-^{13}C]aspirin has been studied using 2D NMR methods. Studies performed in a 50:50 H_2O:D_2O medium show doubling of the acetyl carbonyl resonances, indicating all of the stable adducts formed involved amide linkages. Assignment of the HMQC reso-

nances was accomplished based on comparison of resonance intensities with the results of an Edman degradation analysis, pH titration studies of acetylated ubiquitin, and analysis of two ubiquitin mutants, K33R and K63R. The presence of a single tyrosine residue in close proximity to lysine-48 suggested another assignment strategy. Nitration of tyrosine-59 resulted in a small, pH-dependent shift of the resonance assigned to lysine-48, with a pK of 7.0, close to that expected for the nitrotyrosyl hydroxyl group. An additional adduct resonance with very low intensity is also observed, and tentatively assigned to the acetylated N-terminal methionine residue. The relative rates of acetylation of the various lysine residues were obtained from time-dependent HMQC studies. Since no sample preparation artifacts are introduced, the levels of modification of the various residues can be determined with relatively high accuracy as compared to other methods; the relative degree of acetylation of ubiquitin by aspirin under the conditions of the study was: K6 > K48 > K63 > K11 ~ K33. These results are in much better agreement with predictions based on the crystal structure of the ubiquitin monomer, than with predictions based on the ubiquitin structure in the cystallized dimeric and tetrameric forms. This approach provides a useful basis for understanding how local environmental factors can influence protein adduct formation, as well as for comparing the extent and specificity of various acetylation reagents.

46 HEMOGLOBIN ADDUCT DOSIMETRY OF ACRYLONITRILE FOLLOWING CHRONIC ADMINISTRATION IN THE MOUSE.

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Acrylonitrile (AN) is widely used in the manufacture of plastics and fibers. Hemoglobin adducts can provide a marker of exposure and internal dose, and can verify the extent of exposure in long-term animal studies. The objective of this study was to measure adducts formed by reaction of AN with the N-terminal valine residue in hemoglobin from B6C3F1 mice administered AN by gavage for up to 89 days. Blood samples were obtained from a study conducted by the Acrylonitrile Group, Inc. at Southern Research Institute, in which mice were administered 1.2, 4.8 or 9.6 mg AN/kg/day, 5 days/week for up to 89 days. Blood was collected on days 1, 5, 12, 26, 40, 68, and 89. Globin was isolated from erythrocytes, and the cyanoethylvaline (CEVal) hemoglobin adduct was derivatized by a modified Edman procedure for analysis by gas chromatography/mass spectrometry. Dose response evaluations at days 68 and 89 indicated a sublinear relationship between administered dose and CEVal. This behavior is consistent with saturable metabolism of AN to cyanoethylene oxide. With repeated administration of 9.6 mg AN/kg/day, CEVal reached a plateau between 40 and 68 days. Comparison of these data with simulations of CEVal accumulation conducted with a model for erythrocyte turnover suggests that CEVal is stable. These studies indicate that the animals on this long-term study were exposed to AN. This data set provides an important validation of CEVal as a biomarker for AN exposure.

47 ENVIRONMENTAL EXPOSURE OF CHILDREN LIVING IN MONTREAL TO PAH.

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The aim of study was to assess the intake of polycyclic aromatic hydrocarbons (PAH) by children living in a city. Two groups of children (n=11 and 13; 3-6 years old) were chosen: (1) a group from a kindergarten situated near a road with a high traffic density ("polluted" area); (2) a group from a kindergarten situated in a green zone ("non-polluted" area). Food consumption was recorded in all children and PAH intake from foodstuffs was estimated. Ambient air samples were collected on the playground and indoor of kindergartens during 3 days in summer 1997. Urine samples were collected in the morning and in the evening. Mean outdoor total PAH concentration (sum of 12 individual PAH) in "polluted" area was 10 times higher than that in "non-polluted" area (20.7 vs 1.9 ng/m^3). However, indoor concentrations were similar (2.2 vs 1.7 ng/m^3). The same trend was observed for pyrene concentrations. The contribution to the total pyrene intake from the food consumption (daily intake of 2.8 and 3.4 µg, respectively, in "polluted" and "non-polluted" area) was much more important than that from inhalation (0.008 and 0.002 µg, respectively) in both areas. No statistical difference between the two groups was found in urinary concentrations of 1-OHP, used as a biomarker of the total internal dose of pyrene or the PAH. In conclusion, the food seems to be a main source of the total pyrene and total PAH intake in Canadian children, even under a relative high PAH air exposure in the city.

48 COAL TAR WASTES (CTWs) IN SOIL: A QUALITATIVE EVALUATION OF HAZARDS POSED BY DERMAL CONTACT.

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A literature review was performed to assist in determining hazards to individuals whose activities resulted in dermal contact with coal tar wastes (CTW) in soil. The primary focus areas were the potential for (1) systemic effects related to percutaneous absorption of polycyclic aromatic hydrocarbon (PAH) constituents present in CTWs and (2) direct dermal toxicity. In vivo and in vitro percutaneous absorption studies in animals and humans were evaluated. Factors considered in the evaluation were: experimental design, role of vehicles, and relevance to potential human exposures to contaminated soils. Also, the extent of human effects associated with exposures to CTWs and PAHs under a variety of exposure situations was assessed. The latter was considered in the context of plausible exposures to the public, both children and adults, to CTW-contaminated soils. The data indicate that the percutaneous absorption of soil-bound PAHs is likely to be less than indicated by experimental protocols in which PAHs are applied to the skin in vehicles solvents such as acetone. Thus, risk assessments using default assumptions for dermal absorption of PAH components of CTWs in soils, without consideration of factors that influence absorption and plausible exposure conditions, are likely to overestimate risk. ([1]The opinions expressed are those of the authors and do not necessarily reflect the opinions or policies of the ATSDR or IDPH.)

49 NEUROTOXIC IMPLICATIONS FOR THE TRANSPLACENTAL DISPOSITION OF BENZO(A)PYRENE: CARBON BLACK AEROSOLS.

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A novel model for the illumination of potential neurotoxic mechanisms as a result of the transplacental disposition of desorbed benzo(a)pyrene [B(a)P] from a particulate aerosol is presented. Sprague Dawley timed-pregnant rats were exposed for four hours on gestation day 15 to an acute dose of 100 ug/m3 B(a)P : Carbon black aerosol. Controls received carbon black only. Nominal and chamber concentrations of the particulate aerosol were determined gravimetrically with a seven stage cascade impactor and analyzed for the: mean mass aerosol diameter (MMAD), cumulative frequency, cumulative percent, and differential particle-size distribution using a Windows Cascade Impactor Data Reduction System. The MMAD for the B(a)P : carbon black aerosol was 0.4 ± 0.02 µm. Mobility gel shift analysis of nuclear extract incubations from various brain regions of the resulting pups on postnatal days 3, 5, 7, 10, and 15 with an Sp1 consensus sequence oligonucleotide revealed perturbations in the developmental profile of Sp1 as a result of nose-only particulate aerosol exposure to the timed-pregnant dam. The data suggest that Sp1 DNA-binding is developmentally regulated and expressed very highly in actively developing brain regions. We conclude that "in utero" exposure to B(a)P may alter the DNA-binding and possible levels of critical transcription factors involved in growth and differentiation in the brain. The results from these studies contribute to an understanding of the biological mechanisms by which particulate matter hazardous constituents mediate adverse acute health effects associated with particulate matter exposure. (Supported by Cooperative Agreement MHPF/ATSDR U50/ATU398948, NIH G12 RRO 3032, and NIEHS ES00287.)

50 COMPARATIVE BIOAVAILABILITY OF BENZO(a)PYRENE IN RATS DOSED ORALLY VS. INHALATION.

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The focus of the present study was to evaluate the bioavailability of blood Benzo(a)pyrene [B(a)P], subsequent to oral and inhalation exposure. Eight week old F-344 rats were dosed either orally with 0.5 and 1.0 mg/kg body weight B(a)P or via nose-only inhalation with 0.1, and 1.0 mg/m^3 of B(a)P

and sacrificed at 0, 0.5, 1.0, 2.0, 3.0, 4.0, 8.0, 10.0 and 12.0 hours post exposure. Blood, lung, liver, testes, and ovaries were removed at necropsy and was analyzed for parent B(a)P by HPLC with fluorescence detection. The results demonstrate that subsequent to inhalation exposure at 0.1 mg/m^3, (0.857 m g/kg body weight equivalence) mean blood B(a)P concentrations were maximal 30 minutes post exposure with females exhibiting approximately a 1.65 fold increase in blood B(a)P levels compared to males within the exposure group. At 4.0 hours post exposure, greater than 95% of the parent B(a)P compound was cleared from the blood in these animals when compared to the 30 minute post exposure group. In contrast, subsequent to oral dosing at 1 mg/kg body weight, mean blood B(a)P concentrations were maximal at 8.0 hours post exposure. (Supported by MHPF/ATSDR U50/ATU398948 and NIH G12 RRO 3032, and NIEHS ES00287.)

51 ESTIMATES OF THE *IN VITRO* PERCUTANEOUSABSORPTION OF TWO PAH CONTAMINATED SOILS AND THEIR ORGANIC EXTRACTS.

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Sorption of polynuclear aromatic hydrocarbons (PAHs) on soil can impede their penetration through skin. Previous *in vitro* dermal penetration experiments of PAHs in crude oil and coal tar sorbed onto soil (Roy et al.,1998) showed dramatic reduction of PAH absorption through skin relative to unbound materials. The present study evaluated the dermal penetration properties (flux rates) of two PAH contaminated soils (149 and 228 ppm project-specific PAHs) and compared them with those of the solvent extracts of the respective soils (16,000 and 14,900 ppm project-specific PAHs). *In vitro* percutaneous absorption experiments (96 hr) were performed with the two soil samples (sieved to < 150 μ m) and two soil extracts. Infinite doses (25 mg/cm^2) were applied to the donor side of human abdominal skin sections mounted in Franz *in vitro* diffusion cells to provide flux rates for risk assessment. In order to facilitate the measurement of skin penetration, all doses were spiked with ^{3}H-benzo(a)pyrene (^{3}H-BaP) prior to the study. Based on ^{3}H-BaP, the dermal flux rates for the infinite dose soil extracts were 501 (Soil 102) and 504 (Soil 80) ng/cm^2/hr. The dermal flux rates for the contaminated soils were 0.072 and 0.088 ng/cm^2/hr, respectively. The large reduction in target PAH dermal absorption rate from the two soils in comparison to soil extracts was a consequence of PAH concentration and soil binding. Concentration differences account for a 107 and 65 fold reduction in PAH dermal flux rate from Soil 102 and Soil 80, respectively. Soil binding phenomena accounted for a 65-fold (equivalent to a 98.5%) reduction for Soil 102 and 88-fold (equivalent to 98.9%) reduction for Soil 80 compared to their extract. The combined effects resulted in an overall 5700-7000 fold reduction in PAH dermal penetration from soils as compared to unbound PAHs. These data are in accord with the results of previous experiments (Yang et al., 1989) which indicate significantly reduced risk from dermal exposure to soil-bound PAHs.

52 COMPARATIVE ANALYSIS OF CHEMICAL:DNA ADDUCT FORMATION IN MOUSE LUNG FOLLOWING THE INGESTION OF NEAT MGP TAR AND SOILS CONTAMINATED WITH MGP TAR.

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The types of chemical:DNA adducts formed in mouse lung following the ingestion of neat manufactured gas plant (MGP) tar and soils contaminated with MGP tar were evaluated. B_6C_3F1 mice were fed diets containing neat MGP tar or contaminated soil for 14 days and the types of chemical:DNA adducts formed in lung tissue were determined using ^{32}P-postlabeling and HPLC analysis. DNA adduct profiles were qualitatively similar between the four neat MGP tar samples evaluated. However, the extent of adduct formation varied with the PAH content of each tar sample. Three major adducts were observed with each tar sample. Two of these adducts were determined to be derived from B[b]F and B[a]P. The third adduct corresponded to the unidentified chemical:DNA adduct detected in previous studies. The ingestion of soil contaminated with MGP tar also resulted in detectable levels of chemical:DNA adducts in lung tissue. The extent and types of adducts formed varied considerably between soil samples. DNA adducts derived from B[b]F and the unidentified component of MGP tar were formed with each of the three soil samples evaluated. The most abundant adduct was derived from the unidentified component of MGP tar. In contrast, B[a]P:DNA adducts were detected with only one soil sample. These results demonstrate that the adsorption of MGP tar to soil significantly influences the type of adducts formed *in vivo*. In addition, the presence and abundance of the unidentified chemical:DNA adduct suggests that it may play a critical role in the carcinogenic potential of neat MGP tar and soil contaminated with MGP tar. (EPRI Grant WO2963-06)

53 EVALUATION OF 3-HYDROXYBENZO[A]PYRENE AS A POTENTIAL BIOMARKER OF PAH BIOAVAILIBILITY FROM MGP TAR.

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Recent studies have used 1-hydroxypyrene (1-OH pyrene) levels in urine and chemical DNA adduct formation in lung as biomarkers to estimate the bioavilability of PAH from complex mixtures following ingestion. However, there is considerable concern that pyrene may not adequately represent the absorption, distribution, metabolism, and excretion of carcinogenic PAH (cPAH) present within these mixtures. In the present study, the urinary excretion of 3-hydroxybenzo[a]pyrene (3-OHB[a]P) was evaluated as a biomarker for estimating PAH bioavailability. Urine previously collected from mice fed diets containing MGP tar contaminated soil and extracts of soils were evaluated for 3-OHB[a]P content. Urine samples were treated with β - glucuronidase/aryl sulfatase and 3-OHB[a]P was extracted using C_{18} sep-pak cartridges. 3-OHB[a]P levels were determined using reverse-phase HPLC with fluorescence detection. 3-OHB[a]P was detectable in urine of animals ingesting diets containing B[a]P concentration ranging from 5-50 ppm. Detection and quantitation limits were determined to be 10 pg and 50 pg respectively under the conditions used in this study. Detectable levels of 3-OHB[a]P were observed with soils at all levels evaluated. 1-5% of ingested B[a]P was excreted as 3-OHB[a]P in urine. The detection of 3-OHB[a]P in urine of animals ingesting soils and the corresponding extracts provides the ability to calculate a bioavailability estimate of PAH. These estimates appear to be similar to those calculated based on chemical:DNA adduct formation. The results of this study suggest that 3-OHB[a]P urinary excretion may provide a better biomarker relative to 1-OH pyrene, to assess the bioavailibility of cPAH within a mixture. (EPRI Grant WO2963-06)

54 BENZO[A]PYRENE BIOAVAILABILITY FROM RESIDENTIAL SOILS.

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Eight soil samples were collected from four residential yards in the vicinity of a waste site. A chemical concentration screen was used to guide selection of the samples to use in the animal experiments. This involved using micro-solvent extraction and GC/FID analysis to obtain initial PAH concentrations in unsieved soil. The three samples chosen for the animal study were sieved to <250 μm and Soxhlet extracted with dichloromethane. Soil samples, soil extract samples, and extract-ed soil samples were chemically analyzed for PAHs and then fed to female B6C3F1 mice (7 weeks of age) for two weeks in dietary vehicles. Total PAH concentrations for the three soils ranged from 66 mg/kg to 388 mg/kg, and benzo[a]pyrene-toxic equivalent con- centrations ranged from 9 mg/kg to 70 mg/kg. Two measures were employed to determine the relative oral bioavailability of benzo[a]- pyrene (B[a]P). First, B[a]P urinary metabolite excretion was studied. Relative oral B[a]P bioavailability as estimated by dividing the fractional urinary excretion (FUE) of 3-hydroxy-benzo[a]pyrene in animals treated with contaminated soil by the FUE in animals treated with the soil extract ranged from 0.07 to 0.27. The average relative bioavailability of 0.15 is equivalent to the Oral-Soil Absorption Adjustment Factor (AAF) required for use in risk assessment, because the cancer slope factor for B[a]P is based on a rodent dietary study. Second, levels of chemical:DNA adducts in lung tissue were measured. The level of chemical:DNA adducts was higher in the animals fed the soil extracts compared with animals fed unextracted, sieved soil. Relative oral PAH bioavailability on the basis of chemical:DNA adducts ranged from 0.08 to 0.36. The average Oral-Soil AAF based on DNA adduct results was 0.21. This experimental approach is a useful way to obtain site-specific data to support realistic risk assessment of potentially carcinogenic PAHs.

55 TRANSFER OF SPERMATOZOA BENZO(A)PYRENE-DNA ADDUCTS TO FERTILIZED EGGS ASSOCIATED WITH EMBRYOTOXICITY.

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In epidemiological studies, men who smoke have children with lower birth weight and increased incidence of childhood cancer even if the mothers do not smoke. Smoking may result in formation of spermatozoa benzo(a)pyrene-DNA adducts and DNA damage, which are then transferred to fertilized embryos. Previously, we identified a sperm B(a)P-DNA adduct [(+)-anti-BPDE-deoxyguanosine] in B(a)P-treated mice in vivo and trout sperm treated with BPDE in vitro, using 33P-postlabeling and an anti-B(a)P-DNA antibody. In the present studies, trout sperm were incubated with 10 (g/ml benzo(a)pyrene diol epoxide (BPDE) or saline for 1h, washed, and used to fertilize untreated trout eggs. The eggs from each group were placed in separate hatching trays covered with running water. After 24h , several hundred eggs from each group were removed for DNA isolation while the rest (several thousand/ group) were left in the trays for daily observation, removal/counting of dead eggs, and weighing of the trout fry at hatching and 14d of age. DNA from the saline or BPDE-treated sperm and from the two groups of fertilized eggs was isolated and postlabeled with 33P-ATP. HPLC of postlabeled DNA from sperm incubated with BPDE and eggs fertilized with those sperm revealed a major DNA adduct with the same retention time as purified (+)-anti-BPDE-deoxyguanosine. No adducts were detected in control sperm or eggs. Eggs from BPDE-treated sperm had significantly increased mortality (% dead eggs: Control = 25.8%, BPDE-treated sperm = 55.9%) while trout fry from BPDE sperm had significantly decreased wts (24h wt: Control=0.12832g, BPDE=0.11365g 14d wt: Control= 0.198821g(0.000024, BPDE=0.192843g(0.000024, p(0.005). Conclusion: B(a)P-DNA adducts are present in BPDE-treated sperm and can be detected in eggs fertilized with those sperm. Those sperm B(a)P adducts are associated with increased embryomortality and decreased hatching weights. (Support by March of Dimes grant No. 96-0555 & American Cancer Society grant IRG 17-35 and CA40228(WB).)

56 QUALITATIVE AND QUANTITATIVE CHARACTERIZTION OF 7,12-DIMETHYLBENZ(A)ANTHRACENE (DMBA) HEMOGLOBIN (HB) ADDUCTS.

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Previous studies have demonstrated that the carcinogens benzo(a)pyrene, 4-aminobiphenyl, and the tobacco specific nitrosamines bind covalently to hemoglobin and the adducts can be quantitatively determined by numerous methods, including gas chromatographic and mass spectrometric tools (GC/MS). In this study, we demonstrate the use of Hb adducts as a biomarker of the potent mammary carcinogen, 7,12-dimethylbenz(a)anthracene (DMBA). DMBA is one of the most potent chemical carcinogens and displays extreme potency at inducing mammary tumors in animal models. In this study, female Sprague Dawley rats were administered DMBA at doses ranging from 0.6 mg to 12 mg. 48 hours to 7 days later, animals were sacrificed and the red cells removed by cardiac puncture for determination of Hb adducts. Adducts were determined by enzymatic digestion of the protein, followed by analysis by GC/MS and LC/MS. Additionally, we examined the effect of various antioxidants, including 5,6-benzoflavone, butylated hydroxyanisole, and 1,2-dithiolthione on the formation of Hb DMBA adducts. Rats treated with DMBA alone (12 mg, 7 days) yielded a hemoglobin adduct level of 41.5 ± 7.25 pmol DMBA / mg globin. Rats treated with DMBA and with either 5,6-benzoflavone (1650 mg/kg diet), butylated hydroxyanisole (5000 mg/kg diet) or 1,2-dithiolthione (600 mg/kg diet) resulted in a decrease of DMBA detected hemoglobin adducts from 5.89 ± 2.52, 28.6 ± 9.23, and 12.4 ± 6.43 pmol/mg globin respectively. These results demonstrate that hemoglobin adducts of DMBA are potential biological markers of carcinogen exposure and that the modulation of adduct seen by treatment with antioxidant may be a potential marker for changes in levels of DNA damage caused by this potent mammary carcinogen.

57 THE EFFECT OF A COMPLEX MIXTURE OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) ON PAH ACTIVATION.

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Human exposure to PAH occurs through complex mixtures such as coal tar. To determine how a complex mixture of PAH affects the metabolic activation, PAH-DNA binding and carcinogenicity of individual PAH, mice were treated topically with Standard Reference Material 1597 (SRM), a complex mixture of PAH extracted from coal tar, and either benzo[*a*]pyrene (B[*a*]P) or dibenzo[*a,l*]pyrene (DB[*a,l*]P). At various times after treatment, the epidermal DNA was analyzed for PAH-DNA adducts by ^{33}P-postlabeling and HPLC. At early times after exposure, SRM decreased the total level of PAH-DNA adducts and B[*a*]P-DNA adducts compared with mice treated with B[*a*]P alone. However, after 24 hours, there was no significant difference in the levels of PAH-DNA adducts between these groups. After 16 weeks of promotion in a mouse skinn tumor initiation-promotion study, the SRM plus B[*a*]P-treated mice have only slightly more papillomas per mouse than the B[*a*]P-treated group. In contrast, mice treated with SRM and the potent carcinogen DB[*a,l*]P had a significantly lower level of PAH-DNA adducts after 24 hour exposure compared to mice treated with DB[*a,l*]P alone. The co-treated group has less than half the number of papillomas per mouse than mice treated with DB[*a,l*]P alone after 13 weeks microsomes indicate that the major effect of a complex mixture on the carcinogenic activity of a PAH within it may be related to the ability of the mixure to inhibit the activation of that PAH by the cytochrome P450s that are induced. (Supported by grant CA 28825, DHHS.)

58 PURIFICATION SCHEME FOR A POLYCYCLIC HYDROCARBON-BINDING PROTEIN FROM RAINBOW TROUT LIVER CYTOSOL.

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Feeding rainbow trout a diet containing 5-15 parts per million of dieldrin, increases the biliary excretion of a challenge dose of the tritiated polycyclic hydrocarbon 7,12-dimethylbenz[a]anthracene (DMBA). This increase in biliary excretion appears without evidence of induction of cytochrome P450, UDP-glucuronyl transferase or glutathione -S-transferases. (Fund. Appl. Toxicol.30:187). Induction of hepatic cytosolic binding protein may play a role in xenobiotc trafficking to smooth endoplasmic reticulum for oxidative and conjugative metabolism.Size exclusion chromatography indicated dieldrin increases the activity of [3H] DMBA and [3H] benzo[a]pyrene (BP)binding protein(s) in rainbow trout hepatic cytosol over untreated cytosol by 2-3 fold. A purification scheme was designed to purify this BP-binding protein. Ammonium sulfate fractionation was performed on the control and dieldrin-treated cytosol: 0-20%, 20-40%, 40-60% and 60-80% saturation. These fractions were then assayed for BP binding. The 20-40% fraction retained approximately 80-100% of the total BP binding and retained approximately 15-30% of the original cytosolic protein. This 20-40 % ammonium sulfate fraction was then loaded onto a hydrophobic interaction chromatography (HIC)column. The gradient was run with decreasing concentrations of ammonium sulfate supplemented with 0 .1% Triton X-100. The fractions containing the highest protein concentrations were then assayed for binding. Protein recovery off the HIC column was between 60-80 % and total activity retained was between 80-110%. Purification was between 5-12 fold over the ammonium sulfate fraction. The HIC fractions retaining binding activity were loaded onto an anion exchange column after removal of the Triton X-100 method. The gradient run on this column was increasing NaCl concentration. The fractions with the highest protein concentration were then assayed for binding. Enrichment in specific binding for the final purification was up to 300 fold higher than the original cytosol. 4-20 % SDS-PAGE gels yielded approximately 4-6 bands ranging from 100 Kd to 22 Kd in the most purified and enriched fraction. (Supported by NIEHS R15ESO8817.)

59 EFFECTS OF ACENAPHTHENE, ACENAPHTHYLENE AND 1,2:5,6-DIBENZANTHRACENE ON IMMUNE FUNCTIONS AND CYP450 IN ADULT MALE DEER MICE (*PEROMYSCUS MANICULATUS*).

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Polynuclear aromatic hydrocarbons (PAHs) are among the most common chemicals found in uncontrolled waste site. These chemicals produce diverse of toxic and biochemical responses in most species. Immune function alterations caused by PAHs are often overlooked, however, the objectives of this study were to determine the effects of three PAHs, acenaphthene, acenaphthylene and 1,2:5,6-dibenzanthracene, on immune function and CYP450 activity in a non-traditional mammalian species. In this study, adult male deer mice (*Peromyscus maniculatus*) were dosed with acenaphthene, acenaphthylene or 1,2:5,6-dibenzanthracene every other day for 12 days. Five concentrations of these chemicals (0.3, 1, 3, 10, and 30 mg/kg) and a corn oil vehicle (control) were used. Immune function was assessed using both T- and B- cell proliferation, antibody plaque forming cell response (PFC) and macrophage phagocytosis to determine potential effects on cell-mediate immunity, humoral immunity, and non-specific immunity. Treatment with acenaphthylene or 1,2:5,6-dibenzanthracene resulted in stimulation that appeared to be dose-responsive for both T- and B- cell proliferation. CYP450 activity was measured using ethoxyresorufin-O-deethylase (EROD) and pentoxyresorufin-O-deethylase (PROD). Differences were observed within chemical treatments and among treatments. The PFC assay exhibited a dose-dependent reduction for all three chemicals tested. There was no obvious effect noted on macrophage phagocytosis in this study

60 ALTERATIONS IN IMMUNE FUNCTION AND CYP450 ACTIVITY OF ADULT MALE DEER MICE FOLLOWING EXPOSURE TO EITHER CHRYSENE, PYRENE, OR BENZO-A-PYRENE.

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The realization that wildlife immunity can be affected by environmental contaminants has stimulated the use of immunotoxicity measurements in the field of environmental toxicology for determining the deleterious effects of contaminants on wildlife health as a basis for environmental risk assessment. The use of immunotoxicity parameters as biomarkers of environmental exposure has great potential once they are validated in non-traditional sentinel species. The objective of this study was to determine the effects of two PAHs on immune function and cytochrome P450 (CYP450) activity in a nontraditional murine species. Adult male deer mice were exposed every other day for a period of twelve days to the chemicals (0.3,1,3,10, and 30 mg/kg) or the corn oil control. Immune endpoints assessed were lymphoproliferation, macrophage phagocytosis, and the antibody plaque-forming cell response (PFC). Significant differences from control were observed in the PFC response for all chemicals tested. Other immune endpoints also exhibited differences from control for all of the chemicals tested. CYP450 activity was measured using EROD and PROD assays. Differences were observed within chemical treatments and among treatments. The plaque forming cell response was observed to be the most sensitive of the immune endpoints which were assessed and was also more sensitive than those observed from CYP450.

61 POTENTIATION OF DNA ADDUCT FORMATION IN RAT LIVER AND LUNG SLICES INCUBATED WITH BENZO(A)PYRENE (BAP) IN DYNAMIC ORGAN CULTURE FOLLOWING PRETREATMENT WITH 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) *IN VIVO*.

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Chemical-DNA adducts are a useful biomarker of exposure/effect that integrate absorption, metabolic activation/detoxification, and DNA repair following exposure to a genotoxic chemical. The covalent binding of chemicals to DNA (adduct formation) is generally accepted as an initial necessary step in the carcinogenic process. Precision cut rat liver and lung slices maintained in dynamic organ culture were utilized as an *in vitro* model to assess DNA adduct formation under various conditions. Rats were pretreated *in vivo* with TCDD (5 μ g/kg, po), to induce phase I and phase II metabolizing enzymes, or vehicle alone. Two days following pretreatment, rat liver and lung slices were incubated for 4 or 24 hrs in medium containing 0, 10, or 80 μ M BaP. At 4 hrs, 2.38 % of the BaP was associated with liver slices (~ 52 mg/3slices/vial), while 5.98 % of the compound was retained in lung slices (~ 26 mg/3 slices/vial). At 24 hrs, 4.29 and 11.16 % of the BaP was associated with liver and lung slices, respectively. DNA was isolated from tissue slices and DNA adduct formation was measured by ^{32}P-postlabeling. Liver slices contained at least 2 adducts while lung slices contained 2 or 3 adducts. One of the prominent adducts in both tissues was the BPDE-I-DNA adduct (BaP-7,8-diol-9,10-epoxide bound to guanine). Rat liver slices incubated with 0 and 80 μ M BaP for 24 hrs contained 0.49 and 1.60 fmol adduct/μ g DNA, respectively. Following pretreatment with TCDD *in vivo*, rat liver slices incubated with 80 μ M BaP for 24 hrs contained 5.23 fmol adduct/μ g DNA. Rat lung slices pretreated with TCDD *in vivo* and incubated for 24 hrs with 10 or 80 μ M BaP contained 8.23 and 12.28 fmol adduct/μ g DNA, respectively. Thus, *in vivo* exposure to TCDD potentiated DNA adduct formation. The preliminary data suggest this model is useful for the quantitation of DNA adduct formation under these conditions and may be a suitable model for other target tissues and species, including humans.

62 LIGHT-INDUCED FORMATION OF BENZO[A]PYRENE QUINONES: ROLE IN ALTERED CALCIUM HOMEOSTASIS IN HUMAN MCF10A MAMMARY EPITHELIAL CELLS.

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Previous studies in this laboratory have shown that benzo[a]pyrene (B[a]P) and B[a]P-7,8-diol increase basal levels of intracellular Ca^{2+} in human lymphocytes and human mammary epithelial cells. Recently, we have observed that simultaneous exposure of the human mammary epithelial cell line, MCF10A, to near ultraviolet (nuv) light (350-400nm) and B[a]P results in alterations intracellular Ca^{2+} homeostasis and cell morphology. In the present studies we show that short exposures (5-30 min) to light activates B[a]P to form BaP-quinones, as determined by measuring ^{3}H-B[a]P conversion to 1,6- 3,6- and 6,12-quinones separated by reverse phase HPLC. The amounts of quinone produced were dependent on the time exposed to light . Exogenously added 1mM glutathione (GSH) attenuated the light-induced conversion of B[a]P without the appearance of an HPLC peak corresponding to a B[a]P-GSH conjugate. B[a]P(3μ M) + light (5min) produced a near total inhibition of thapsigargan induced Ca^{2+} release and capacitative Ca^{2+} entry, as compared to MCF10A cells kept in the dark and treated with B[a]P. These results indicate that formation of B[a]P quinones may induce changes in cellular redox status leading to the oxidation of critical protein sulfhydryls involved in maintaining intracellular Ca^{2+} homeostasis. (Supported by NIH ES07259.)

63 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN AND BENZO(a)PYRENE DIFFERENTIALLY REGULATE CELL ATTACHMENT AND EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR, E-CADHERIN, AND PROSTAGLANDIN H-SYNTHASE IN HUMAN UTERINE ENDOMETRIAL CELLS.

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This study investigated mechanisms whereby 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and benzo(a)pyrene (BaP) may contribute to uterine disease through alteration in cell adhesion molecules and cell surface receptors. Exposure of human uterine endometrial adenocarcinoma RL95-2 cells to 10 μM BaP for 48 hours completely inhibited their ability to attach to matrigel-coated membranes, whereas 10 nM TCDD treatment had no effect. Immunohistochemistry and western blot analysis showed that BaP caused a marked loss of plasma membrane epidermal growth factor receptor (EGF-R) along intercellular boundaries, while TCDD produces no change in the localization of EGF-R immunostaining. Western blot analysis of E-cadherin, a key cell adhesion molecule, showed a significant decrease with BaP treat-

ment with no change for TCDD. In comparison, prostaglandin H-synthase (PGSH-1), a pathway for the biotransformation of BaP, is constitutively expressed in RL95-2 cells and is significantly increased 150% by BaP exposure, but not by TCDD. These results indicate that the BaP-mediated loss of cell attachment involves decreased localization of molecules important for cell-cell and cell-extracellular matrix interactions. Data further suggest that constitutive and induced PGHS may be a pathway for BaP bioactivation and toxicity. (Supported by NIEHS Superfund Grant 07375.)

64 ESTRADIOL ENHANCES THE EFFECT OF 2,3,7,8-TETRA CHLORODIBENZO-*P*-DIOXIN ON 7-ETHOXYRESORUFIN-*O*-DEETHYLASE (EROD) ACTIVITY IN A MOUSE OVARIAN EPITHELIAL CANCER CELL LINE.

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2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a ubiquitous environmental pollutant, is a well known toxin and promoter of carcinogenesis. TCDD induced the activity of EROD (representing mainly CYP1A1) dose-dependently in a mouse ovarian epithelial cancer cell line (ID8). The K_m and V_{max} of induced EROD activity were 180 *p*M and 1085 *p*M/min/mg, respectively. TCDD did not induce the activity of methoxyresorufin-*O*-demethylase (MROD) in the same cell line, which is in accordance with a lack of expression of CYP1A2 in extrahepatic tissues. Induction of EROD by TCDD was inhibited by alpha-naphthoflavone, an aryl hydrocarbon receptor (AhR) antagonist. Interestingly, estradiol increased the induction of EROD by TCDD dose-dependently. The K_m and V_{max} of EROD activity in the presence of estradiol (1μM) were 200 *p*M and 2304 *p*M/min/mg, respectively. The effect of estradiol on EROD induction by TCDD was at maximum in the concentration range of 1-5 μM. Estradiol did not increase the number of ID8 cells during 48 hr in culture. Progesterone and gonadotropins (FSH and LH), which are important hormones for ovarian function, had no effect on the induction of EROD by TCDD. In conclusion, estradiol enhanced the induction of EROD by TCDD in ovarian epithelial cancer cells suggesting that TCDD might affect the manifestation of ovarian cancer.

65 ESTROGEN RECEPTOR-MEDIATED ACTIVITIES OF BENZO[A]PYRENE: RECEPTOR BINDING, INDUCTION OF GENE EXPRESSION, AND UTEROTROPHIC EFFECTS.

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Polycyclic aromatic hydrocarbons (PAHs) are a class of contaminants that are widely dispersed in the environment, and that are suspected of having the ability to mimic endogenous estrogen. The ability of the PAH benzo[a]pyrene (B[a]P) to: 1) competitively displace [^{3}H]17β-estradiol (E2) from a bacterially expressed GSThERαdef fusion protein, 2) induce gene expression in MCF-7 breast cancer cells transiently transfected with a Gal4-hER chimeric receptor and a Gal4-regulated luciferase reporter gene, and 3) cause a uterotrophic response in ovariectomized (OVX) DBA\2 mice was assessed. Binding assay results indicate that B[a]P was unable to significantly displace E2 from the recombinant hER protein at concentrations as high as 10 μM. However, B[a]P was able to induce gene expression in the *in vitro* gene expression assay; though a full dose response was not observed due to insolubility at high concentrations, 10 μM B[a]P, the highest dose studied, was able to cause a 35-50% increase in hER-mediated gene expression relative to 10 nM E2. Potency appeared to be approximately 10^5-fold lower than that of E2. *In vivo*, increases in uterine wet weight in OVX DBA\2 mice caused by doses of 6-25 mg /kg B[a]P were statistically significant ($p < 0.001$; n=10), but not dose-dependent. Since competitive binding data indicates that B[a]P does not significantly bind to the hER, metabolites may be responsible for the observed *in vitro* induction of hER-mediated gene expression and *in vivo* uterotrophic activity of B[a]P.

66 METABOLISM AND RECEPTOR BINDING OF ANTIESTROGENIC POLYCYCLIC AROMATIC HYDROCARBONS.

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In an earlier study we demonstrated that eight PAHs (benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, indeno[1,2,3-cd]pyrene and dibenz[a,h]anthracene) detected in sediment samples from two rivers in upstate New York were antiestrogenic in human breast cancer cells (MCF-7 cells). In the present study we demonstrate that the antiestrogenicity was due to both increased metabolism of 17β-estradiol (E_2) by enzymes induced by the PAHs and by displacement of E_2 from the estrogen receptor (ER). Radiometric analysis showed that benz[a]anthracene, benzo[k]fluoranthene, benzo[a]pyrene and dibenz[a,h]anthracene significantly increased the metabolism of E_2 in MCF-7 cell cultures. Competitive binding assays showed that none of the PAHS directly bound human recombinant ERα or β. However, seven of the PAHs (all except dibenz[a,h]an-thracene) significantly displaced ^{3}H[E_2] in a whole-cell binding assay in MCF-7 cells only under conditions in which the PAHs could be metabolized by endogenous or induced enzymes. In conclusion, PAHs can be antiestrogenic in metabolically intact human breast cancer cells through at least two mechanisms, one involving competition by PAH metabolites for the ER and the other involving depletion of E_2 through induction of metabolism. (NIH ES04913-07.)

67 HUMAN PROSTATE CANCER LNCaP CELLS DERIVED FROM LOW AND HIGH PASSAGE NUMBERS DISPLAY DIVERGENT EXPRESSION OF CYP1A1 IN RESPONSE TO ENZO[A]PYRENE.

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This study examined the expression of cytochrome P450 (CYP1A1) in two strains of the androgen responsive LNCaP human prostate cell line. Cells at a low passage number (LP), 20 to 30, or a high passage number (HP), 50 to 60 were grown in the presence of 20μM benzo(a)pyrene BaP with 0.1% DMSO as control. Western immunoblot analyses showed induction of CYP1A1 protein by BaP in LP, but not in HP LNCaP cells. In the absence of CYP1A1 induction in HP cells, however, BaP inhibited cell proliferation by 75%, as well as significantly decreased levels of EGF-R by 85% and c-myc immunoreactive proteins by 50%. We further evaluated whether prostaglandin H synthase (PGHS) is expresses in LNCaP cells which could serve as an alternative pathway for the biotransformation of BaP. PGHS-1 immunoreactive proteins were found to be constitutively expressed at a high level in both HP and LP cells. In addition, PGHS-1 expression over 48 hours appears to be regulated by 10μM BaP and 10nM TCDD. In summary, the inhibitory effects of BaP on proliferation and growth factor expression in the HP cells in the absence of CYP1A1 may be mediated by PGHS-1 through the peroxidase pathway for bioactivation. Furthermore, effects of BaP on LP cells may involve dual metabolism by the constitutive pathway through PGHS and induced CYP1A1.

68 REPAIR OF DNA LESIONS: RELATIVE EFFICIENCIES OF REPAIRING POLYCYCLIC AROMATIC HYDROCARBON ADDUCTS IN HUMAN CELL-FREE EXTRACTS.

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous, genotoxic compounds. DNA lesions induced by these compounds are normally removed by excision repair mechanisms. In contrast to the extensively studied repair mechanisms, much less is known about the relative repair efficiencies of various DNA lesions. Since DNA repair is a crucial defense against carcinogenesis, it may constitute an important factor affecting the carcinogenicity of DNA damaging agents. We have adopted a human cell-free system for measuring relative DNA repair efficiencies based on the concept of repair competition between acetylaminofluorene adducts and other DNA lesions of interest. Using this *in vitro* system, we determined the relative repair efficiencies of PAH adducts induced by: anti-(±)-benzo[*a*]pyrene-*trans*-7,8-dihydrodiol-9,10-epoxide (BPDE), anti-(±)-benz[*a*]anthracene-*trans*-3,4-dihydrodiol-1,2-epoxide (BADE-I), anti-(±)-benz[*a*]anthracene-*trans*-8,9-dihydrodiol-10,11-epoxide (BADE-II), anti-(±)-benzo[*b*]fluoranthene-*trans*-

9,10-dihydrodiol-11,12-epoxide (BFDE), anti-(±)-chrysene-*trans*-1,2-dihydrodiol-3,4-epoxide (CDE), and anti-(±)-dibenzo[*a,l*]pyrene-*trans*-11,12-dihydrodiol-13,14-epoxide (DBPDE). While damage by BPDE, DBPDE, CDE, and BFDE were repaired by nucleotide excision repair as efficiently as AAF adducts, the repair of BADE-I and BADE-II adducts were significantly slower in human cell extracts. Damage by DBPDE at 3 μM *in vitro* yielded ~5-fold higher DNA adducts than BPDE as determined by quantitative PCR. This potent DNA reactivity may account in part for the potent carcinogenicity of dibenzo[*a,l*]pyrene. The correlation of these results to the carcinogenic properties of the PAH compounds is discussed. Furthermore, we show that NER plays a role in AP site repair *in vivo* in the eukaryotic model organism yeast.

69 ANALYSIS OF THE SUBCELLULAR DISTRIBUTION AND CYTOTOXICITY OF BENZO(a)PYRENE IN RAT LIVER CELLS.

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The subcellular distribution and effects of benzo(a)pyrene (B(a)P) exposure on cellular homeostasis in a rat liver cell line (Clone 9) was monitored in cells exposed for 30 min or overnight (12 - 16 hr) to graded doses (0, 0.1, 1.0, 2.5, 5.0 and 10 μg/ml) of B(a)P. The uptake and distribution of B(a)P into cells combined with analysis of a number of vital fluorescence bioassays for intracellular glutathione (GSH) content, gap junction-mediated intercellular communication (GJIC), intracellular Ca^{2+} levels, intracellular pH, reactive oxygen species (ROS) generation, and mitochondrial and plasma membrane potentials (MMP and PMP) were performed with a laser cytometer. A rapid partitioning of B(a)P into cytoplasmic membranes within 30 min was verified using organelle-specific fluorescence probes, however, there were no significant alterations in the above referenced functional endpoints by 30 min. In cells treated overnight with B(a)P, there was a significant reduction in both PMP and GJIC at the lowest dose tested (0.1 μg/ml). Elevation of basal intracellular Ca^{2+} and suppression of signaling induced by 0.5 nM vasopressin, in the form of Ca^{2+} oscillations, were also significant beginning at the 1.0 μg/ml doses. Significant effects on intracellular GSH levels, pH, ROS production, and MMP were not detected at the time points examined. There appears to be a direct relationship between the partitioning of B(a)P into cellular membranes and effects on PMP and GJIC. These effects were similar in Clone 9 cells synchronized by serum deprivation (0.5%) for 24 hr, however, the effects were pronounced at lower B(a)P concentrations. These studies indicate that the partitioning of B(a)P into cytoplasmic membrane systems contributes to the alterations in plasma membrane and intracellular membrane functions. The time course needed to detect these effects is consistent with the involvement of cytochrome P450-mediated metabolism in these responses. (Supported by NIH P42-ES04917 and P30-ES09106.)

70 USE OF MUTATION SPECTRA TO COMPARE ONE-ELECTRON AND TWO-ELECTRON PATHWAYS OF BENZO[A]PYRENE (BAP) ACTIVATION.

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Two metabolic pathways have been proposed for the generation of mutagenic metabolites from polycyclic aromatic hydrocarbons (PAHs): one-electron oxidation and two-electron oxidation. One-electron oxidation generates PAH-radical cations that can form unstable DNA adducts that yield abasic sites in DNA. Two-electron oxidation produces PAH metabolites such as diolepoxides that can form stable DNA adducts. To better understand the role of these two pathways in benzo[a]pyrene (BP)-induced mutagenesis we compare the *lacI* mutation spectrum from Big Blue animals exposed to BP with mutation spectra from mammalian cells exposed to BP or BP-diolepoxides (BPDE). Additional comparisons are made with mammalian cells transfected with shuttle vectors modified at specific sites to contain BPDE/DNA adducts or abasic sites. The data indicate that the *lacI* mutations in the liver of Big Blue mice exposed *in vivo* to BP reflect mutagenesis by BPDE/DNA adducts, not by abasic sites. Therefore, we conclude that two-electron oxidation of BP has greater significance for BP mutagenesis than one-electron oxidation. Nevertheless, further research is warranted because appropriate mutation spectra for PAHs other than BP are not yet available. Similarly, mutation spectra are not available for the skin or mammary gland of PAH-exposed animals, two organs where one-electron oxidation may be important.

71 THE DOUBLE-STAINING OF RAT FETAL SKELETONSUSING ALIZARIN RED S AND ALCIAN BLUE IN AN INDUSTRIAL LABORATORY.

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The present regulatory guidelines on the conduct of rodent developmental toxicity studies recommend that fetal skeletal evaluations include an assessment of both bone and cartilage. Current methods to double-stain the fetal skeleton using Alizarin Red S and Alcian Blue are typically described for small sample sizes. In an industrial setting there is a need for an effective means to double stain fetal skeletons on a large scale (i.e., hundreds of fetuses simultaneously). This paper describes a method utilized in our laboratory to stain both fetal bone and cartilage using solutions and procedures on an industrial scale. Specifically, following cesarean section, fetuses retained for skeletal evaluation are eviscerated, skinned, and placed in 250 ml jars containing 150 mls ethanol (70% or greater) for at least three days but no longer than four weeks. Fetuses are double stained in 70% ethanol containing: 0.0015% Alizarin Red S, 0.0005% Alcian Blue, and 2% Potassium Hydrogen Phthalate, for 72 hours and should be agitated at least once during this step. Following staining, the fetuses are rinsed with deionized water and then macerated with 0.75% potassium hydroxide [w/v] in deionized water for 72 hours with agitation at least once during this step. Following maceration, the fetuses are rinsed with deionized water and then cleared with a 2:2:1 solution of 70% ethanol:glycerin:100% benzyl alcohol for 24 hours. Following clearing, the fetuses are kept in holding solution (glycerin 50% [v/v] in 70% ethanol) for a minimum of 6 days prior to examination. Fetuses may be stored in 100% glycerin (with thymol) indefinitely. Using the aforementioned procedure up to 20 litres of each solution may be prepared for each step, enabling one to process approximately 1000 fetuses simultaneously.

72 DEVELOPMENTAL TOXICITY STUDY OF PENTANENITRILE,3-AMINO (3-APN) IN RATS.

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The developmental toxicity of pentanenitrile, 3-amino (3-APN) was assessed in rats by orally administering solutions of the test material in water over days 6-20 of gestation. Groups of 25 time-mated female Crl:CD® BR rats were gavaged at daily dose levels of 0, 5, 30, 100 or 300 mg/kg. The dams were euthanized on day 21 and examined grossly. The fetuses were weighed, sexed, and examined for external, visceral, and skeletal alterations. Evidence of maternal and developmental toxicity was seen at 100 and 300 mg/kg. Regarding maternal toxicity, there were compound-related, statistically significant reductions in maternal body weight, weight change, and food consumption. The incidence of alopecia was significantly increased at these levels as well. Regarding developmental toxicity, mean fetal weight was significantly reduced at 100 and 300 mg/kg. In addition, at 300 mg/kg, there were significant increases in several skeletal variations consistent with developmental delay. There was no evidence of either maternal or developmental toxicity at 5 or 30 mg/kg. Thus, the maternal and developmental no-observed-effect level (NOEL) was 30 mg/kg.

73 DEVELOPMENTAL NEUROTOXICITY SCREEN - A COMBINED DEVELOPMENTAL TOXICITY ASSESSMENT WITH RATS FROM A MULTI-GENERATION REPRODUCTION STUDY.

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The U.S. EPA guideline for a developmental neurotoxicity (DNT) study (OPPTS 870.6300, 1998) allows the option to perform the study "as a separate study, as a follow-up to a standard developmental toxicity and/or adult neurotoxicity study, or as part of a two-generation reproduction study, with assessment of the offspring conducted on the second (F2) generation." This paper outlines an experimental design for DNT study that uses rats from a multi-generation reproduction study and discusses the resulting advantages (e.g., fewer animals, less labor, a more comprehensive assessment, and potential cost savings) for conducting both as a combined study at the first tier of assessment. With this approach, the registrant assumes responsibility for the conduct of the DNT study, which is currently only required on a conditional basis. On the other hand, the registrant may benefit by avoiding an additional "FQPA" uncertainty factor for infants and children in lieu of the

results from a DNT study. The DNT study can utilize certain offspring from either the F1 or the F2 generation. These animals will have been exposed continuously to the test substance from conception, during gestation (via the placental circulation), continuing after birth (via the milk) and lactation (via the milk and feed), through postnatal day 21. Exposure may then be discontinued at weaning. This time period exceeds the duration of exposure that is outlined in the existing EPA guideline but this extended period of exposure provides for the possibility of a change in the EPA requirement or a new requirement (e.g., OECD). This paper outlines the advantages of this approach for both the registrant and the responsible regulatory agencies.

74 DEVELOPMENTAL TOXICITY OF SELEXOL® SOLVENT BY CUTANEOUS DOSING OF CD® RATS.

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Selexol® solvent is a mixture of polyethylene glycol dimethyl ethers (CAS No. 24991-55-7) used extensively in the natural gas treatment industry. Timed-pregnant CD rats, 25/group, were dosed with Selexol® solvent by occluded skin contact for 6 h/d on gestation days (gd) 6-15. Doses employed were 0, 1, 2 and 4 ml/kg/d and were selected on the basis of maternal and fetal effects observed in a range-finding study with similarly dosed pregnant rats. Monitors for maternal toxicity included body weight and food consumption measurements and clinical observation including skin irritation scores. At necropsy on gd 21, the liver, kidney, and uterus were weighed. Fetuses were evaluated for body weight and for external, visceral and skeletal anomalies. The pregnancy rate was 96-100%. No treatment-related mortality occurred during the study. Clinical signs observed in the 4 ml/kg group included exfoliation, excoriation, and crusting of the skin. Microscopic evaluation revealed acanthosis and epidermitis. There were no effects on maternal body weight, body weight gain, food consumption, or organ weights. There were also no effects on gestational parameters including the number of ovarian corpora lutea, pre- and post-implantation losses, or sex ratio. However, the fetal body weight of the high dose group was significantly decreased. There were no significant increases in the incidences of any malformations, thus Selexol® solvent was not teratogenic to rats. However, statistically significant increases in the incidences of variations in several skeletal districts (cervical centra, thoracic centra and sternebrae) were observed in the high dose group. In conclusion, occluded cutaneous application of Selexol® solvent to pregnant rats during organogenesis resulted in maternal effects and developmental delay at 4 ml/kg/day. The NOEL for developmental toxicity was 2 ml/kg/day.

75 DEVELOPMENTAL TOXICITY OF STRUCTURALLY RELATED DISUBSTITUTED HALOACETIC ACIDS IN EMBRYO CULTURE.

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Disinfection of water for drinking results in numerous byproducts. One important class of disinfection byproducts is the haloacetic acids. Recent studies have shown several of the haloacetic acids to be developmentally toxic in mouse embryos. We chose representative haloacetic acids (dichloro-(DCA) and dibromo-(DBA) acetic acid) for comparative toxicity testing on rat embryos in whole embryo culture (WEC) and included bromochloro acetic acid (BCA) as a mixed haloacetic acid. Gestational day (GD) 9 embryos were exposed to 50-300 μM BCA, 10-400 μM DBA or 500-5000 μM DCA for 48 hours and then evaluated for dysmorphology, developmental score (DEVSC), head length (HL), somite number (SN), crown rump length (CRL) and embryo lethality. All three acids were dysmorphogenic in rat WEC, and DBA and DCA were effective at concentrations less than or comparable to those reported previously in mice. DCA was dysmorphogenic at concentrations ≥ 2500 μM which is only half the concentration necessary to cause dysmorphology in the mouse. DEVSC, HL, SN and CRL were significantly reduced at concentrations ≥ 1000 μM. DBA was dysmorphogenic in rat WEC at concentrations ≥ 200 μM which is similar to the 250 μM reported in mice. DEVSC, HL, SN and CRL were significantly affected at concentrations ≥ 300 μM. In general, these LOEL's are similar to those reported in the mouse, however, a striking difference between the rat WEC data and those from mice was the absence of significant effects on neural tube closure as reported in mice. The mixed haloacetic acid, BCA, was dysmorphogenic at 300 μM and HL, SN and DEVSC were significantly reduced at concentrations ≥ 200 μM. Following the 300 μM exposure, there was a significant incidence of delayed embryonic caudal development with apparent normal development anterior to the 2nd visceral arch. This is a rare observation in our lab and suggests selective inhibition of segmentation or gastrulation. (This abstract does not reflect EPA Policy.)

76 SHORT TERM REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF TRIBROMOACETIC ACID, BROMOCHLOROACETIC ACID, AND BROMODICHLOROMETHANE IN THE S-D RAT WHEN ADMINISTERED IN THE DRINKING WATER.

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Three drinking water disinfection byproducts (DBP), Tribromoacetic Acid (TBA), bromochloroacetic acid (BCA), and bromodichloromethane (BDCM), were evaluated using a modified short-term reproductive/ developmental toxicity protocol (Harris *et al.*, 1992). TBA (10, 70, and 400 ppm), BCA (60, 200, and 600 ppm), and BDCM (100, 700, and 1300 pm) were administered in drinking water over a 35-day period to one group of male rats (10/group) and two groups of female rats, Group A (10/group for effects of exposure during conception and early gestation) and Group B (13/group for effects of exposure during gestation and birth). Group A female endpoints included no. of corpora lutea, live and dead fetuses, and implantation sites. Group B female endpoints included maternal body wt.; feed and water consumption; no., wt., and anogenital distance of pups; visceral evaluation of hearts and brains, and no. of uterine implantations. Male endpoints included clinical pathology, organ weights, sperm analysis, and histopathology. Compound consumption (mg/kg/d) was: TBA-1, 7, and 39; BCA-7, 20, and 50; and BDCM-11, 53, and 88. TBA marginally reduced water consumption at 400 ppm with no general toxicity. In contrast, BCA and BDCM produced decreases in water consumption by 17-86% and general toxicity in both sexes at doses at and above 600 and 700 ppm, respectively. BDCM also decreased body wt. by 5-13%, and feed consumption by 14-47% at doses at and above 700 ppm. TBA and BDCM produced no reproductive toxicity in either sex while BCA reduced litter size and implant no. at 600 ppm. Of the 3 DBPs administered to S-D rats in the drinking water, TBA produced no toxicity, BDCM produced only general toxicity, and BCA produced general and potential reproductive and developmental toxicity.

77 THE EFFECTS OF PERINATAL TEBUCONAZOLE EXPOSURE ON ADULT NEUROLOGICAL, IMMUNOLOGICAL, AND REPRODUCTIVE FUNCTION IN RATS.

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Studies are underway to address concerns of potential persistent immunotoxic, reproductive, and neurotoxic effects of perinatal exposure to several pesticides. Tebuconazole, a triazole fungicide, was evaluated as part of this project. Sprague-Dawley dams were administered tebuconazole (0, 6, 20, or 60 mg/kg) by oral gavage daily from gestational day 14 to postnatal day (PND) 7; the pups were then dosed at the same levels from PND 7 to 42. Separate groups of rats were used for testing of immuno-logical parameters, and neurobehavioral testing using a screening battery of functional tests, including cognitive evaluations. Other groups of rats were evaluated for repro-ductive development and function, while others were sacrificed at designated times for histological analyses. Pup viability and body weight were decreased (13-17%) at the high dose. Histological analyses were generally unremarkable. There were no differences in the fertility indices. Although spleen weights were increased in high-dose rats immunized with sheep RBCs, there were no biologically significant changes in the rats evaluated for immunotoxicity. Acquisition of learning the platform location in a water tank (*i.e.*, Morris water maze) was impaired in high-dose rats, but there were no differences in neuromuscular ability, motor activity, or swim speed to account for this finding. Furthermore, there was no effect on recall of the position during a free-swim trial. In rats trained on a multiple fixed-ratio (FR) fixed-interval (FI) schedule of operant responding for food reinforcement, the high-dose rats showed faster FR responding but FI responding was not altered. Thus perinatal exposure to tebuconazole produced specific neurobehavioral deficits in rats, but did not alter immunological or reproductive function.

78 THE EFFECT OF ELIPRODIL ON EMBRYONIC DEVELOPMENT USING THE RAT POST-IMPLANTATION WHOLE EMBRYO CULTURE SYSTEM.

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Eliprodil is an experimental neuroprotectant which mediates its effects by antagonism of NMDA and possibly sigma receptors in the brain. Recent studies suggest that sigma receptors are enzymes in the post-squalene cholesterol biosynthetic pathway. Eliprodil and other sigma receptor binding compounds are, therefore, likely to be inhibitors of cholesterol biosynthesis. The developing embryo/fetus is dependent upon *de novo* cholesterol biosynthesis, only a small proportion is derived from the maternal supply. The purpose of this study was to determine the effects of eliprodil on developing embryos and investigate the mechanism of any observed toxicity using rat post-implantation whole embryo culture. Rat embryos were explanted and cultured for 48 hours during early/mid organogenesis (9.5-11.5 days). After this time embryos were evaluated microscopically and scored for lethality, growth, development and incidence of malformation. Eliprodil at 0.5μM induced growth and developmental retardation in addition to forebrain and eye abnormalities. Total embryolethality was observed at 5μM. In order to determine whether the observed effects were attributable to cholesterol biosynthesis inhibition, further studies were conducted in the presence of cholesterol, which was added to the culture media at 12.5μg/ml (in an appropriate vehicle). The presence of cholesterol had no effect upon eliprodil-induced growth and developmental retardation, however, the incidence of malformations was inhibited at concentrations of 1.5μM eliprodil and below. Furthermore, embryolethality was reduced to 44% at 5μM eliprodil. These findings suggest that inhibition of cholesterol biosynthesis is at least partially responsible for the observed developmental toxicity of eliprodil.

79 FREE RADICAL-MEDIATED OXIDATIVE DNA DAMAGE IN THE MECHANISM OF THALIDOMIDE TERATOGENICITY.

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Our preliminary rabbit studies suggested that thalidomide oxidises embryonic DNA, which along with its teratogenicity was inhibited by the free radical trapping agent alpha-phenyl-N-t-butylnitrone (PBN). In this completed study, New Zealand white rabbits (thalidomide sensitive) and mice (resistant) were treated on gestational day (GD) 12 with PBN (40 mg/kg) or its vehicle iv 15 min prior to thalidomide (400 mg/kg, rabbits; 1200 mg/kg, mice) or its vehicle po. Six hr later, in rabbits, thalidomide enhanced DNA oxidation 3.8-fold in embryos, and 5.5- to 63-fold in various maternal tissues ($p<0.05$), while mice were unaffected. In rabbits treated as above, but from GDs 8-12, controls had no birth defects, while thalidomide caused a 35 % incidence of phocomelia ($p<0.005$), 10% omphalocele and 10% adactyly, and enhanced fetal resorptions, postpartum lethality ($p<0.005$) and fetal weight loss ($p<0.05$). PBN pretreatment inhibited thalidomide-initiated DNA oxidation by 73% in embryos and 61-86% in maternal tissues, virtually abolished phocomelia ($p<0.001$) and other anomalies, and substantially reduced all other embryopathies ($p<0.05$). These results suggest a free radical-mediated mechanism of thalidomide teratogenesis, and provide the first direct insight into a potential embryonic determinant of species-dependent susceptibility. (Support: Medical Research Council of Canada.)

80 DEVELOPMENTAL CARDIOTOXICITY OF BETA-ADRENERGIC AGONISTS: UNIQUE RECEPTOR SIGNALING MECHANISMS.

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Beta-adrenergic agonists are potentially cardiotoxic but the adult heart is protected from excessive adrenergic stimulation by receptor desensitization. In contrast, fetal and neonatal hearts sensitize in response to repeated stimulation. We have examined the mechanisms underlying the developmental regulation of cardiac beta-adrenoceptor responsiveness by assessing adenylyl cyclase activity, receptor/G-protein coupling and BARK1 activity. Two hours after a single s.c. injection of isoproterenol to adult rats, homologous receptor desensitization was demonstrable as a loss of the ability of isoproterenol, but not glucagon, to stimulate adenylyl cyclase in isolated cell membranes. At later time points, adults showed heterologous desensitization, a loss of the response to glucagon, which shares the same transduction protein (Gs) but not the same receptor. There was also a decrement in the ability of receptors to couple to Gs after isoproterenol treatment in adults, demonstrated as the loss of the ligand binding response to GTP. When isoproterenol was given to neonates, neither homologous nor heterologous desensitization was seen. Instead, the treatment enhanced the coupling of beta-receptors to Gs and increased the adenylyl cyclase response to either isoproterenol or glucagon. The absence of desensitization in neonates did not reflect a deficiency in the activity or subcellular distribution of BARK1, the enzyme that initiates receptor phosphorylation and consequent desensitization. The absence of desensitization in the fetus and neonate, and the presence instead of desensitization, is likely to play a role in cardiotoxicity of adrenergic agents such as those used to arrest premature labor. Furthermore, as fetal/neonatal adrenergic signals establish the proper set-point for future receptor responsiveness, exposure to adrenergic agonists or antagonists during this period may result in long-lasting changes in cardiac responses to physiological or environmental challenge. (Supported by USPHS HD-09713 and a fellowship from the American Heart Association.)

81 EFFECTS OF ACETAMINOPHEN ON PREIMPLANTATION EMBRYO GLUTATHIONE CONCENTRATION AND DEVELOPMENT IN VIVO.

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This study investigated whether high doses of acetaminophen (APAP) adversely affect preimplantation embryos in vivo. Previous studies indicate that cleavage stage embryos cannot synthesize reduced glutathione (GSH) de novo and may as a result be sensitive to GSH depleting toxicants. Alternatively, there may be maternal mechanisms that protect the embryos from the adverse effects of these toxicants. To address these possibilities, we measured GSH in embryos, livers, and ovaries of female mice after treatment with either vehicle (C) or APAP. Treatment with 800mg/kg/day APAP (A800) from day -8 to day 3 (d3) of gestation (day 0 being the day of detection of vaginal plugs) did not affect embryo (E) or liver (L) GSH concentration on d1 or d3 of gestation (d1E C 0.58 pmol/embryo, A800 0.51; d3E C 0.10, A800 0.24; d1L C 8.2mM, A800 8.9; d3L C 5.0, A800 5.0). Embryos also did not exhibit developmental effects in vitro (% blastocyst C 70, A800 80) or in vivo (% blastocyst C 76, A800 75). Two hours (h) after 1 dose of 800 or 1430mg/kg/day (A1430) on d2 of gestation, GSH concentration in the liver had decreased (C 7.7mM, A800 1.8, A1430 1.2; $p<.01$) but it recovered by 16h (C 7.2, A800 6.6, A1430 8.1). There was no effect on embryo development (% morula C 81, A800 93, A1430 99) or embryo GSH concentration at 2h (C 0.39 pmol/embryo, A800 0.37, A1430 0.33) or at 16h (% morula C 88, A800 73; C 0.51 pmol/embryo, A1430 0.39). Ovary GSH concentration did not decrease at 2h or 16h (2h C 2.5mM, A800 3.3, A1430 2.2; 16h C 1.2, A800 1.1, A1430 0.71). Preliminary data indicate no significant difference in the number of fetuses (C 9.3, A800 7.5), the number of resorptions (C 5.3, A800 1.5) or the number of gross malformations (C 0, A800 0) on d17 of gestation after treatment with 800mg/kg/day from d -8 to d3 of gestation. In summary, large doses of APAP depleted liver GSH temporarily but did not affect the development of preimplantation embryos or their GSH concentration. In conclusion, preimplantation embryos appear to be protected from GSH depleting toxicants in vivo.

82 GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G6PD): EMBRYONIC GENOTYPE AND PHENOTYPE CONTROL TERATOLOGIC SUSCEPTIBILITY TO ENDOGENOUS OXIDATIVE STRESS AND PHENYTOIN.

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While the major risk from hereditary G6PD deficiencies is thought to be red blood cell hemolysis, we have shown in mutant pregnant mice that both heterozygous (+/–) and homozygous (–/–) G6PD–deficient dams are more susceptible to embryopathies and teratogenicity caused by oxidative stress arising endogenously, or enhanced by phenytoin (Fund. Appl. Toxicol. 30[S1,P2]: 197, 1996; 36[S1,P2]: 98, 1997 In this study, compared to G6PD–normal littermates on gestational day 19, G6PD activity was 48% in +/– G6PD deficient fetuses and 18% in combined –/– and –/y G6PD deficient fetuses ($p<0.05$). There was an 11–fold difference between the highest (spleen) and the lowest (heart) activities in various maternal organs. G6PD–deficient fetuses with a mutation in all G6PD alleles (–/–, –/y) had more embryopathies, including teratologic anomalies, with +/– G6PD–deficient fetuses showing intermediate risk ($p<0.05$). This gene dose–dependent pattern was observed in both vehicle– and phenytoin–exposed fetuses, with

greater, drug dose–dependent teratogenicity in the latter ($p<0.05$). Genotyped fetal resorptions were primarily G6PD–deficient. This is the first direct evidence that embryonic G6PD is a critical determinant of susceptibility to the embryopathic and teratogenic effects of developmental oxidative stress and phenytoin. (Support: Medical Research Council of Canada.)

83 THE STRESS GENE HSP90 FUNCTIONS IN NORMAL VERTEBRATE MYOGENESIS, IN ADDITION TO ITS FUNCTION AS AN INDICATOR OF BIOLOGICAL STRESS.

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The accumulation of Hsps (heat shock proteins) is a universal cellular response to chemical and environmental insults, elevated temperatures, and disease states. The presence of Hsps in every living organism examined, and in every tissue type, make them an extremely sensitive indicator of cellular stress. In addition to an examination of *hsp90* gene regulation following stress, this work examined the in vivo regulation of the *hsp90a* gene during zebrafish embryogenesis. Whole mount in situ hybridization analysis using gene specific DIG-riboprobes was used in order to examine the in vivo spatial expression pattern of *hsp90a* and *hsp90b*. Constitutive accumulation of *hsp90b* mRNA was visible in a wide range of embryonic tissues, whereas *hsp90a* mRNA was localized specifically to the skeletal myogenic tissue of the trunk and pectoral fin. This suggests that *hsp90a* and *hsp90b* expression is uncoupled during embryogenesis. Similar experiments performed on mid-somitogenic chicken embryos gave similar results, suggesting that the stage- and tissue-specific expression of *hsp90a* is indicative of an evolutionarily conserved function in vertebrate myogenesis. In order to examine the cell-specific localization of *hsp90a*, whole zebrafish embryos were sectioned following hybridization with antisense riboprobe. Results confirmed high level expression of *hsp90a* within the myogenic cells, prior to segmentation and somite formation. Zebrafish mutant embryos which exhibit aberrant myogenesis were analysed, and results confirmed the localization of *hsp90a* within the cells fated to form striated trunk muscle. This work demonstrates, for the first time, the independent regulation of *hsp90a* and *hsp90b*, and the uncoupling of stress-induced and constitutive expression of *hsp90a*.

84 CONSTRUCTION OF A BIOLOGICALLY BASED DOSE-RESPONSE MODEL FOR DEVELOPMENTAL TOXICITY OF 5-FLUOROURACIL IN THE RAT.

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Biologically based dose-response modeling is an emerging approach to improve human health risk assessment. For a developmental toxicant, these models provide an integrated picture of the sequence of events from exposure of the pregnant dams, to molecular interactions within the embryonal cells, interference with organogenesis, and ultimately defective anatomical structures and/or deficient organ functions. Our laboratory has attempted to construct such a model using the chemotherapeutic agent 5-fluorouracil (5-FU) as a prototype developmental toxicant. A single sc injection of 5-FU (doses ranging from 1 mg/kg to 40 mg/kg) was given to pregnant Sprague-Dawley rats on gestational day 14, and various biochemical parameters were measured. 5-FU had a short half-life in maternal serum and direct incorporation of 5-FU into embryonic nucleic acids was low. However, activity of thymidylate synthetase and deoxyribonucleotide (dNTP) pools in the whole embryos, cell cycle profiles of embryonic tissues, total embryonic DNA content and fetal weights were profoundly altered by 5-FU in a dose-dependent manner. Frank terata were noted at doses above 25 mg/kg. The relationship between dNTP pool imbalance and incidence of anatomical defects as well as fetal weight deficits were evaluated by mathematical simulation. These models indicated different associative patterns between dNTP alterations and the two endpoints of toxicity, perhaps reflecting different underlying mechanims. (This abstract does not reflect US EPA policy.)

85 ENDOTHELIN-A RECEPTOR ANTAGONISM PRODUCES DEFECTS OF NEURAL CREST-DERIVED TISSUES IN WHOLE EMBRYO CULTURE.

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Endothelin-1 (ET-1) is a potent vasoconstrictor and has recently been shown to be important in development of the cranial neural crest (CNC) derivatives. Furthermore, recent evidence suggests that the role of ET-1 in CNC development is mediated by the ET_A receptor. Whole embryo culture was used to determine whether ET_A receptor antagonism in gestation day (GD) 8-10 embryos produced defects in CNC-derived tissues. CD-1 mouse embryos at GD8 (4-5 somite) were cultured for approximately 52 hours in the presence of 0 or 100 μM BQ-123 (an ET_A selective antagonist). Embryos exposed to the antagonist had abnormalities of the pharyngeal arches, especially the first and second arches. Also, preliminary data suggest that treatment with 200 μM BQ-123 does not cause malformations that are obviously more severe than those resulting from exposure to 100 μM. To determine the period of greater sensitivity, embryos exposed for the full culture period were compared with those exposed for either the first or second half of that period only (i.e., day 8 or 9). The effect on arch development appeared greater in day 8- than day 9-exposed embryos. Therefore, blockade of the ET-1/ET_A pathway interferes with development of the pharyngeal arches with the greater effect apparently occurring on GD8. This further implicates ET-1 and the ET_A receptor in the early events of CNC cell development, such as emigration, migration, proliferation, and/or differentiation. (This abstract does not necessarily reflect US EPA Policy and was partly funded by EPA/UNC Toxicology Research Agreement CT902908.)

86 REPRODUCTIVE AND DEVELOPMENTAL TOXICITY IN RATS TREATED ORALLY WITH 2-AMINO-6-CHLOROPURINE.

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Reproductive studies were undertaken with 2-amino-6-chloropurine (ACP), a pharmaceutical intermediate (CAS RN 10310-21-1), because it was found to be an *in vitro* and *in vivo* mutagen. Male fertility was assessed at 10, 50 and 100 mg/kg/day after 32 and 67 days of dosing by mating with untreated females. Effects on female fertility were examined at 10, 50 and 75 mg/kg/day by dosing prior to mating, throughout mating (with untreated males), and during pregnancy, delivery and lactation. Developmental toxicity was assessed at 10, 50 and 100 mg/kg/day by dosing females from days 6 to 17 of pregnancy and sacrificing day 20 for examination of their uterine contents. In the male fertility study severe toxicity and lethality occurred at 100 mg/kg/day. All animals died or were terminated prior to mating, but no morphological changes were detected in the testes and there was no reduction in sperm counts. Male fertility was unaffected at lower doses and there were no changes in the testes or sperm counts. In the female fertility study even with adult toxicity at 75 mg/kg/day there was no effect on estrous cycles and mating performance but embryo and fetal lethality occurred. At 50 mg/kg/day adult toxicity was minimal with a reduction in litter size, birth weight, growth and viability during lactation. No treatment related effects were observed at 10 mg/kg/day. In the developmental toxicity study at 100 mg/kg/day there was maternal toxicity, embryolethality and reduced fetal and placental weights. The surviving fetuses showing visceral and skeletal anomalies. At 50 mg/kg/day there was no maternal toxicity, but placental weights were decreased and there was an increase in fetuses with supernumerary ribs. No adverse fetal effects were seen at 10 mg/kg/day. The reproductive NOEL of ACP for both sexes was 10 mg/kg/day in this study.

87 TERATOLOGICAL RELEVANCE OF EMBRYONIC GENOTYPE IN BENZO[A]PYRENE (B[a]P)–TREATED KNOCKOUT MICE.

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B[a]P teratogenicity is enhanced in knockout mice, suggesting serves a teratological suppressor role with DNA–damaging teratogens (Nature Genet. 10: 181–185,1995). We report here by embryonic genotype that pooled –/– fetuses exposed in vivo to B[a]P had about a 60% higher incidence of col-

lective anomalies than either +/+ or +/– fetuses (p>0.05), while +/– fetuses from +/– dams had a 2.8–fold higher incidence than those from +/+ dams p<0.003).Conversely, +/+ fetuses were more susceptible to 2 anomalies (omphalocele, red nevÍ 7ÛI ´Ís) (p=0.09), and +/+ fetuses from +/+dams had a 4–fold higher incidence of postpartum lethality (p<0.002) than +/– littermates, with a similar but lesser gene pattern in +/– dams (p<0.02). Also, +/+ and +/– fetuses from +/– dams had 3.2– and 8.8–fold higher postpartum lethality than their respective genotypes in +/+ dams (p<0.0001). In staged embryos analyzed by Nile blue sulfate staining during organogenesis, a pattern of cell death was evident 3 hr after B[a]P exposure, independent of genotype. Our results suggest that the protective role of p53 for most B[a]P anomalies and in utero death may involve DNA repair, while postpartum lethality and 2 anomalies involve dependent apoptosis, and some embryopathies are independent. Maternal genotype remains an important risk determinant, but the embryonic genotype is teratologically relevant, and determines whether B[a]P–exposed fetuses will die in utero or postpartum. (Support: Medical Research Council of Canada.)

88 PROTEIN KINASE C ONTOGENY IN THE DEVELOPING PALATE AND THE EFFECT OF SECALONIC ACID-D ON ITS EXPRESSION IN MICE.

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Protein Kinase C (PKC) is a central player in signaling pathways mediating cell proliferation. Since cell proliferation is critical in palatal shelf growth and since PKC-inhibition may play a role in secalonic acid-D (SAD)-induced cleft palate, this study was undertaken to establish the normal ontogeny of Ca^{2+}-dependent (-α , -β I, -β II and -γ) and independent isozymes (-δ , -ε , -ζ) in developing murine palate during gestation days (GD) 12 through 15 and to evaluate the effects of SAD on PKC ontogeny. Palatal tissue extracts from control and SAD-exposed offspring were subjected to western blot analysis using isozyme specific antibodies and quantitative densitometry. The Ca^{2+}-dependent isozymes ε and ζ were expressed in controls at high levels on GD 12 and increased further throughout palatogenesis. The isozyme δ was expressed at constant but lower levels with a ~60 Kd band of equal intensity being also present. The expression of Ca^{2+}-dependent isozymes β I and γ was negligible. The expression of β II progressively decreased between GD 12 and 14.5 and was noticeably reduced by SAD on GD 14. PKC-α was expressed moderately between GD 12 and 14.5 but was drastically reduced on GD 15. The observed ontogeny of PKC isozymes may suggest isozyme-specific roles for PKC in palate development. Lack of effects of SAD on isozyme expression suggests a greater role for inhibition of PKC activity by SAD in its teratogenic effect. (Supported by NIH grant # DE/OD 11822.)

89 DEVELOPMENTAL, PHYSIOLOGICAL, AND CHEMICAL REGULATION OF RAT HEPATIC STEROID SULFATASE.

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Steroid sulfatase is a microsomal enzyme that mediates the hydrolysis of sulfate esters formed in part through sulfotransferase-mediated sulfation. The expression and activity of steroid sulfatase in proximity to sulfotransferase enzymes may effectively negate cellular sulfation of drug and chemical moieties. To understand the role of steroid sulfatase in sulfation we characterized the effect of gender, age, physiological hormones, and chemicals on the expression and activity of steroid sulfatase. Western-blot analyses and enzymatic activity with estrone-3-sulfate as substrate indicated that there were no significant differences in steroid sulfatase expression and activity between male and female Spraque-Dawley rats. Steroid sulfatase was present at day-1 post-partum and remained relatively constant during development (1-90 days of age). Manipulation of endogenous endocrine hormones by surgical hypophysectomy (25-day-old rats) did not significantly alter expression of steroid sulfatase in male and female rats. In addition, male and female rats were treated with phenobarbital, 3-methylcholanthrene, Aroclor 1254, and pregnenolone-16α-carbonitrile for 7 days to determine whether steroid sulfatase is responsive to chemical induction. In contrast to many microsomal drug-metabolizing enzymes, steroid sulfatase is refractory to induction by these classical enzyme-inducing chemicals. Our results suggest that steroid sulfatase is fully developed at birth, is not sexually divergent, and is not modulated by either hormonal status or chemical treatment. Therefore, the activity of steroid sulfatase must be accounted for when assessing sulfation-mediated detoxication or bioactivation of sulfotransferase substrates. (Supported by USPHS grants ES-07079 and ES-03192, and Kansas Health Foundation grant KHF-411606.)

90 DEVELOPMENTAL ANALYSIS OF GENE EXPRESSION IN THE MALE REPRODUCTIVE TRACT DURING *IN UTERO* EXPOSURE TO DI-*N*-BUTYL PHTHALATE.

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Di-*n*-butyl phthalate (DBP), a plasticizer and solvent with widespread commercial use, produces severe developmental abnormalities of the male reproductive tract via a nonreceptor-mediated mechanism following *in utero* high-dose exposure. The testis is postulated to be the primary target of DBP toxicity. Therefore, the objective of this study was to examine the steady-state levels of critical genes expressed in the testis that are involved in the development of the male reproductive tract. Pregnant Sprague-Dawley rats were administered 500 mg/kg/day DBP in corn oil by gavage from gestation days 12 to 21, the period of reproductive organ development in the fetus and, consequently, the critical window of exposure for induction of DBP-mediated reproductive tract malformations. Steady-state message levels of steroidogenic factor-1 (SF-1) and androgen receptor (AR) were analyzed from combined testes and epididymides total RNA of individual DBP- and corn oil-treated offspring using semi-quantitative PCR. Flutamide-treated animals (50mg/kg/day) served as positive controls. Preliminary studies showed a slight (10-20%) decrease in SF-1 mRNA expression in combined DBP-treated testes and epididymides and a similar increase in flutamide-treated animals at GD 21. However, AR message levels were unaltered by DBP or flutamide treatment during the same period of development. More recently, immunohistochemical analysis of AR protein levels has shown an apparent increase in AR protein staining in DBP-treated testes and decreased AR protein staining in DBP-treated epididymides. These findings suggest that changes in gene expression may be tissue-specific in the reproductive tract of DBP-treated males, and therefore, additional analysis of individual tissues will be necessary to observe significant changes in gene expression that may lead to the observed reproductive tract malformations.

91 DI(*n*-BUTYL) PHTHALATE INDUCES CHANGES IN MORPHOLOGY AND ANDROGEN RECEPTOR LEVELS IN THE FETAL TESTIS.

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Fetal exposure to di(*n*-butyl) phthalate (DBP) causes epididymal agenesis, decreased sperm production, and interstitial cell hyperplasia and adenoma in adult male rats. Similar effects are produced by the potent androgen receptor (AR) antagonist flutamide (FLU). Although these effects are indicative of disruption of male sexual differentiation via an antiandrogenic mechanism, DBP is not an AR antagonist. Because AR-mediated signaling pathways are essential for male sexual differentiation, changes in fetal AR levels may occur in response to DBP. Pregnant CD rats were dosed from gestation day (GD) 12 to 21 with DBP at 500 mg/kg/day (n = 5) or FLU at 100 mg/kg/day (n = 2). On GD 21, AR levels in the fetal testis and epididymis were evaluated by immunohistochemistry. In the testicular interstitial cell compartment, DBP caused an increase in the number of cells and an apparent increase in AR immunostaining. Seminiferous tubules were also enlarged and contained multinucleated, undifferentiated germ cells. AR staining in interstitial cells was decreased by FLU with no change in testis morphology. In the epididymis, fewer ducts were apparent in DBP- and FLU-exposed fetuses. An overall decrease in AR staining was seen with FLU whereas DBP caused decreased staining in some epididymal ducts. Unlike FLU, the antiandrogenic activity of DBP is not mediated through an overall decrease in AR levels and suggests that DBP disrupts sexual differentiation through a different mechanism. The early morphological changes induced by DBP in the fetal testis are likely to be associated with the testicular lesions seen in adults.

92 TOXIC RESPONSES OF MEDAKA (d-Rr STRAIN) TO POLYCHLORINATED NAPHTHALENE (PCN) MIXTURES AFTER EMBRYONIC EXPOSURE BY IN OVO NANOINJECTION: A PARTIAL LIFE CYCLE ASSESSMENT.

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PCNs are compounds with some chemical properties/uses similar to polychlorinated biphenyls (PCBs), and have been detected in biota from some aquatic environments. The toxicities of several PCN technical mixtures (Halowax) to medaka (*Oryzias latipes*), were determined using an embryo nanoinjection method. Medaka eggs (early gastrula) were injected 0.5 nl of triolein (vehicle control) or 0.5 nl of 4-5 graded doses (0.3-30 ng/egg) of Halowax 1014, Halowax 1013, or Halowax 1051 in triolein. Following exposure, embryos developed and fry were reared for approximately 4 mo (sexual maturity), at which time they were euthanized. Responses were evaluated as early life stage (ELS), and early adult life stage (EALS) assessments. For ELS, lethality and sublethal alterations in embryos and larvae (< 16-d old) such as craniofacial, cardiovascular, and myoskeletal deformities, and abnormal or delayed hatch were monitored for the first 9 d, and a severity index computed. EALS assessment examined survival of 16 d-old larvae until early adulthood (122 ± 2 d-old), including gonadosomatic index (GSI) and morphometry. Halowax 1014 was found to be the most toxic mixture (LD_{50} 4.2 μg/g egg), while Halowax 1013 and 1051 were significantly less toxic. GSI of females was significantly less in fish dosed with Halowaxes 1014 (dose response manner) or 1051. The LD_{50} for embryos nanoinjected with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is approximately 0.8 ng/g. Thus, Halowax 1014 was 5250-fold less potent than TCDD. ELS assessments accurately predicted the results of EALS assessments.

93 EFFECTS OF DEVELOPMENTAL EXPOSURE TO AROCLOR 1254 ON LEARNING AND SUSTAINED ATTENTION IN RATS.

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Perinatal exposure to polychlorinated biphenyls (PCB) in humans is suspected of causing behavioral and developmental deficits in infancy and childhood. In addition, *ortho*-substituted PCBs accumulated selectively in brain during *in vivo* exposure and these PCB congeners altered Ca^{++} homeostasis *in vitro* in neuronal cultures. The present studies were designed to determine whether perinatal exposure to Aroclor 1254 (Lot #124-191, AccuStandard), a commercial PCB mixture containing a high proportion of *ortho*-substituted congeners, would affect cognitive function. We assessed the ability of perinatally-exposed rats to learn a lever press response and a two-choice, discrete-trial visual signal-detection task (SDT). The SDT was then used to assess sustained attention. Thirty-five dams received daily oral doses of 0, 1, or 6 mg/kg Aroclor 1254 in corn oil from gestational day 6 through postnatal day 21. One male and one female pup were selected from each litter for behavioral assessment. Assessments began at 110 days of age and included 1) autoshaping a lever press for food reinforcement; 2) learning the SDT; and 3) manipulation of the inter-trial interval (ITI), from constant to variable, in the SDT. Control male rats learned the lever press response faster during autoshaping than did the PCB treated males, while the high-dose females learned more quickly than the control and low dose females. No significant differences were seen in learning or performing the SDT on measures of probability of a hit [P(hit)], or probability of a false alarm [P(fa)]. However, when the ITI was made variable, P(hit) of the high dose PCB animals fell less than that of the control or low-dose animals. These results provide little support for reports that perinatal exposure to PCB causes deficits in cognitive function in children. Here, Aroclor 1254 appeared to exert a gender-specific, transient effect on learning, but no persistent effects on sustained attention.

94 PERINATAL EXPOSURE TO AROCLOR 1254 SUPPRESSES THE ONTOGENETIC PEAK OF MOTOR ACTIVITY IN LONG-EVANS RATS.

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Previously, this laboratory has shown that Long-Evans rats have a reproducible pattern of motor activity during development which peaks between postnatal days (PND) 14 and 16 followed by a decline in activity until stasis is reached at approximately PND 21. In this study we examined the effect of perinatal Aroclor 1254 exposure on the established ontogeny of motor activity in developing rats. Maternal dosing via oral gavage was carried out daily from gestational day six through weaning (PND 21). The doses of Aroclor 1254 chosen were 0, 1, and 6 mg Aroclor 1254/kg body weight/day; thus pups were exposed only via the dams. One male and one female each from eight litters were tested for each dose level. Each animal was placed in a Motron chamber and horizontal activity was measured for 30 minutes. There was no effect of Aroclor 1254 on motor activity in the males. However, there was a significant depression of the peak in motor activity in females (-34.4%, p=0.02) at the 6 mg/kg/day dose level. This experiment was repeated twice, once using the same male and female from each litter and in the second cohort choosing one male and one female at random each day. This suppressive gender-specific effect was robust to be observed in both protocols. Thus it appears ontogeny of motor activity, a recognized developmental milestone, is suppressed in a gender-specific manner by perinatal Aroclor 1254 administration. (Supported in part by USEPA/UNC Training Agreement, CT 901915.)

95 PERINATAL EXPOSURE TO AROCLOR 1254 ALTERS MOTOR ACTIVITY IN ADULT LONG-EVANS RATS CHALLENGED WITH NICOTINE.

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Previously, this laboratory has shown that Long Evans rats have a reproducible pattern of motor activity during development which peaks between postnatal days (PND) 14 and 16 followed by a decline in activity until stasis is reached at approximately PND 21 and that perinatal Aroclor 1254 exposure suppresses the peak of this ontogenetic pattern of motor activity in females. In this study, we examined the alteration of responses to drug challenges using motor activity as an easily measured endpoint of drug exposure. Maternal dosing via oral gavage was carried out daily from gestational day six through weaning (PND 21). The doses of Aroclor 1254 chosen were 0, 1, and 6 mg Aroclor 1254/kg body weight/day; thus pups were exposed only via the dams. At PND 90, pharmacological challenges were administered to probe alterations of GABAergic, cholinergic, or dopaminergic neurotransmitter systems. Specific agonist and antagonists were used on a Tuesday/Friday dose schedule to allow at least 2 days wash-out time between drugs. Each animal was treated with each drug in a pseudorandom order, placed in a Motron chamber, and horizontal and vertical activity was measured for 30 minute sessions. Notably, all drugs chosen affected motor activity. The only drug which showed any significant interaction of drug treatment and Aroclor 1254 exposure was nicotine. Changes in motor activity induced by nicotinic agonists were altered by prior perinatal exposure to Aroclor 1254. (Supported in part by USEPA/UNC Training Agreement, CT 901915.)

96 CULTURED RAT NEURAL CREST CELLS RELEASE A MACROPHAGE REGULATORY FACTOR IN RESPONSE TO POLYCHLORINATED BIPHENYL (PCB) EXPOSURE.

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Neural crest cells (NCCs) are essential progenitors for many developmental structures. They are a highly migratory population of differentiating cells that are sensitive to toxic insults. The mechanisms that mediate NCC toxicity are not fully understood. Inflammatory cells (e.g. neutrophils, macrophages, mast cells) play a role in the mechanisms of various toxicities. Our recent data has suggested that conditioned medium collected from NCCs exposed to a single dose of PCBs is chemotactic for macrophages. Accordingly, the present study was designed to determine if PCBs, in a dose/time dependent manner, cause NCCs to release a factor(s) that regulates macrophage activity. To conduct this study, NCC cultures were treated with

various doses of Aroclor 1248 (a standard PCB mixture) and incubated for 8, 16, or 24 hours. The resulting NCC-derived conditioned media (NCC-CM) was collected. The ability of NCC-CM to induce macrophage chemotaxis (expressed as % positive control) and cytokine release was determined using a two-chambered chemotaxis system and spectrophotometric detection assays, respectively. NCC-CM derived from treatment with 25 and 50 uM Aroclor 1248 demonstrated chemotactic activity for macrophages and caused them to release both tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1). The production of NCC-CM that caused macrophage chemotaxis and cytokine release was dose and time dependent. NCCs required atleast 16 hours incubation with 25 or 50 uM Aroclor 1248 to produce stimulatory NCC-CM. These data suggest that the PCB mixture, Aroclor 1248, causes NCCs to release a factor(s) that is chemotactic for macrophages and induces their release of cytokines. (Supported by the Paul Cornely Fellowship and NIH grant ES05235.)

97 LONG-TERM EFFECTS OF POLYCHLORINATED BIPHENYLS (PCBS) IN THE NEONATAL BALB/C MOUSE MODEL.

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The neonatal mouse model has been a valuable tool in determining the long-term effects of early exposure to estrogenic agents in mammals. Using this model our specific aim was compare the effects of two hydroxylated polychlorinated biphenyls (HO-PCBs): 2Ô4Ô6Ô-trichloro-4-biphenylol (TRI) and 2Ô3Ô4Ô5-tetrachloro-4-biphenylol (TETRA) alone, and in combination (BI) to the effects of 17b-estradiol (E2). Treatment was administered via 5 subcutaneous injections beginning within 16 hrs after birth, days 1 - 5. The treatment groups were as follows: a) Control = vehicle, sesame oil b) E2 = [5 mg/g]; c) E2/Tri = [2.5 mg/g & 105 mg/g,]; d) Tri-Hi = [210 mg/g], e) Tri-Lo = [21 mg/g]; f) Tetra-Hi = [400 mg/g]; g) Tetra-Lo = [40 mg/g]; h) Bi-Hi = [100 mg/g Tri and 100 mg/g Tetra]; i) Bi-Lo = [10 mg/g Tri and 10 mg/g Tetra]. Vaginal openning results indicate that both HO-PCB congeners are estrogenic in the neonatal mouse model. The average day of vaginal opening in mice treated with these compounds were as followings: a) 25.4, b) 12.2, c)11, d) 12.6, e) 23.6, f) 12.6, g) 19.3, h) 11.1, and i) 25.1. These results also indicate that lower doses of HO-PCB3, HO-PCB4 or a mixture of these congeners exhibit no effect. At 20 month of age percent of mice which developed solid tumors from these neonatal treatments are as follows: a) 0, b) 5, c)5, d) 0, e) 30, f) 20, g) 26, h) 18, and i) 24. Additional results indicate that at the high dose TRI can induce an increase in uterine wet weight similar to E2 (141% and 140% of control, respectively). However, E2 was more effective in inducing persistent vaginal cornification (71% versus 53%). Based on these results in the neonatal mouse model, the PCBs tested are strongly estrogenic when exposure occurs during development. Such results may have very important implications for risk assessment in determining the effects of xenoestrogens exposure early versus later in life.

98 ARNT KNOCKOUT MICE EXPRESS VEGF INTROPHOBLASTIC GIANT CELLS AND PLACENTA.

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The aryl hydrocarbon nuclear translocator protein (ARNT) is a transcriptional regulator that heterodimerizes with other members of the basic helix-loop-helix/ Per-ARNT-Sim family, the Ah receptor, and hypoxia inducible factor 1α (HIF-1α). Under hypoxic conditions HIF-1α and ARNT upregulate vascular endothelial cell growth factor (VEGF). VEGF regulates vasculogenesis and plays a critical role in embryonic development. Transgenic mouse embryos null for ARNT expression die in utero and lethality is attributed to placental abnormality. This study compares VEGF expression of the wild type, heterozygous and homozygous ARNT conceptus. ARNT heterozygotes were mated; pregnant females sacrificed on gestation day 9.5; conceptuses removed from the uterus and a sample of embryonic tissue collected for genotypic analysis by PCR. The conceptuses were fixed in 3% paraformaldehyde, embedded in paraffin, and sectioned for immunohistochemical localization of VEGF. The polyclonal antibodies were prepared with either amino acids 1-20 of the amino terminus of mouse VEGF (P-20, sc-1836; peptide sc-1836P, Santa Cruz Biotechnology, Inc, Santa Cruz, CA) or with recombinant mouse VEGF, expressed in Sf-21 insect cells using a baculovirus expression system (AF-493-NA, peptide 493-M, R&D Systems, Inc, Minneapolis, MN). Antibody specificity was confirmed by competition for binding with excess purified VEGF peptide. VEGF was expressed strongly in trophoblastic giant cells and in decidual cells in contact with maternal blood lacunae at the periphery of the placenta. The localization did not differ across genotypes and was completely eliminated by competition with excess peptide. It has been hyopothesized that hypoxia is a factor in developmental regulation of VEGF, particularly during placentation. If ARNT were obligatory for this process, then a difference would be expected between ARNT null and wild type embryos and this was not the case. (Disclaimer: This abstract does not necessarily reflect EPA policy.)

99 DETERMINATION OF THE DEVELOPMENTAL EXPRESSION AND ACTIVATION OF THE ARYL HYDROCARBON RECEPTOR IN TCDD- RESPONSIVE *LacZ* MICE.

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The Aryl-hydrocarbon Receptor (AhR) is a ligand-dependent transcription factor that mediates the toxicity of halogenated aromatic hydrocarbons including 2,3,7,8-tetra-chlorodibenzo-p-dioxin (TCDD). These compounds are potent immunologic, reproductive and developmental toxicants due to their ability to alter gene expression and affect cell differentiation and proliferation. We do not yet know which tissues are most sensitive to these compounds nor do we know what genes are modulated to produce toxicity. The determination of the tissue-specific transcriptional activity of the AhR complex *in vivo* will allow the identification of tissues where the AhR may have some functional role and where exposure to TCDD may be disruptive. A transgenic mouse model has been developed in our lab that can delineate the temporal and spatial context of active AhR in a cell- and region-specific manner. This reporter gene based model has recently provided evidence for the presence of transcriptionally active AhR by *in situ* staining for β-galactosidase activity in the genital tubercle, facial regions, paws and other tissues after 24 hour exposure *in utero* to 30μg/kg TCDD. Specifically, in the genital tubercle, a pattern of staining was observed with intense staining at GD 14, a bifocal pattern on GD 15 and a single line of staining on GD 16. Staining is also observed in the genital tubercle without TCDD treatment and preliminary gel shift data points to the potential for ligands to exist in this tissue. On the paw, a circular pattern of staining was observed in the palm on GD 15. This mouse model will prove useful in the examination of the developmental targets of the dioxin-like compounds and for the identification of potential endogenous roles for the AhR in development. (Supported in part by NIEHS Grant ES04862 and Training Grant ES07026.)

100 ALTERED CELLULAR PROLIFERATION IN THE SEMINAL VESICLES OF RATS GESTATIONALLY EXPOSED TO TCDD.

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) has been shown to alter male reproductive development in gestationally exposed laboratory animals. The accessory glands of the male reproductive tract, including the seminal vesicle, are decreased in size as determined by total weight of the tissue. Analysis of seminal vesicles over time suggests that the changes in weight may be transient (Mably *et al.*, 1992a and Gray *et al.*, 1997). However, Gray *et al.* (1995) reported that gestational exposure to 1.0 mg/kg of TCDD caused a significant decrease in seminal vesicle weights of 8-11 month old offspring. Therefore, we examined the proliferation of TCDD exposed rat seminal vesicle in the early postnatal period until the time of puberty. Pregnant Long-Evans rats were exposed to 1.0 mg/kg TCDD on GD 15 in corn oil. Male pups were sacrificed on postnatal days (PND) 14, 25, 32, and 49 and whole body, liver, and paired seminal vesicle, epididymal, and testis weights were recorded. In addition, seminal vesicles were fixed in 10% neutral buffered formalin for histological examination. No significant differences were seen in body weights throughout the study. Seminal vesicle weights were not significantly different between control and exposed animals at PNDs 14 (10.3± 1.5 versus 9.8± 2.2 mg) or 25 (16.9± 1.7 versus 16.1± 1.8 mg), by PND 32 exposed animals had lower seminal vesicle weights (59.6± 11.1 versus 36.7± 9.4 mg). By PND 49 total seminal vesicle weight was determined along with fluid content. While there appeared to be less of a difference in total seminal vesicle weight, seminal vesicle fluid content of exposed males was approximately 2/3 that of control animals. Differences in proliferation were examined histologically with identification of cell types using immunohistochemical markers. Data suggest that prenatal TCDD exposure alters seminal vesicle epithelial development. (This abstract does not necessarily represent USEPA policy. JTH, EPA, CT 902908).

101 TREATMENT OF RATS DURING PUBERTAL DEVELOPMENT WITH 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ALTERS BOTH SIGNALING KINASE ACTIVITIES AND EPIDERMAL GROWTH FACTOR RECEPTOR BINDING IN THE TESTIS AND THE MOTILITY AND ACROSOMAL REACTION OF SPERM.

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Different doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (0.1, 1, 5 and 10 mg/kg body weight) were administered ip to 21day old male Sprague-Dawley rats. Control animals received the same volume of the vehicle (acetone: corn oil, 1:19). Body weight and daily food intake were recorded during the 90 day time course of the study. Random samples of five rats were sacrificed at 34, 49, 62 and 90 days of age. Epidermal growth factor receptor (EGFR) in whole testis was measured, as were the activities of c-Src kinase, protein tyrosine kinase (PTK), mitogen activated protein 2 kinase (MAP2K also termed as Erk2), protein kinase A (PKA) and protein kinase C (PKC). Testicular tissue from 90 day old rats was evaluated for histopathology, and sperm numbers in whole testis were counted to estimate daily sperm production. The motility of sperm in the vas deferens and caudal segments of the epididymis of 90 day old rats was measured by computer assisted sperm analysis (CASA) and the function of the sperm was tested by assessment of acrosome reactions. A dose of 10 mg/kg resulted in testicular atrophy and histopathologic examination revealed a decrease in the diameter of the seminiferous tubules. Sertoli cell nuclei were clearly seen, but the spermatogonial population was totally absent. Lower doses of TCDD did not affect testicular histology, but doses as low as 1 mg /kg significantly decreased testicular sperm numbers and effected some sperm functions (motility parameters and acrosome reactions) in 90 day old rats. Significant decreases in EGFR were found in 34 day old rats and this effect on EGFR was sustained until the end of the experiment (90 days). Although TCDD significantly increased c-Src kinase activity in immature and mature rats, opposite effects of TCDD on activities of PTK, PKA and PKC were found in 34 day old rats vs 49, 62 and 90 day old rats. These findings provide evidence for involvement of signaling kinases and EGFR in the mechanism by which TCDD disrupts testicular development and subsequently affects testis function.

102 ACUTE AND SUBCHRONIC ADMINISTRATION OF TCDD IN LONG EVANS RATS: COMPARISON OF FETAL TISSUE LEVELS AND ADVERSE DEVELOPMENTAL EFFECTS.

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Prenatal exposure to TCDD interferes with fetal development at doses lower than those causing maternal toxicity. Studies have demonstrated that exposure to TCDD during development produced alterations in the reproductive system of developing pups, such as reduced sperm counts in males and malformations in the external genitalia of females. The objective of this study was to determine whether tissue concentration is the appropriate dose metric by relating fetal concentrations of TCDD after acute and subchronic exposure to the development of adverse effects in LE pups. In the acute studies, pregnant LE rats were dosed orally with 1.0 μ g [^{3}H]TCDD/kg on gestation day (GD) 8 or 0.05, 0.20, 0.80 and 1.0 μ g [^{3}H]TCDD/kg on GD15. In the subchronic study, LE rats received 1, 10 or 30 ng [^{3}H]TCDD/kg, 5 days/wk for 13 weeks. After 13 weeks, females were mated and dosing continued. Dams were sacrificed on GD16 and GD21 and analyzed for [^{3}H]TCDD in maternal and fetal tissues. A dose of 1.0 μ g TCDD/kg on GD8 produced fetal concentrations of TCDD on GD16 of approximately 18-22 pg/g. Exposure on GD15 to 0.05, 0.20, 0.80 or 1.0 μ g TCDD/kg produced fetal concentrations ranging from 7-58 pg/g on GD16. Repeated administration of 1, 10, or 30 ng TCDD/kg/d resulted in fetal tissue concentrations of 1.3, 7.2 and 14.8 pg/g, respectively. For the end-points examined (ejaculated sperm counts and puberty delay in males and urethra-phallus distance and vaginal thread incidence in females), GD16 fetal concentrations after acute exposure on either GD8 or GD15 result in comparable adverse responses. Furthermore, subchronic administration of 10 ng/kg/d produced similar fetal concentrations on GD16 as well as a similar incidence of vaginal thread formation as a single dose of 0.20 μ g TCDD/kg on GD15. These dispositional studies after different exposures demonstrate that tissue concentration, during a critical window of sensitivity, is the appropriate dose metric to predict adverse reproductive and development effects. (This abstract does not necessarily represent US EPA policy. CHH supported by EPA, CT 902908.)

103 DOSE-DEPENDENT INTERACTION OF SIMPLE CHEMICAL MIXTURES.

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The risk assessment for chemical mixtures requires several assumptions including that the nature of the aggregate toxicity at low total doses is similar to that at high doses. High to low dose extrapolation is a fundamental issue of toxicology and the experimental assessment of interactive toxicity is typically performed at dose levels which produce significant toxicity. In ongoing studies through a cooperative agreement with the ATSDR, issues related to refinement and development of methods for the toxicity assessment of mixtures are being explored. A series of experiments were performed to investigate the dose-dependent interactive toxicity of simple mixtures of toxic metals. Cultured Rhesus renal cortical cells, LLC-MK2, target cells for Cd and Hg toxicity, were treated with 12 concentrations of $CdCl_2$ (0 to 40 μM), $HgCl_2$ (0 to 80 μM), or binary mixtures of Cd and Hg (1:2 molar ratio) as to bracket ED_{50}s estimated for each toxicant. The cytotoxicity was determined at 24 hr by lactate dehydrogenase release. The data were analyzed four times; first as a complete data set, then using only the lowest, the middle, or the highest six metal concentrations. The data were analyzed statistically and graphically by the linear model of null interaction of Kodell and Pounds. Analysis of the full data set and the highest six concentrations revealed Response Additivity ($p \leq 0.0003$ and 0.02), while the middle dose set was neither Dose nor Response Additive but was within the envelope of additivity ($p \leq 0.27$), while the low dose data set was Dose Additive ($p \leq 0.0004$). These results suggest that (a) an elaborate data set is needed to identify and define interactive toxicity at low doses, and (b) illustrate the complexity of integrating toxicity from exposures to multiple chemicals in the environment. (Supported by ATSDR U61/ATU581481.)

104 DEVELOPMENT OF ISOBOLOGRAMS FOR THE ACUTE LETHAL INTERACTION OF PYRIDOSTIGMINE BROMIDE, PERMETHRIN, AND DEET IN THE LABORATORY RAT.

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In order to better understand the interactive effects caused by concurrent exposure to certain chemicals associated with Gulf War Illness, a study was conducted which examined the lethal effects in the male laboratory rat when given various two-compound combinations of pyridostigmine bromide (PB), permethrin, or DEET orally. The study was conducted in two phases. The first phase determined the lethal response to varying doses of individual compounds and a dose-response curve with 95% confidence intervals was generated to allow prediction of responses for a given dose. The second phase, using the isobologram approach, determined the interactive effect of two-compound combinations. Each compound was given at a minimum of three fixed doses, less than its median lethal dose (LD_{50}), with four varying doses of one of the other two-compounds. The LD_{50} for the second compound in the presence of the first fixed dose compound was determined. All LD_{50}s for the combination of two-compounds were plotted on the isobologram and compared to the hypothesized LD_{50}s. The combination of PB and DEET as well as permethrin and DEET produced 100% lethality at the lowest dosage levels (LD_{10} X LD_{10}, additive LD_{20}) and isoboles could not be developed for these combinations. An isobole was developed for the combination of PB and permethrin. The results of this study indicate that two-compound combinations of PB, permethrin or DEET, given orally to male rats, produce a significant increase in lethality when compared to expected results. Preliminary follow-up experiments suggest that the increased lethality may result from changes in the absorption of these materials from the gut following concurrent oral administration.

105 AN EMPIRICAL APPROACH TO PREDICTING BIOLOGICAL RESPONSES FOLLOWING EXPOSURES TO MIXTURES.

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In prior years, we developed an empirical method to calculate relative potencies (REP) for dioxin-like compounds (SOT, 1997) and validated the model by comparison to a mixture experiment (SOT, 1998). The REP is commonly calculated using a ratio of reference to test compound ED50s. That approach assumes that the test compound behaves as a dilution of the reference compound. The empirical method was developed because experimental data do not always result in an estimable ED50. This might be because the response is not saturable or because the maximum dose in the experiment did not result in a saturated response. A characteristic of the empirical method, therefore, is that it makes no assumptions about the biological relationship of test compounds to the reference compound. For this reason, the empirical method can be applied to any mixture of compounds. The empirical model is developed from dose-response experiments for a reference compound and as many test compounds as would be in the mixture. First, a calibration curve is fit for the reference compound. Second, inverse prediction is used to express the test compound data in the dose-metric of the reference compound. Third, a regression is fit using the dose-metric as the dependent variable and the test compound dose as the independent variable. This regression provides information about the biological relationship between the responses generated by the test and reference compounds. For example, if the regression is a straight line through the origin, then the slope will be identically equal to the REP calculated using ratios of ED50s. Because the method is based on statistical curve fitting, the variability estimates may be used in conjunction with Monte Carlo techniques to perform uncertainty analyses. The focus of this presentation will be to describe 1) appropriate experimental design to fit a 'good' calibration curve and 2) the information concerning biological relationships that can be determined from the regression relationships. (This abstract does not reflect US EPA policy.)

106 MULTIPLE CHEMICAL EXPOSURES SHOULD ALWAYS BE CONSIDERED BUT MIGHT NOT BE A CONCERN.

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National attention has been focused recently on health risks from air pollutants produced in urban areas where concentrated industrial activity coexists with high population density. In this study, ATSDR collected air monitoring data from the interior of houses of one such community and evaluated the potential for health effects from exposure to various chemicals. Detectable levels of 25 volatile organic compounds, 7 semi-volatiles, and three metals were found including benzene, chloroform, methylene chloride, polycyclic aromatic hydrocarbons, and lead. Benzene, methylene chloride and tetrachloroethylene were the most noteworthy chemicals, being found at levels ranging from 1.3 to 33.9 ug/m^3 (mean 4ug/m^3), 2 to 1194 ug/m^3 (mean 161ug/m^3), and 0.5 to 13 ug/m^3 (mean 2.6ug/m^3), respectively. Comparison of the indoor air levels of the chemicals found in these houses with levels reported in other studies, evaluations of exposure to individual chemicals using health-based comparison values developed by ATSDR/EPA, and assessment of multi-chemical exposures for noncarcinogenic effects were conducted. For the multi-chemical assessment, a 3-tier paradigm was designed using the hazard index (HI) approach. If the HI was<1, no threat was perceived. When the HI was >1, in the second tier, target-organ-specific toxicities were determined. If for the target-organ-specific toxicities the HI was >1, in the third tier, issues related to pharmacokinetics, bioavailability and mechanism of action of the chemicals were considered. The results indicate that residents in these houses were being exposed to concentrations of indoor air pollutants comparable to concentrations found in houses elsewhere in the United States. Also, the levels of contaminants detected, either singly or in combination, would not be expected to cause adverse health effects.

107 SUBCHRONIC TOXICITY OF AIDS COMBINATION THERAPIES 3'-AZIDO-3'-DEOXYTHYMIDINE (AZT) AND NITAZOXANIDE (NTZ) IN B6C3F1 MICE.

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Combination therapy with anti-HIV drugs and opportunistic infection therapies is a common practice in treatment of AIDS patients. Toxic effects of most individual therapies are available, but toxic consequences of most combination therapies are not known. To understand the toxicity of AIDS combination therapies, the commonly used anti-HIV drug AZT and an anti-cryptosporidial drug NTZ, either alone or in combination, were evaluated by 13 week gavage studies in B6C3F1 mice. The doses were AZT 200 and 400; and NTZ 250, 500, and 1000 mg/kg/day. AZT alone did not cause any mortality but caused dose-related hematological toxicity that was manifested as decreases in erythrocyte and hemoglobin values. NTZ alone did not cause mortality or significant changes in hematological parameters. Co-administration of NTZ with AZT caused a dose-related reduction in body weight gain in both sexes and markedly increased the hematological toxicity of AZT. All mice in the highest dose combinations and all female mice in the middle dose combinations died during the course of the study due to treatment-related hematological toxicity. Female mice were more sensitive to hematological toxicity with mortality at lower dose combinations. NTZ alone caused dilatation of the cecum. AZT did not appear to influence the dilatation of cecum caused by NTZ. However, NTZ potentiated the hematological toxicity of AZT even at the doses closer to the therapeutic dose. Results of our studies indicate that the toxic effects of combination therapies could be severe and different than the toxicity of individual therapies.

108 ALTERATIONS IN EXPRESSION OF TGFα, TGFβ, AND *C-MYC* IN HUMAN KERATINOCYTES EXPOSED TO ARSENIC OR A CHEMICAL MIXTURE OF FOUR METALS.

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A number of cellular and molecular biomarkers along the carcinogenic pathway in human keratinocytes were studied following exposures to arsenic (As) and an arsenic-containing metal mixture. To this end, two immortalized human keratinocyte cell lines, RHEK-1 and HaCaT, were exposed to arsenic alone and arsenic in a mixture of the environmentally relevant heavy metals, cadmium, lead, and chromium. Alterations in expression of the important keratinocyte growth regulatory factors, TGFα, TGFβ, and *c-myc* in treated versus control cells were analyzed by RT-PCR. In both HaCaT and RHEK-1 cells, exposure to increasing concentrations of As for 4 hr induced expression of both TGFα and TGFβ in a linear manner. Maximum induction of TGFα (12-fold over control) and TGFβ (5-fold) occurred at 0.05 μM As. At higher As concentrations (up to 2 μM), expression levels for both genes dropped substantially to control values. In HaCaT, *c-myc* mRNA was induced at all As concentrations tested (maximum induction 5-fold). In contrast, there was no detectable effect on *c-myc* expression in RHEK-1 cells under any conditions examined. We are currently comparing these results with alterations in RHEK-1 exposed to an arsenic-containing metal mixture. Although the exact significance of our findings is unclear at this time, these or other changes in gene expression may mediate, in part, the observed carcinogenic effect of As. This study was supported by ATSDR (Cooperative Agreement U61/ATU881475), and NIEHS Superfund Basic Research Program (P42 ES05949).)

109 DIFFERENTIAL TOXICITY OF ARSENIC, CADMIUM, CHROMIUM, AND LEAD SINGLY AND IN COMBINATION IN HUMAN KERATINOCYTES.

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As an initial phase of a project exploring the carcinogenic potential of As, Cd, Cr, and Pb alone or in combination in human keratinocytes, cytotoxicity studies were conducted. Three immortalized keratinocyte cell lines (RHEK-1, HaCaT, and NM-1) and primary human keratinocytes were exposed to increasing concentrations of the individual metals or to a mixture containing equimolar concentrations of all four metals. Cell viability was determined by MTT and clonogenicity assays after 24 hr treatment. The overall range of concentrations studied was from 0.001 μM to 100 μM and

at least 8 concentrations were used for each dose-response cytotoxicity curve. These studies demonstrated that the four types of keratinocyte cells exhibited different sensitivities to the four metals. Estimated LD_{50}s for As, Cd, or Cr in RHEK-1 were 4.5μM, 20μM, 6.4μM, respectively. Those for HaCaT were determined to be 4.6μM, 58μM, 5.6μM, respectively. The lead toxicity in RHEK-1 was low (only 12% cytotoxicity with the highest concentration). We are comparing these metal sensitivities in NM-1 cells and primary keratinocytes. Toxicity of the four metal mixture was studied in RHEK-1 and HaCaT cells; preliminary results suggest additive or antagonistic effects as compared to the individual metals. Cellular mechanisms conferring this differential sensitivity to heavy metals could potentially involve alterations in intracellular glutathione and/or expression of metallothionein. (This study was supported by ATSDR (Cooperative Agreement U61/ATU881475), and NIEHS Superfund Basic Research Program (P42 ES05949).)

110 THE EFFECT OF MIXING RATIO ON THE HEPATOTOXICITY OF MIXTURES OF TRIHALOMETHANE (THM) DISINFECTION BYPRODUCTS.

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Chlorination of water containing organic matter results in the production of a variety of halogenated byproducts, including the THMs, chloroform (CHCl3), bromodichloromethane (BDCM), chlorodibromomethane (CDBM) and bromoform (CHBr3). Water treatment processes that involve ozonation/chloramination decrease the total amount of THMs formed but alter their relative distribution. Thus, the objective of this investigation was to determine the effect of mixing ratio on mixtures of the 4 THMs. Female CD–1 mice (~65–70 days old) were gavaged daily for 14 days with the THM mixtures in 10% Alkamus El–620. Hepatotoxicity was assessed on day 15 by serum sorbitol dehydrogenase (SDH) and by histopathology. Relevant mixing ratios were selected from a study in which a high bromide water was treated either by pre-chlorination/post–chlorination (CL) or by pre–ozonation/post chloramination (OZCM). The proportions of the CL mixture were: CHCl3, 0.319; CHBr3, 0.049; BDCM, 0.342; and, CDBM, 0.290. The proportions of the OZCM mixture were: CHCl3, 0.655; CHBr3, 0.029; BDCM, 0.187; and, CDBM, 0.129. Three experiments were conducted, each testing a different total dosage of these two mixtures: 0.05, 1.50 and 3.0 mmol/kg/day. At each total mixture dosage, the SDH values for the CL and OZCM mixtures did not differ significantly. Similarly, the proportion of mice with centrilobular hepatocellular necrosis did not differ significantly between the two mixing ratios. In conclusion, at the same dosage, mixing ratio, or at least the two mixing ratios tested here, had little/no apparent effect on the hepatotoxicity of THM mixtures. Data such as these may help in selection of optimal water treatment strategies. As the total mixture dose was held constant in each experiment, differences due to the amount of THMs produced by each treatment are not reflected in these experiments, but are worthy of future investigation. (Abstract may not reflect EPA policy.)

111 CO-EXPOSURE TO EPICHLOROHYDRIN ON THE ELIMINATION OF URINARY METABOLITES OF DIMETHYLFORMAMIDE.

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Co-exposure of dimethylformamide (DMF), an organic solvent and epichlorohydrin (ECH), an ingredient of epoxy-resins not only occurs in industries but, to a lesser degree, also in the use of household products. Chronic effects of exposure to DMF include changes in liver function and reproduction. The detoxification of both DMF and ECH involves a metabolite conjugation with glutathione. This study was designed to determine the effect of a co-exposure to ECH on the elimination of urinary metabolites of DMF. Male Wistar rats, 4 per dose group, were gavaged consecutively with a dose of ECH (0, 4.5, 9.0, & 18.0 mg/kg) and a dose of DMF, 72 mg/kg. Urinary DMF and demethylated DMF (NMF) were analyzed by GC/FID. DMF-OH was analyzed by the method of Chang and Lin (1991) and creatinine, by Jaffe reaction. The results showed that at the dose levels studied, no effect of ECH co-exposure on elimination of urinary DMF, DMF-OH, NMF, creatinine, and urine volume was observed. It was concluded that in the presence of ECH, the biological exposure threshold limits of DMF required no change. (Supported by a grant NSC 86-2621-B-182-001Z, ROC.)

112 COMPENSATORY HYPERTROPHY AND CELLULAR FUNCTION AND TOXICITY IN PRIMARY CULTURES OF RAT RENAL CELLS.

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Primary cultures of rat renal proximal tubular (PT) and distal tubular (DT) cells from control and uninephrectomized (NPX) Sprague-Dawley rats were established to study whether the biochemical properties and toxicological responses identified in freshly isolated cells are maintained in culture. Cells were grown in serum-free, hormonally-defined DMEM: Ham's F12 (1:1) media and measurements of cellular protein and DNA contents, markers of cellular differentiation, activities of GSH-dependent enzymes, and transport activities were performed on days 1, 3 and 5 of primary culture. Epithelial cell differentiation was maintained in all cell types as evidenced by cytokeratin staining. PT and DT cell differentiation were maintained, as judged by γ-glutamyltransferase and hexokinase activities. PT cells from NPX rats retained their hypertrophic properties, as evidenced by increased cellular size and protein content. Previously, we demonstrated that freshly isolated PT cells from NPX rats were more susceptible to injury from environmental toxicants such as $HgCl_2$ than cells from control rats, even though they exhibited no difference in the cellular accumulation of $^{203}HgCl_2$. In this study, no significant differences in cellular accumulation of $^{203}HgCl_2$ were observed. However, PT cells from NPX rats were more susceptible than PT cells from control rats to cellular injury induced by either $HgCl_2$, KCN, or t-butyl hydroperoxide. Active transport of GSH was 3-fold higher in acivicin-pretreated PT cells from NPX rats than in those in control rats, suggesting that this could be an important defensive response to the increased susceptibility to toxicants. These results are in agreement with earlier *in vitro* and *in vivo* studies, demonstrating that primary cell culture provides a useful model to study the biochemistry and toxicology of uninephrectomy and compensatory renal growth.

113 NEPHROTOXIC AGENTS AND EXTRACELLULAR MATRIX PROTEINS INHIBIT CELL PROLIFERATION OF RENAL PROXIMAL EPITHELIAL CELLS UNDERGOING GROWTH FACTOR-INDUCED BRANCHING TUBULOGENESIS.

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Previous studies have shown that LLC-PK1 cells seeded into collagen type I matrix and treated with specific growth factor combinations undergo *in vitro* tubulogenesis. The present studies were conducted to determine if nephrotoxic agents or extracellular matrix proteins modulate cell proliferation of RPTEs undergoing branching tubulogenesis. In initial studies, cells were grown to post-confluence, treated with cisplatin or S-1,1',2,2'-tetrafluoroethyl-L-cysteine (TFEC) and seeded into collagen matrix. Both cisplatin and TFEC inhibited cell proliferation of LLC-PK1 cells undergoing branching tubulogenesis in a concentration-dependent fashion. In parallel experiments, cells were seeded into collagen gels, stimulated to undergo branching morphogenesis and then treated with cisplatin or TFEC. Under these conditions, only cisplatin inhibited cell proliferation of LLC-PK1 cells undergoing branching tubulogenesis. The effects of extracellular matrix on LLC-PK1 cell proliferation was then evaluated. Prior to seeding cells in collagen matrix, various concentrations of laminin, fibronectin, collagen type IV and poly-L-lysine were added to the matrix. LLC-PK1 cells were then seeded in the matrix and stimulated to undergo branching tubulogenesis. Laminin enhanced growth factor-induced proliferation of LLC-PK1 cells, while fibronectin, collagen IV and poly-L lysine caused a concentration-dependent inhibition in cell proliferation. In conclusion, growth conditions can profoundly effect the way specific nephrotoxicants modulate the proliferation of LLC-PK1 undergoing branching tubulogenesis *in vitro*. Furthermore, extracellular matrix components differentially regulate the ability of LLC-PK1 cells to undergo cell proliferation during growth factor-induced branching tubulogenesis. (These studies were supported by a grant from NIDDK (F32 DK09253-03).)

114 GLYCINE, STRYCHNINE, MUSCIMOL AND THAPSIGARGIN BLOCK INCREASES IN CYTOSOLIC FREE Ca^{2+} LEVELS IN RENAL CELL INJURY.

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We have shown previously that the cytoprotectants glycine, strychnine, muscimol and thapsigargin block $^{45}Ca^{2+}$ influx in the late stage of cell injury in renal proximal tubules (RPT) exposed to the mitochondrial inhibitor antimycin A. The goal of the current study was to determine the effects of glycine, strychnine, muscimol and thapsigargin on cytosolic free Ca^{2+} concentrations in rabbit RPT suspensions during antimycin A exposure. RPT were loaded (30 min) with the fluorescent Ca^{2+} indicator Fura-PE3-2AM (2 μM) in the presence of 0.1% pluronic acid. Thapsigargin (5 μM) was added 5 min prior to antimycin A (10 μ M) while glycine (2mM), strychnine (1 mM), or muscimol (100 μ M) was added simultaneously with antimycin A. Cytosolic free Ca^{2+} concentrations were 266 ± 14 nM in controls and increased to 515 ± 36 nM 30 min after antimycin A addition. Addition of glycine, strychnine, muscimol or thapsigargin prevented the antimycin A-induced increase in cytosolic free Ca^{2+} (273 ± 10 nM, 250 ± 5 nM, 277 ± 3 nM and 289 ± 28 nM, respectively). LDH release, a marker of cell death, was 8% in controls and 21% in antimycin A exposed RPT after 30 min. Glycine, strychnine, muscimol and thapsigargin prevented antimycin A-induced LDH release (8%, 7%, 8% and 11%, respectively). These experiments provide evidence that glycine, strychnine, and muscimol are cytoprotective through their ability to prevent a rise in cytosolic free Ca^{2+} and subsequent cell death. The inhibition of the rise in cytosolic free Ca^{2+} and cytoprotection produced by prior depletion of endoplasmic reticulum Ca^{2+} stores with thapsigargin provides further evidence that endoplasmic reticulum Ca^{2+} release may mediate extracellular Ca^{2+} influx and cell death.

115 CYTOTOXICITY OF 4-AMINOPHENOL IN LLC-PK_1 CELLS.

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4-Aminophenol (PAP) is nephrotoxic *in vivo*, causing morphological damage to the proximal tubule. However, we were unable to reproduce cytotoxicity *in vitro* using renal tubule suspensions unless hepatic post-mitochondrial supernatant was included in the incubation medium. These results were at variance with previous reports that PAP was directly cytotoxic in suspensions of renal tubular cells. Therefore, the present experiments were designed to determine if PAP produced cytotoxicity in an established cell line. LLC-PK_1 cells were seeded in 24 well plates and incubated in DMEM F12 medium until log phase growth was attained. Cells were incubated with medium containing various concentrations of PAP for 4 hr at 37 °C under 5% CO_2. PAP-containing medium was removed and cells were incubated for an additional 24 h. Viability was determined using MTT, Alamar Blue, and lactate dehydrogenase release. Below 0.1 mM PAP, cell viability was unchanged. At 0.1 mM, viability in PAP-treated cells was 50% of viability in untreated control cells. At 0.2 mM PAP, viability was less than 15%. Thus, PAP induces cytotoxicity in LLC-PK_1 cells. One mechanism by which PAP may induce cytotoxicity involves autoxidation (nonenzymatic oxidation). To determine if autoxidation of PAP contributed to cytotoxicity, we incubated cells with 0.1 mM PAP in the presence or absence of various antioxidants, including glutathione (GSH), ascorbate (Vit C), butylated hydroxytoluene (BHT), α-tocopherol (Vit E), NADPH, and NADH. GSH, Vit C and NADPH significantly attenuated the cytotoxicity of PAP. BHT, Vit E and NADH were ineffective. To determine if intracellular antioxidants exerted cytoprotection, cells were preincubated with GSH, Vit C, or NADPH for 1 h. Cells were washed, incubated with 0.1 mM PAP for 4 h, then incubated in DMEM F12 medium for 24 h as previously described. GSH and Vit C significantly prevented PAP-induced cytotoxicity, whereas NADPH was ineffective. These data suggest that PAP cytotoxicity involves nonenzymatic oxidation in both the medium and intracellular compartments. (Supported by NIH GM51361.)

116 EXAGGERATED PHARMACOLOGY OF THE ORALLY ACTIVE IRON CHELATOR ICL 670 IN THE KIDNEY.

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ICL 670 is an orally active iron chelator entering Phase I clinical trials. Its potent and specific ability to mobilize tissue iron and promote its excretion has been demonstrated in several animal models. In subchronic studies in rats and marmosets treatment with ICL 670 at high dosages was associated with changes in the renal tubular epithelium. In the present study the mechanism of this effect, which most likely is caused by iron deprivation, was investigated. LLC-PK1 and RPTEC human proximal tubular epithelial cells were incubated with ICL 670 for 48 h in the presence or absence of equimolar amounts of Fe^{3+} (iron citrate). ICL 670 induced a concentration-dependent increase of lactate dehydrogenase (LDH)-leakage, which was not detected when an equimolar amount of Fe^{3+} was added. Treatment with the stable iron complex CGP 82813 (ICL 670 : Fe^{3+} = 2 : 1) did not increase LDH-leakage significantly. Rat kidney slices were treated with 1 mM ICL 670 for 4.5 h in the presence or absence of 0.1, 0.5 and 1 mM Fe^{3+}. ICL 670 increased LDH-leakage and decreased the ATP-dependent para-aminohippuric acid (PAH)-uptake. In parallel ATP-content and energycharge were also reduced significantly. All effects were prevented in the presence of an equimolar amount of Fe^{3+}. In the present in vitro systems ICL 670 induced renal effects only in the absence of Fe^{3+}. These results support the hypothesis that the compound exerts the changes in the renal tubular epithelium by sequestering the vitally important iron which will be less likely to occur in iron overloaded patients.

117 INVESTIGATION OF VANCOMYCIN (VAN)–INDUCED RENAL REGENERATION: ROLE OF GROWTH FACTORS.

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Vancomycin (VAN) is a nephrotoxic glycopeptide antibiotic used in the treatment of gram-positive infections. Previous studies in our laboratory have suggested that a regenerative response occurs following VAN exposure. Epidermal growth factor (EGF) has been demonstrated to enhance regenerative hyperplasia, and studies on tubular injury and regeneration have shown an elevation in soluble EGF. The purpose of this study was to demonstrate the induction of a VAN-induced regenerative response in vitro and to investigate the role that growth factors might play. To examine the role of growth factor release in nephrogenic repair, conditioned media postulated to contain elevated levels of released growth factors was utilized. $LLCPK1_1$ cells were exposed for 6 hours to medium containing 5mM VAN. The VAN medium was then removed and replaced with fresh medium which was harvested and stored for use as conditioned medium. Serum-deprived quiescent $LLCPK1_1$ cells were then treated with the conditioned medium for 24 hours. Cell number, total protein, and ^{3}H-thymidine incorporation were analyzed to investigate a possible regenerative or hyperplastic response. SDS gel electrophoresis was also performed for the detection of protein expression levels of the 6kd entity of epidermal growth factor (EGF) after conditioned media exposure. A 15% increase in cell number and 10% in total protein were both observed when comparing VAN and control conditioned media. A 19% statistically significant increase in ^{3}H-thymidine uptake was also observed. Exposure to VAN-conditioned media was suggestive of increased expression of the 6kd protein when comparing samples from control conditioned medium to VAN treated conditioned medium. This data demonstrates that the induction of a regenerative/hyperplastic response is occurring and suggests the involvement of EGF in this process. (Supported by NIEHS Training Grant T32–ES–07290[DWK] andGM47549[MAS].)

118 INHIBITION OF CELLULAR RESPIRATION IN NONPERMEABILIZED LLC-PK1 CELLS: A POSSIBLE MECHANISM FOR PENTAMIDINE NEPHROTOXICITY.

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Pentamidine is a nephrotoxic antiprotozoal drug used in the treatment of Trypanosomiasis and AIDS-associated Pneumocystis Carinii Pneumonia.

Previously, we have demonstrated a nephrotoxic effect induced by pentamidine in vivo and in vitro in renal cortical slices and cell culture. The mechanism of antiprotozoal action is unknown, but has been proposed to involve changes in oxidative phosphorylation in some organisms. Inhibition of mitochondrial ATP production could be related to pentamidine–induced toxicity. The purpose of this study was to examine the effects of pentamidine on basal cellular respiration, as well as nystatin-induced and uncoupled respiration in LLC-PK1 cells. LLC-PK1 cells were grown to confluence in T75 flasks. Oxygen consumption was measured polarographically in a magnetically stirred chamber at 37° C. A pentamidine dose–response curve was constructed by adding the drug at 0.05, 0.1, 0.2, and 0.5mM to the cell suspension in the oxygen consumption chamber and recording oxygen consumption immediately. Pentamidine significantly reduced basal respiration at a concentration of 0.5mM. Cells were incubated for 5 minutes with pentamidine 0.5mM before oxygen consumption was recorded, to determine if the effect was time dependent. To examine the effect on maximal (nystatin-induced) and uncoupled respiration (2,4 Dinitrophenol-induced), pentamidine was added before and after each of the respiratory stimulants. Nystatin-induced respiration initially increased as expected, but pentamidine inhibited oxygen consumption by almost 33% compared to control. This suggests that pentamidine may inhibit ATP production rather than utilization. Pentamidine inhibited uncoupled respiration within 5 minutes compared to control, which suggests an effect occurs within the respiratory chain or prior to it. (Supported by NIEHS Training Grant T32–ES–07290 [RLB].)

119 MODULATION OF THE PERIPHERAL BENZODIAZEPINE (BZD) RECEPTOR AS A POSSIBLE MECHANISM FOR LINDANE (LIN) TOXICITY IN MADIN DARBY CANINE KIDNEY (MDCK) CELLS.

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Previous research in our laboratory has shown that LIN can produce distal tubular toxicity in renal cortical slices, and this toxicity may be attenuated by the addition of BZD/GABA receptor agonists and the peripheral BZD antagonist PK11195. We have also demonstrated LIN induction of apoptosis in MDCK and not in LLC–PK1 cells, which are models for distal and proximal tubular cells. In addition, LIN induce increased apoptosis in cells subjected to volume challenge. The purpose of these experiments is to further investigate the mechanism by which LIN induces toxicity in volume challenged and non–volume challenged MDCK cells. We are also interested in the role the peripheral BZD receptor plays in LIN's mechanism of toxicity. MDCK cells were pretreated with LIN ($1x10^{-5}$ M) in DMSO or DMSO alone (vehicle control) for 1hr. The media was removed and 500 mOsm media was added. The cells were harvested at 4, 8, 12, 24, and 36 hrs. after volume challenge. Western blot analysis for stress protein HSP70 showed no induction of the stress protein in either control, vehicle control, or treated cells at 4 and 8 hrs. Control and vehicle control showed modest induction of the stress protein at 12 hrs and increased induction at both 24 and 36 hrs. Modest induction of HSP70 was seen in LIN treated cells at 24 hrs followed by increased induction at 36 hrs. No effect was seen in LLC–PK1 cells. In order to investigate the involvement of the peripheral BZD receptor MDCK cells were treated with LIN ($1x10^{-5}$ M), Flunitrazepam (FLU, $1x10^{-5}$ M), a BZD receptor agonist, LIN+ FLU, or DMSO alone for 1 hr and then subjected to volume challenge (500 mOsm). DNA ladders showed an apparent amelioration of apoptosis in the LIN + FLU treated cells. These results suggest an involvement of the peripheral BZD receptor in LIN induced apoptosis in MDCK cells.

120 DIFFERENTIAL EXPRESSION OF HSP 70A, B AND C GENES IN HUMAN PROXIMAL TUBULE CELLS IN RESPONSE TO STRESS.

S Somji, J H Todd, S H Garrett and D A Sens. *Department of Pathology, West Virginia University, Morgantown, WV, USA.*

Heat shock protein (hsp) genes are induced by a variety of agents such as heat shock, heavy metals and chemical agents. This study investigates the expression of hsp 70 genes in cultured human proximal tubule cells exposed to elevated temperature, sodium arsenite and cadmium chloride. Reverse transcriptase - polymerase chain reaction was used to determine the expression of inducible hsp 70 genes (hsp 70A, B and C) and the cognate hsc 70 gene. In unstressed cells, there was no expression of hsp70B mRNA, whereas hsp 70A and C were expressed at low levels. Upon exposure to elevated temperature of 42.5° C for 1 hour or sodium arsenite for 4 hours followed by a recovery period, the expression of hsp 70 mRNA and protein increased. There was transient expression of hsp 70B mRNA while 70A and C mRNA remained elevated during most of the recovery period. There was a slight increase in hsc 70 mRNA without any induction of protein. Acute exposure to cadmium for 4 hours followed by a recovery period resulted in increased expression of hsp 70A, B and C mRNA and hsp 70 protein. Chronic exposure to Cd^{+2} over a 16 day time course resulted in an increase in mRNA and protein during the first 24 hours only. Exposure to Cd^{+2} did not increase hsc 70 mRNA or protein levels. This study demonstrates variation in the expression pattern of hsp 70 genes under normal and stress conditions.

121 HEAT SHOCK PROTEIN 27 IS INCREASED IN HUMAN PROXIMAL TUBULES CELLS BY ACUTE, BUT NOT CHRONIC, LETHAL EXPOSURE TO $CdCl_2$.

J H Todd, S Somji, S H Garrett and D A Sens. *Department of Pathology, West Virginia University, Morgantown, WV, USA.*

Cadmium is a potent poison responsible for acute and chronic toxicity in man. This heavy metal causes nephrotoxicity due to a slow accumulation of Cd^{+2} in the proximal tubules of the kidney. Heavy metals can induce a response know as the heat shock response which results in the expression of heat shock proteins (hsp). These proteins are believed to protect cells during periods of stress. In this study, the expression of hsp 27 mRNSA and protein was determined in cultured human proximal tubule cells following exposure to lethal concentrations of Cd^{+2} under both acute and chronic conditions. Acute exposure to Cd^{+2} involved a 4 hour exposure followed by a 48 hour recovery period in Cd^{+2}-free growth media. Chronic exposure to Cd^{+2} was modeled by continuous treatment with Cd^{+2} at lethal and sub-lethal concentrations over a 16 day time course. Acute exposure to 53 μ M $CdCl_2$ resulted in an increase in hsp 27 mRNA and protein and a shift to the more phosphorylated protein isoform. This increase in hsp 27 mRNA and protein occurred in the presence of Cd^{+2}. Chronic exposure to Cd^{+2} failed to increase either hsp 27 mRNA or protein expression in human proximal tubule cells, even at lethal Cd^{+2} concentrations. Overall, these results demonstrate that human proximal tubules have different patterns of hsp 27 expression when exposed to Cd^{+2} under acute versus chronic conditions.

122 THE IN VITRO CYTOTOXICITY OF HYDROQUINONE AND SELECTED HYDROQUINONE METABOLITES TO ISOLATED RAT RENAL PROXIMAL TUBULAR CELLS.

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The cytotoxicity of hydroquinone (HQ) and several metabolites was studied using rat proximal tubular (PT) kidney cells. Incubations were conducted for up to 4 h at 37° C with cytotoxicity measured either as increased leakage of lactate dehydrogenase (LDH) or as a decreased energy status, as determined by adenine nucleotide (ATP and ADP) concentrations. Incubation atmospheres consisted of either 95% O_2/5% CO_2, to promote cell viability *in vitro*, or 5% O_2/5% CO_2/90% N_2, to more closely simulate physiological (*in vivo*) conditions. Incubations were performed with either the addition or exclusion of 2% bovine serum albumin (BSA). In the presence of BSA, PT cells were unaffected by high (1 mM) concentrations of HQ, 2-(glutathion-S-yl)hydroquinone, 2,3,5-(triglutathion-S-yl)hydroquinone, or 2-(N-acetylcystein-S-yl)hydroquinone. 2-(Cystein-S-yl)hydroquinone (Cys-HQ) at 1 mM displayed some minimal toxicity affecting primarily ATP levels late (4 h) in the incubation period. Similar results were obtained regardless of the oxygen atmosphere used. In the absence of BSA, Cys-HQ and HQ had pronounced effects on both cell viability and ATP. Under 95% O_2, PT cells from male F344 rats were significantly more sensitive to HQ than those from male SD rats with decreases in ATP concentrations seen as early as 1 h at a concentration of 0.5 mM. Increased LDH leakage occurred later in the incubation and was less sensitive as an indicator of toxicity. Under 5% O_2, toxicity was observed later (4 h) in the incubation period and was more similar in magnitude for the two strains. These results show that variations in the *in vitro* incubation conditions of atmosphere and media can dramatically influence the toxicity of hydroquinone and its metabolites. Current study results are discussed in relation to the known toxicity of HQ and its glutathione-derived metabolites. (Supported by the Chemical Manufacturers Association Hydroquinone Panel.)

123 NEPHROTOXICITY ASSESSMENT OF SELECTED POLYCLIC AROMATIC HYDROCARBONS.

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Polycyclic aromatic hydrocarbons (PAHs) are widespread contaminants present in multiple forms of environmental pollution. Although hydrocarbon-induced nephropathies have been recognized for some time, the nephrotoxic potential of individual members of this class of chemicals remains undefined. The present study was conducted to define the kidney cell-specific responses to anthracene (ANTH) and chrysene (CHRY) relative to those elicited by benzo(a)pyrene (BaP). Measurements of mitochondrial membrane permeability, protein synthesis, cytoplasmic enzyme leakage, and trypan blue exclusion were conducted in established cultures of rat glomerular mesangial cells (rGMCs) and porcine tubular epithelial kidney cells (LLCPK-1) challenged with hydrocarbons at concentrations ranging from 0.03 - 30 mM for 24 hr. Dose-related injury to mitochondrial membranes and reduced rates of protein synthesis were observed in rGMCs treated with BaP. Modest rates of cell death were measured in rGMC cultures challenged with BaP at the highest concentration. BaP was not overtly toxic to LLCPK-1 cells at any of the concentrations examined, but increased mitochondrial permeability in a concentration-dependent manner. ANTH challenge with concentrations > 3 mM induced mitochondrial membrane injury and reduced rates of protein synthesis in rGMCs, while LLCPK-1 cells remained unaffected. In contrast to BaP, CHRY was devoid of toxicity in rGMCs, but increased mitochondrial injury and inhibited protein synthesis in LLCPK-1 cells challenged with concentrations ≥ 3 mM. On the basis of these data we conclude that ANTH and CHRY exhibit nephrotoxic potentials different from BaP, with rGMCs being preferentially injured by BaP and ANTH and LLCPK-1 cells by CHRY. (Supported in part by ATSDR Grant TU68450502 and NIEHS Center Grant 09106.)

124 EFFECTS OF TAURINE ANALOGS ON QUINONE FORMATION AND CYTOTOXICITY

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Taurine is an abundant amino acid present in mammalian tissues. Recent evidence has supported a role for taurine as an antioxidant and cytoprotective agent. Endogenous catecholamines such as dopamine can spontaneously oxidize or be metabolized to cytotoxic quinones. Studies were conducted to determine how various structural analogs of taurine affect the metal-stimulated oxidation of 3,4-dihydroxyphenylalanine (DOPA) in the presence and absence of DNA. Studies were also conducted in renal epithelial cells (LLC-PK_1) to test the ability of taurine analogs to prevent or cause cellular injury in the presence or absence of DOPA and $FeCl_3$. DOPA (100 μ M), $FeCl_3$ (60 μ M) and H_2O_2 (2.8 mM) were incubated for 15 minutes in the presence or absence of calf thymus DNA with taurine or taurine analogs (homotaurine or hypotaurine) present. Quinone formation was monitored spectrophotometrically at 480 nM. Taurine or taurine analogs (homotaurine, aminomethane sulfonic acid, hypotaurine, or isethionic acid) were incubated with LLC-PK_1 cells for 60 minutes in the presence of DOPA (250 μ M) and $FeCl_3$ (75 μ M) and cell death was monitored 24 hours later using a tetrazolium-based assay. Quinone formation in the presence of DNA was enhanced about 3 fold above the levels seen when DNA was not present. Taurine, homotaurine and hypotaurine significantly ($p<0.05$) inhibited quinone formation in the presence of DNA. Homotaurine alone was not cytotoxic to LLC-PK_1 cells, but in the presence of DOPA/$FeCl_3$ it significantly potentiated cytotoxicity. Aminomethanesulfonic acid alone (10 mM) was extremely cytotoxic to LLC-PK_1 cells. Taurine, hypotaurine, and isethionic acid reduced the cytotoxic response to DOPA/$FeCl_3$ in LLC-PK_1 cells. These studies suggest that taurine and certain taurine analogs can prevent cell death and DNA damage associated with quinones generated from catecholamine oxidation. (Supported by a grant from the American Heart Association-Florida Affiliate.)

125 TRANSPORT OF CHLOROTRIFLUOROETHYLGLUTATHIONE (CTFG) BY PRIMARY CULTURES OF RABBIT PROXIMAL TUBULE CELLS (RPTC) AND SUSPENSIONS OF RABBIT PROXIMAL TUBULES.

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Organic anion transport has been proposed as the excretory pathway for many toxic xenobiotics. We have demonstrated that CTFG inhibits fluorescein secretion and accumulation in primary cultures of RPTC, but its direct transport has yet to be measured. Thus, the goal was to characterize the direct transport of CTFG by proximal tubule cells. The uptake of [^{35}S]-CTFG into tubule suspensions was reduced ~50% by 2 mM CTFG, 5 mM PAH, 1 mM α-ketoglutarate and 25 μM ochratoxin A. In cells pre-incubated for 15 min with 250 μM acivicin to inhibit γ-glutamyl transpeptidase, 5 mM PAH reduced the basal-to-apical flux and basolateral uptake of [^{35}S]-CTFG ~ 65% and 60%, respectively, after 2 hr. The organic cation TEA at a concentration of 10 mM also had no effect on the basolateral uptake of [^{35}S]-CTFG by primary cultures. In contrast, 5 mM PAH had no effect on the apical-to-basal flux and apical uptake of [^{35}S]-CTFG, but was reduced ~ 70% by 2 mM CTFG, which is consistent with carrier-mediated [^{35}S]-CTFG uptake in the apical membrane. Thus, although the reabsorptive flux and accumulation of CTFG appear to involve a pathway other than organic anion transport, basolateral [^{35}S]-CTFG flux and accumulation appears to involve the organic anion pathway. (Supported by NIEHS 08860 and AHA Faculty development award.)

126 EFFECTS OF ANTIVIRAL DRUGS CIDOFOVIR AND FOSCARNET ON THE SODIUM-GLUCOSE COTRANSPORTER IN CULTURED RABBIT KIDNEY PROXIMAL CONVOLUTED (PCT) AND STRAIGHT (PST) TUBULAR CELLS.

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Rabbit kidney proximal convoluted (PCT) and proximal straight tubular (PST) cells were independently isolated and cultured on plastic support. The kinetics of the Na^+ / glucose transporter (SGLT) present in these epithelial cells were characterized by determining the uptake of the glucose analog alpha-methylglucoside (AMG). Results indicated the presence of two distinct transporters; a high capacity-low affinity (Vmax ≅ 3 μ M, Km ≅ 6 mM) transporter in PCTs and a low capacity-higher affinity (Vmax ≅ 0.5 μ M, Km ≅ 2 mM) transporter in PSTs. Both transporters discriminated between fructose (a pentose) and glucose and galactose (hexoses). Further, galactose transport was similar in PSTs and in PCTs. Phlorizin inhibited the uptake of AMG in PCTs to a much greater extend than in PSTs. Effects of antiviral drugs cidofovir (30 μ M to 3 mM) and foscarnet (500 μ M to 3 mM) on the SGLT were evaluated after exposures up to 96 hours under the same culture conditions. Results from these experiments indicate that both antiviral drugs reduce AMG uptake in PCTs and PSTs. However, PCT transporters appear more sensitive to exposure than PSTs transporters. This is the first report of a kinetic characterization of the sodium-glucose cotransporter in cultured PST cells compared to PCT cells. Results suggest that intrinsic cellular attributes between proximal tubular cell types, independent of blood flow or passive concentrating mechanisms, are important in determining vulnerability to drug effects.

127 ENDOTOXIN POTENTIATES MERCURY-INDUCED NEPHROTOXICITY.

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Both endotoxin (LPS) and mercury are nephrotoxic compounds of food safety concern. The effects of either toxicant alone on the kidney have been well investigated but their toxic interaction has not. Endotoxin is a product of gram negative bacterial cell walls. Humans are constantly exposed to LPS through contaminated food and air. Mercury is a food contaminant and the highest risk of human exposure is by consumption of contaminated fish. Endotoxin has recently been shown to potentiate the toxicity of a number of xenobiotics *in vivo*. In this study we tested the hypothesis that endotoxin enhances mercuric chloride-induced nephrotoxicity. Forty 6 weeks old C3H/HeN male mice were randomly allocated to 4 groups of 10 mice each

as follows: Group I received 0.9% normal saline IV (Control), Group II received 2.0 mg of E.Coli 0128:B12 LPS/kg once IV, Group III received 1.75 mg mercury/kg once IV, and Group IV received E.Coli 0128:B12 LPS/kg once IV 4 hours prior to receiving 1.75 mg mercury/kg once IV. Mice were monitored for 48 hours. Twelve hours prior to termination, they were placed in metabolic cages individually for urine collection. Monitored end-points included body and renal weights, renal histology and ultrastructural pathology, blood urea nitrogen and creatinine, serum and urine IL-1β, IL-6, and TNF-α, and renal mercury concentrations. Results showed that serum creatinine, urea, IL-1β, IL-6, proteinuria, and urine IL-6 in Group IV mice were all significantly greater than the rest of the groups ($p \leq 0.05$). Histologically, kidneys from mice given endotoxin alone were normal but the most severe lesions were found in mice given both endotoxin and mercury. Endotoxin significantly increased renal mercury concentration ($p \leq 0.05$). In conclusion, LPS potentiates mercury induced nephrotoxicity in mice.

128 EFFECT OF INDUCIBLE NITRIC OXIDE SYNTHASE INHIBITOR L-IMINOETHYL-LYSINE ON RENAL TOXICITY AFTER LIPOPOLYSACCHARIDE.

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The effects of lipopolysaccharide (LPS) on the kidney during sepsis are poorly understood. The role of nitric oxide (NO) generated from inducible nitric oxide synthase (iNOS) in LPS-induced renal injury was evaluated using the iNOS inhibitor L-iminoethyl-lysine (L-NIL). Male rats (250 g) were divided into three groups. One group received LPS (S. minnesota) 2 mg/kg iv. A second group received LPS plus an ip injection of L-NIL (3 mg/kg) at the time of LPS administration followed by a second dose 3 hr later. A third group received saline iv. At 6 hr blood, liver and kidneys were collected. iNOS was induced in both liver and kidney. Serum nitrate/nitrite levels (n=4) were increased from 4 ± 2 nmole/ml in the saline group to 431 ± 23 nmol/ml in the LPS group. Values for the LPS + L-NIL group was significantly reduced to 42 ± 9 nmol/ml. Tissue maldondialdehyde levels, a marker of oxidant stress, were increased in the kidney from 0.16±0.01 nmol/mg (n=4) in the saline group to 0.30±0.03 (n=7) in the LPS group. L-NIL significantly reduced values to 0.22±0.02 nmol/mg (n=7). Similar results were observed in the liver. LPS increased blood urea nitrogen levels from 13±2 mg/dl to 43±5 mg/dl (n=8). L-NIL reduced these levels to 36±4 mg/dl (n=8) but this reduction was not significant. Hematocrit was unchanged in all groups. These data indicate that LPS-induced NO generation can result in oxidant stress in the kidney and inhibiting iNOS may have therapeutic benefit.

129 TOXICITY OF A SEVOFLURANE DEGRADATION PRODUCT TO RAT LIVER AND RENAL CORTICAL SLICES.

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Compound A (2-fluoromethoxy-1,1,3,3,3-pentafluoro-1-propene) produced by reaction of the inhalation anesthetic, sevoflurane, with CO_2 absorbents. Past *in vivo* and *in vitro* studies have demonstrated that Compound A is nephrotoxic, possibly via bioactivation through the β-lyase pathway. Although glutathione (GSH) conjugates of Compound A have been reported, it is not clear if they are formed enzymatically or via direct reaction with GSH. To determine if these metabolites are produced and toxic, a tissue slice system that first exposes male Fischer 344 rat liver slices to volatilized Compound A, followed by exposure of rat kidney slices to the liver incubate was employed. Liver slices exposed to volatilized Compound A (6-12 uM medium conc ; ~50 ppm air space) exhibited a loss of K+ by 6 hr, which was not seen in kidney slices exposed to Compound A. Aminobenzotriazole, a P-450 suicide inhibitor, initially inhibits the cytotoxicity of Compound A to liver slices at these times and concentrations. As yet, the sequential liver/kidney slice experiments using Compound A have not demonstrated nephrotoxic results. However, both of the saturated synthetic Compound A-GSH conjugates have proven to be nephrotoxic at concentrations above 91 uM (24 hr), with higher concentrations showing toxicity at earlier times. Non-enzymatic reactions of Compound A with GSH or sulfhydryl-containing medium produce nephrotoxic products. These studies show that Compound A is directly toxic to the liver, possibly via P-450 activation, and Compound A can react with sulfhydryls directly to produce a nephrotoxic product. (Supported by Baxter Pharmaceutical Products Inc.)

130 2-AMINO-5-CHLOROPHENOL (2A5CP) TOXICITY IN RAT RENAL CORTICAL SLICES: EFFECT OF ANTIOXIDANTS.

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2A5CP is nephrotoxic to the proximal tubules. This study assessed whether the presence of a sulfhydryl group or an antioxidant could reduce 2A5CP toxicity in renal cortical slices. Renal cortical slices were prepared from male Fischer 344 rats (200-245 g). Renal slices were rinsed 3 times for 3 min each in 5-ml renal Krebs buffer. The slices (50-100 mg tissue) were preincubated for 10-30 min with 0.25 or 1 mM dithiothreitol (DTT), 50-100 μ M N, N'-diphenyl-1, 4-phenylenediamine (DPPD) or 1 mM glutathione (GSH) at 37°C under oxygen in 3 ml. An aliquot of 2A5CP was added at a final concentration of 0.25, 0.5 or 1 mM. Loss of membrane integrity was monitored by measurement of lactate dehydrogenase (LDH) leakage. 2A5CP at a concentration of 0.5 and 1 mM induced LDH leakage and lipid peroxidation above control values. Addition of the antioxidant DPPD did not reduce 2A5CP mediated LDH leakage or lipid peroxide formation. A 30-min pretreatment with 1 mM DTT reduced 2A5CP toxicity. DTT pretreatment also reduced lipid peroxidation induced by 2A5CP when compared to tissue not pretreated with DTT. A 30-min pretreatment with 1 mM GSH also reduced the extent of LDH leakage induced by 2A5CP. Lipid peroxidation induced by 2A5CP was also reduced by GSH pretreatment. These results indicate that a sulfhydryl containing antioxidant was more effective in reducing 2A5CP toxicity. The protection associated with GSH and DTT may be related to reduction of lipid peroxidation. (Supported by NIH Grant ES08834.)

131 S-(1,2,2-TRICHLOROVINYL)-L-CYSTEINE SULFOXIDE (TCVCS), A POTENTIAL METABOLITE OF TETRA-CHLOROETHYLENE (TCE), IS A POTENT NEPHROTOXIN.

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Previously we have shown that rabbit liver microsomes and cDNA-expressed rabbit FMO3 catalyzed the sulfoxidation of *S*-(1,2,2-trichlorovinyl)-L-cysteine (TCVC), a known metabolite of TCE, to yield the reactive metabolite TCVCS. In the present study, incubations of male rat liver or kidney microsomes with TCVC (10 mM) and NADPH (2 mM) led to TCVCS detection, with liver microsomes exhibiting much higher activity than kidney microsomes. TCVCS formation in liver microsomes was not inhibited by the addition of superoxide dismutase, KCN, or deferoxamine, but was inhibited by the inclusion of methimazole or 1-benzylimidazole, suggesting the involvement of both FMOs and cytochrome P450s. Rats given TCVC or TCVCS (230 μmol/kg, ip) exhibited acute nephrotoxicity, as indicated by histopathological examinations at 2 and 24 h after treatment and kidney function tests at 24 h. However, TCVCS was a much more potent nephrotoxin than TCVC. No significant liver lesions were observed with either TCVC or TCVCS. The TCVCS treatment decreased the reduced non-protein thiol (NPT) concentrations in the kidneys at 2 h after treatment by nearly 50% compared to saline treated rats without affecting the liver NPT status. An equimolar dose of TCVC did not affect the NPT status of either kidney or liver. Pretreatment of rats with the β -lyase inhibitor, aminooxyacetic acid, enhanced TCVC toxicity. Collectively, the results suggest that both TCVC and TCVCS play roles in TCE-induced nephrotoxicity. (Supported by NIH grant DK 44295.)

132 PHENOBARBITAL POTENTIATES N-(3,5-DICHLORO-PHENYL)-2-HYDROXYSUCCINIMIDE (NDHS) AND N-(3,5-DICHLOROPHENYL)-2-HYDROXYSUCCINAMIC ACID (2-NDHSA) NEPHROTOXICITY IN MALE BUT NOT FEMALE FISCHER 344 RATS.

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The agricultural fungicide, N-(3,5-dichlorophenyl)succinimide (NDPS) induces nephrotoxicity as its primary toxicity. Two metabolites of NDPS [N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS) and N-(3,5-dichlorophenyl)-2-hydroxysuccinamic acid (2-NDHSA)] are four times more potent than NDPS as nephrotoxicants in both male and female Fischer 344 rats. Recent work has shown that pretreatment with phenobarbital , an inducer of CYP2B1, CYP2B2, and UDP-glucuronosyltransferase, potentiates NDHS nephrotoxicity in male Fischer 344 rats via a non-oxidative bio-

transformation pathway. The purpose of this study was to determine if phenobarbital pretreatment also potentiated 2-NDHSA nephrotoxicity in male rats and NDHS and/or 2-NDHSA nephrotoxicity in female rats. Fischer 344 rats (4/group) were pretreated intraperitoneally (ip) with phenobarbital (75 mg/kg/d, 3 d). At 24 hr following the last phenobarbital injection, male rats were administered 2-NDHSA (0.05 mmol/kg, ip) or vehicle, while female rats were treated with NDHS (0.025 mmol/kg, ip), 2-NDHSA (0.025 mmol/kg, ip) or vehicle. Renal function was then monitored for 48 hr. Phenobarbital markedly potentiated 2-NDHSA nephrotoxicity in male rats but did not potentiate NDHS or 2-NDHSA nephrotoxicity in female rats. These results suggest that different metabolites may mediate NDPS metabolite nephrotoxicity in male and female rats. (Supported by NIH grant DK31210.)

133 SODIUM SULFATE POTENTIATES N-(3,5-DICHLORO-PHENYL)-2-HYDROXYSUCCINIMIDE (NDHS) AND N-(3,5-DICHLOROPHENYL)-2-HYDROXYSUCCINAMIC ACID (2-NDHSA) NEPHROTOXICITY IN FISCHER 344 RATS.

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N-(3,5-Dichlorophenyl)succinimide (NDPS) is an agricultural fungicide which induces nephrotoxicity as its major toxicity. Previous studies have shown that NDPS induces nephrotoxicity following oxidation of the succinimide ring to form N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS) and the hydrolysis product of NDHS, N-(3,5-dichlorophenyl)-2-hydroxysuccinamic acid (2-NDHSA). Both NDHS and 2-NDHSA induce nephrotoxicity in male rats at intraperitoneal (ip) doses of 0.1 mmol/kg or greater. Recent work found that sodium sulfate (10 mg/kg, ip) pretreatement potentiated NDPS nephrotoxicity. The purpose of this study was to determine if sodium sulfate also potentiated the nephrotoxicity of the two nephrotoxicant metabolites of NDPS. Male Fischer 344 rats (4-16 rats/group) received an ip injection of sodium sulfate (10 mg/kg) 20 min before a non-nephrotoxicant dose (0.05 mmol/kg, ip) of NDHS, 2-NDHSA or metabolite vehicle (12.5% dimethylsulfoxide in sesame oil). Renal function was then monitored over 48 hr. Sodium sulfate pretreatment potentiated the non-nephrotoxicant dose of NDHS and 2-NDHSA to a nephrotoxicant dose. Nephrotoxicity was characterized by diuresis, increased proteinuria, elevated blood urea nitrogen (BUN) concentration, increased kidney weight and proximal tubular necrosis. These results support the likelihood that sulfate conjugates of NDPS metabolites contribute to NDPS nephrotoxicity. (Supported by NIH grant DK31210.)

134 EVIDENCE FOR THE FORMATION OF PHASE II METABOLITES OF *N*-(3,5-DICHLOROPHENYL) SUCCINIMIDE (NDPS) IN RATS.

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The agricultural fungicide NDPS is nephrotoxic in rats. Previous studies have shown that NDPS undergoes extrarenal biotransformation prior to induction of kidney damage. Although Phase II metabolites of NDPS have been implicated in nephrotoxicity, no direct evidence for their formation has been found yet. The studies described here were therefore conducted to determine if NDPS undergoes Phase II metabolism. Male Fischer 344 rats received NDPS or its nephrotoxic Phase I metabolite, *N*-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS). Urine, plasma, and tissue samples (liver and kidneys) were collected. NDPS and NDHS were also incubated with rat liver S9 fractions, microsomes and cytosol. Samples were analyzed using HPLC coupled with an electrospray tandem mass spectrometer (LC-ESI-MS/MS). Metabolites were identified in negative ionization mode using a combination of Q1, neutral loss, and collision induced dissociation of the pseudomolecular ion $(M-H)^-$ scans. In addition to the known metabolites of NDPS, isomers of several mercapturic acid derivatives (m/z 421) were found in urine. Glucuronide (m/z 452) and sulfate conjugates (m/z 356) of *N*-(3,5-dichlorophenyl)-2- and -3-hydroxysuccinamic acids (2-NDHSA and 3-NDHSA, respectively), were detected in all the *in vivo* samples from rats dosed with NDPS or NDHS. The same conjugates were formed *in vitro* from NDHS, but not from NDPS. In conclusion, we have demonstrated for the first time the formation of Phase II metabolites of NDPS in rats. However, prior Phase I metabolism is required. The role of the Phase II metabolites in NDPS nephrotoxicity requires further investigation.

135 8-,CHLORO-CYCLIC AMP, BUT NOT 2-,FLUORO-8-, CHLORO-CYCLIC AMP IS NEPHROTOXIC IN MICE.

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8-,Chloro-cyclic adenosine monophosphate (8Cl-cAMP) has been shown to inhibit tumor growth, possibly due to modulation of cAMP-dependent protein kinases. However, 8Cl-cAMP is nephrotoxic to animals and man. A fluorinated analog, 2,-fluoro-8,-chloro-cyclic AMP (2Fl-8Cl-cAMP), was synthesized by the National Cancer Institute in an attempt to modulate the toxicity of the parent compound by blocking metabolism at the 2-position. The toxicities of 2Fl-8Cl-cAMP and 8Cl-cAMP were compared in male CD_2F_1 mice following constant subcutaneous infusion using an osmotic pump at a dose rate of 10 mg/kg/hr for up to 7 days. Infusion of 8Cl-cAMP produced lethality by day 5, most likely due to nephrotoxicity (renal tubule necrosis and mineralization). Both serum creatinine and blood urea nitrogen levels were significantly increased 2- and 4-fold, respectively, in the 8Cl-cAMP group. Neither lethality nor nephrotoxicity were produced by 2Fl-8Cl-cAMP. Both 2Fl-8Cl-cAMP and 8Cl-cAMP produced lymphoid depletion in thymus and spleen, with incidence and severity greater in the latter group. Salivary gland and stomach lesions were produced by both compounds, and 8Cl-cAMP produced single cell necrosis in the intestines, and periportal degeneration in liver in a few animals. In conclusion, fluorine substitution at the 2-position of 8Cl-cAMP inhibits a metabolic pathway that results in nephrotoxicity and results in a molecule that is significantly less toxic than the parent. (Supported by NCI Contract No. N01-CM-37838.)

136 SPECIES DIFFERENCES IN *ORTHO*-DIPHENOL MEDIATED NEPHROTOXICITY.

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Para-diphenols such as bromohydroquinone and hydroquinone produce selective nephrotoxicity in rats via a mechanism that involves oxidation, conjugation with glutathione, and delivery of the conjugates to renal proximal tubules. Necrosis is evident in the S3 segment of the outer strip of the outer medulla (OSOM). In contrast, *ortho*-diphenols are nephrotoxic in mice, but not rats. We have evaluated the nephrotoxicity of catechol (CA) and its mono-methyl (Me)-, chloro (Cl)-, and bromo (Br)- substituted derivatives in male ICR mice and Sprague-Dawley rats. Only halogenated CAs induced substantial nephrotoxicity in mice, at doses between 0.56 to 1.0 mmol/kg, i.v. Blood urea nitrogen (BUN) levels were elevated in mice following treatment with 3-ClCA, (1.0 mmol/kg), 4-ClCA or 4-BrCA (0.56 and 0.75 mmol/kg). No significant increases in BUN were seen after treatment of mice with CA, 3- or 4-MeCA in mice at a dose as high as 1.0 mmol/kg. Morphological evaluation of renal tissue from 3-ClCA, 4-ClCA and 4-BrCA treated mice revealed renal tubular necrosis in the cortex, beginning at the lower doses, in the S1 and S2 segments, and extending to the OSOM region at higher doses. The severity of necrosis correlated with increases in BUN. At a dose of 0.75 mmol/kg, none of the *ortho*-diphenols were capable of elevating BUN levels in male Sprague-Dawley rats. In conclusion, rats are sensitive to *para*-diphenol- but not to *ortho*-diphenol-induced nephrotoxicity. In contrast, mice are sensitive to *ortho*-diphenol- induced nephrotoxicity but not to *para*-diphenol. Moreover, the addition of an electronegative substituent (halogen) to *ortho*-diphenols increases nephrotoxic potency, probably by making the corresponding *ortho*-quinone a stronger oxidant. (GM 39338)

137 FUNCTIONAL, MORPHOLOGICAL AND MOLECULAR ALTERATIONS INDUCED BY BENZO[A]PYRENE IN THE ADULT RAT KIDNEY.

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Epidemiological and occupational studies have suggested a link between aromatic hydrocarbon exposures and glomerulo-nephropathies. With this in mind, previous studies in this laboratory have shown that glomerular mesangial cells are preferential targets of benzo[a]pyrene (BaP) relative to the cortico-tubular epithelial cell population. Based on these data we hypothesized

that BaP induces glomerular injury in vivo and compromises renal function leading to the onset of kidney disease. To test this hypothesis, Sprague Dawley rats (175-225 g) were injected with BaP (10 mg/kg, i.p.) once a week for up to 16 weeks and renal function was monitored by measurements of 24 hr urinary albumin levels. Computer-assisted morphometric analysis was conducted to evaluate glomerular cellularity, urinary space, area of Bowman's capsule and glomerular tuft density. Expression of α-smooth muscle (SM) actin was used as an index of mesangial cell injury. Urinary albumin levels were elevated at 16 weeks of BaP treatment relative to 11 week counterparts. Glomerular cellularity increased in BaP-treated rats relative to vehicle controls. No changes in the urinary space, Bowman's capsule and glomerular tuft densities were observed in treated kidneys. Immunohistochemical analysis detected α-SM actin in the glomerulus of BaP-treated rats. These data suggest that the glomerulus is a target of BaP within the kidney and reinforces the need to conduct further research in this area. (Supported by NIEHS 04847, ES 09106 and CVM Enhancement Funds.)

138 SENSITIVITY OF MICE TO PHENACETIN TOXICITY IS STRAIN-SPECIFIC.

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As part of the ILSI effort to evaluate alternatives to the two year rodent carcinogenicity assay, we are testing phenacetin, a group 2A carcinogen, in the p53 hemizygous knockout model and Tg.AC transgenic model. Four-week dose range finding studies were conducted in C57BL/6 and FVB/N mice, the respective background strains of the alternative models. Phenacetin was admixed in the diet at concentrations of 0.3, 0.7, 1.4 and 2.0%. Although phenacetin-associated mortality was not observed in C57BL/6 mice at any dose tested, all FVB/N mice dosed with 1.4% or 2.0% phenacetin were either sacrificed or found dead within the first eight days of dosing. Subsequently, a 0.14% dose group was added to the FVB/N dose range finding study. This differential strain sensitivity can be explained, in part, by the higher systemic phenacetin exposure in FVB/N mice compared to that in C57BL/6 mice at similar doses. However, histopathologic findings indicate that differential susceptibility of C57BL/6 and FVB/N mice cannot be explained solely by differences in systemic exposure. Differences in target organ susceptibilities were found in the kidney and liver. For example, even at the highest concentration of phenacetin (2.0%), histopathologic findings in the kidneys of C57BL/6 mice were limited to minimal to mild pigment accumulation in the tubular epithelium. In contrast, renal papillary necrosis was noted in FVB/N mice following dietary administration of 0.7%, a dose that resulted in AUC values that were approximately 33-75% lower than AUC values determined in C57BL/6 mice administered 2.0% phenacetin. Biotransformation of phenacetin is complex. Metabolic pathways include de-ethylation, *N*-deacetylation and ring hydroxylation. Although the contribution of metabolites in each of these pathways to phenacetin toxicity remains to be fully explored, it is likely that strain-specific differences in phenacetin metabolism could account for strain-specific sensitivity to phenacetin toxicity.

139 PROTEIN DROPLET NEPHROPATHY AND DOSIMETRY OF *t*-BUTYL ALCOHOL IN MALE AND FEMALE F-344 RATS.

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t-Butyl alcohol (TBA) is used as a chemical intermediate with a wide range of applications including solvents for pharmaceutical products, paint removers, and personal care products. A major use of TBA is in the synthesis of methyl *t*-butyl ether (MTBE). In both rodents and humans, TBA is a major metabolite of MTBE. Chronic TBA exposure caused an increase in renal tumors in male, but not female, F-344 rats. The objective of the present study was to determine if TBA induces α2u-globulin (α2u) nephropathy and renal cell proliferation in male, but not female, rats. The dosimetry of TBA in male and female rats and the interaction of TBA with the male rat-specific protein α2u were also investigated. Male and female F-344 rats were exposed to TBA vapors at 0, 250, 450, or 1750 ppm for 6 hr/day for 10 consecutive days. Significant proximal tubule degeneration (450 and 1750 ppm), protein droplet accumulation (450 and 1750 ppm), α2u immunoreactivity (1750 ppm), and an increase in renal cell proliferation (250, 450, and 1750 ppm) were observed in TBA-exposed male, but not female, rats. The renal concentration of α2u increased in male rats exposed only to 1750 ppm TBA. TBA kidney-to-blood ratio was greater in male rats compared with female rats at all time points following TBA exposure. When α2u was purified from male rats treated with 500 mg/kg TBA, TBA coeluted with the protein fraction containing α2u. TBA causes a mild α2u-mediated nephropathy in male rats and it appears to interact with α2u which causes the protein as well as the chemical to accumulate in the male rat kidney. These results suggest that male rat-specific nephropathy from TBA exposure is mediated through its interaction with α2u. (Sponsored in part by the Oxygenated Fuels Association.)

140 RE-EVALUATION OF THE CHLOROFORM 2-YEAR DRINKING WATER BIOASSY IN OSBORNE-MENDEL RATS INDICATES THAT SUSTAINED RENAL TUBULE INJURY IS ASSOCIATED WITH RENAL TUMOR DEVELOPMENT.

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Chloroform is a non-genotoxic compound and a rodent carcinogen. Chloroform produced renal tubule tumors in Osborne-Mendel rats when administered by corn oil gavage or in the drinking water. There is a lack of data on intermediate endpoints that are linked to renal cancer development in this strain of rat. Specifically, the evidence linking chloroform induced renal and hepatic tumors in mice with cytotoxicity and regenerative cell proliferation is very strong, but weak for chloroform-induced rat renal tumors. In the present study, kidneys from the 2-year drinking water cancer bioassy in Osborne-Mendel rats were re-evaluated for microscopic evidence of chloroform-induced cytotoxicity and regeneration. HE stained sections of kidney from rats exposed to at water concentrations of 0, 200, 400, 900, and 1800 ppm for 6, 12, 18 or 24 months were examined. The toxicity associated alterations were characterized by slightly increased cytoplasmic basophilia, cytoplasmic vacuolation, karyomegaly, anisokaryosis, nuclear crowding, and mild tubule hyperplasia. Characteristic changes associated with toxicity were present inproximal tubule epithelial cells from rats treated with 1800 ppm chloroform at all time points and from rats treated with 900 ppm for 18 or 24 months. None of the other treatment groups or controls had these characteristic changes. These data are consistent with other studies indicating that chloroform-induced cancer is secondary to sustained cytotoxicity and regenerative hyperplasia. (This abstract does not reflect EPA policy.)

141 SEQUENTIAL ANALYSIS OF THE HEPATO-RENAL TOXICITY AND CADMIUM ACCUMULATION IN RATS GIVEN MINIMUM TO LARGE AMOUNTS OF CADMIUM CHLORIDE FOR 2 YEARS.

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Sponsor: T Shirai.

To clarify the threshold dose of cadmium (Cd) to induce hepato-renal toxicity, groups of female SD rats were fed diet containing cadmium chloride ($CdCl_2$) at 0, 8, 40, 200 or 600 ppm for 2, 4 or 8 months. All surviving rats given 600 ppm Cd were killed at 4 month because of deterioration of their general condition. Hepatotoxicity was observed after 2 months in the groups treated with 200 ppm or more. By 4 months, the rats in the 600 ppm group had developed periportal liver cell necrosis. Renal toxicity characterized by degeneration of proximal tubular epithelia was apparent in the rats given 200 ppm or more from the 2 month. Liver showed high capacity to accumulate Cd even at the highest dose. In contrast, the renal Cd in the 600 ppm group reached a plateau level within the first 2 months. The renal Cd-concentration in the 200 ppm group when renal toxicity was first detected at 2 months ranged from 104 to 244 μ g/g. Similar level of renal Cd (91-183 μ g/g) was found in the 40 ppm group after 8 months, whereas no renal lesions were detected. The results suggest that renal toxicity would not be induced by exposure with minimum amounts of $CdCl_2$ for periods longer than 8 months, although accumulation of Cd might gradually progress. A further 2-year feeding study of very low amount of Cd, up to 40 ppm in diet, is now in progress. At 1-year interim sacrified, blood chemistry, urinalyses and pathological analyses revealed no indication of Cd-related hepato-renal toxicity.

142 EFFECT OF SODIUM FLUORIDE & VERAPAMIL ON CULTURED HUMAN CELLS USING SYTOX & CALCEIN FLUORESCENT PROBES.

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Male Wistar rats (6 weeks old) were given Cd (as CdCl2) at a dose of 0, 2, 5, 10, 20, 30 and 60 mgCd/kg/day by gastric tube daily for 6 consecutive days a week for 60 weeks. Animals were sacrificed at 0, 1, 3, 5, 10, 30, and 60 week after oral Cd administration. Cd concentration in the kidney, urinary excretion of enzymes (NAG,AAP,GST,ALP), the urinary excretion of Pyridinoline (Pyr) and Deoxypyridinolin (Dpyr) were determined. Bone mineral density of the femur was determined by microdensitometry (MD) method, and the osteoid volume (%) was also determined by the Quantitative Image Analysis method. At low Cd administration groups of 2 and 5 mgCd/kg/day, the prominent renal dysfunction was not observed. On the other hand, in the high Cd dose groups of 20, 30 and 60 mgCd/kg/day, the renal proximal tubular dysfunction was observed, and the decreased bone mineral density was found at before and after the manifestation of renal dysfunction. Osteoid volume (%) in the femur was shown the lower level compared to that of control animal. The increased osteoid volume was not found in all animals except the case of 2 mgCd/kg group at the 5th week. The increased urinary excretion of Pyr, Dpyr and Ca was observed in the high Cd dose groups from the 3rd week after the Cd administration. It was suggested that the bone resorption is activated. These results suggest that the renal dysfunction, the decreased bone mineral density, and the decreased osteoid volume in the femur are resulted at different time corresponding to the Cd accumulation resulted by the different condition of Cd exposure.

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143 SUBACUTE INHALATION TOXICITY OF PERFLUOROETHYL VINYL ETHER (PEVE) IN RATS.

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The toxicity of PEVE was evaluated by exposing Crl:CD® (SD) BR male and female rats to 0, 500, 3000, or 18,000 ppm for 4-weeks. Body weights and food consumption were measured weekly. Clinical pathology and morphological evaluations were conducted at the end of the exposure period and after 2 weeks of recovery. Rats exposed to 18,000 ppm had significantly lower mean body weights, body weight gains, food consumption, and food efficiency during the exposure period. Rats had a diminished response to an auditory alerting stimulus during exposure to 18,000 ppm, which was reversible. Female rats exposed to 18,000 ppm had significantly increased absolute and relative kidney weights at the end of the exposure period. Microscopic changes were observed in the kidneys in the 18,000 ppm males and females at the end of the exposure period, and in the 18,000 ppm females at the end of the recovery period. The renal lesions occurred in the outer stripe of the outer medulla, and were characterized by tubular epithelial cell degeneration, necrosis, sloughing, and basophilia and megalocytosis of regenerative cells. Urine analytical changes which indicated diuresis were seen in rats in the 3000 and 18,000 ppm groups at the end of the exposure period and at the end of the 2-week recovery period. The changes in the 18,000 ppm groups were considered biologically adverse because they were accompanied by histopathologic lesions in the renal tubules. The no-observed-adverse-effect level (NOAEL) for repeated exposure to PEVE in male and female rats was 3000 ppm.

144 CHARACTERIZATION AND EFFECTS OF CRYSTAL DEPOSITS IN RENAL CORTEX AFTER ORAL ADMINISTRATION OF CI-1007 IN MONKEYS.

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CI-1007 (pyridine, (R) 1,2,3,6-tetrahydro-4-phenyl-1-[(3-phenyl-3-cyclohexen-1-yl)methyl]) was administered orally for 13 weeks at 10, 35 and 75 mg/kg to 4 cynomolgus monkeys/sex/group; 3/sex/group were euthanatized after 13 weeks and 1/sex/group after a 4 week non-dosing period (Week 17). Renal lesions were observed at 35 and 75 mg/kg (Weeks 13 and 17), and in one male at 75 mg/kg euthanatized moribund (Week 5). Birefringent, crystalline deposits in the renal cortex were observed in the interstitium, and tubular cells and lumina. Crystals were associated with mononuclear cell infiltrates and tubular epithelial regeneration was observed in moderately affected animals. Ultrastructurally, crystals were electron dense spicular composites ranging in diameter from 2 to 25 m m with individual spicule length of 0.5 to 2.0 μ m. They appeared plate- or flake-like by scanning electron microscopy. Energy dispersive X-ray analyses revealed high sulfur concentrations in the crystals. Concentrations of CI-1007, 3 hydroxylated metabolites, and 5 sulfated conjugates were determined in formalin-fixed renal cortices. At 10 mg/kg, all analytes were below limit of quantification, while at 35 and 75 mg/kg there was a disproportional increase in PD 163639 (sulfated conjugate of a dihydroxylated metabolite) concentrations. In animals with renal crystals, PD 163639 concentrations ranged from 0.32 to 10.4 mg/g compared to <0.0002 to 0.07 mg/g in unaffected animals. Plasma concentrations of PD 163639 increased with dose and were 21 to 66 times higher than those of CI-1007 at all doses. It is concluded that PD 163639 is responsible for the crystal formation with three-dimensional structure that elicit minor tissue response and no effects on kidney function. No crystals in the kidney and high plasma concentrations of PD 163639 at 10 mg/kg indicated elimination of conjugate.

145 EFFECT OF RENAL INJURY ON PHOSPHOROTHIOATE OLIGONUCLEOTIDE (P=S ODN) PLASMA AND TISSUE DISTRIBUTION AND EXCRETION IN RATS.

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The kidney is a major organ for the accumulation of P=S ODN, antisense therapeutic agents; therefore, injury to the kidney may alter the distribution and elimination of these compounds. In this experiment renal injury was induced by a single intravenous administration of 5 mg/kg cisplatin along with a concurrent group of saline treated animals to serve as controls. Three days following cisplatin or saline administration, all animals received P=S ODN by a single intravenous dose of 2 or 10 mg/kg. Blood samples were collected at 1, 2, 4, 6, 24, 48 and 72hr after P=S ODN dosing. Urine was collected at 0-6, 6-24, 24-48 and 48-72 hours. Renal injury was assessed by water consumption, urine output volume, pH, BUN values and histopathology. P=S ODN concentrations in urine, plasma and tissue were determined using capillary gel electrophoresis. There was a significant increase in kidney weights, urine output, and BUN levels in cisplatin treated animals. Histopathologic examination of the kidneys indicated moderate proximal tubule injury consistent with cisplatin nephrotoxicity. In tissue and plasma, distribution and concentrations of P=S ODN were similar between control and cisplatin-treated animals. An exception was the kidney, where concentration of P=S ODN was 30-70% lower in the cisplatin-treated animals than controls. There was approximately a two fold increase in urinary excretion of P=S ODN. However, urinary excretion of P=S ODN was only 0.1-0.4% of dose. This study indicates that injury to kidney proximal tubules does not effect distribution of P=S ODN to tissues other than the kidney or effect excretion significantly.

146 EXPRESSION OF METALLOTHIONEIN-3 IN THE HUMAN KIDNEY.

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The original description of metallothionein-3 (MT-3) indicated that this protein was limited to brain and neural tissues; however, recent reports show a much wider tissue distribution. This study determines the tissue distribution of MT-3 in the human kidney and its expression in cultures of human proximal tubule cells. An antibody was generated against the unique eight amino acid sequence that is not shared by any other MT family member and was used for immunohistochemical staining. Staining with this antibody showed: moderate staining in the parietal epithelial cells of Bowman's capsule and in visceral epithelial cells of the glomerular tuft, as well as the proximal convoluted tubule cells; strong cytoplasmic staining in the distal tubules, particularly in the medullary rays; weak to moderate staining in the medullary collecting ducts; and a general absence of staining in the thin loops of Henle and in the transitional epithelium of the renal pelvis. Expression was also deter-

mined in cultures of human proximal tubule cells exposed to various metals. It was shown that there was an increase in the production of MT-3 in response to metals (Cd and Zn); however, the response was only transient and MT-3 levels decreased after an initial induction, despite continued exposure to metals. The finding that MT-3 is constitutively expressed in several glomerular and tubule epithelial elements of the human kidney warrants consideration of an expanded role for this protein family in maintaining renal homeostasis.

147 NAPROXEN TOXICOSIS IN DOGS: A REVIEW OF FIFTY-SEVEN CASES.

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Naproxen, a propionic acid derivative, is a nonsteroidal anti-inflammatory drug (NSAID) available over the counter as acid and as the sodium salt. Structurally and pharmacologically, naproxen is similar to carprofen and ibuprofen. In humans and the dog, it has been used for its anti-inflammatory, analgesic, and antipyretic properties. The objective of this study was to summarize characteristic clinical signs, alteration of clinical chemistries, duration of illness, and success of treatment, following ingestion of excessive doses of naproxen in dogs. For this purpose, the National Animal Poison Control Center (NAPCC) database was searched for records of dogs ingesting naproxen or naproxen sodium from January 1992 to December 1994. Data were retrieved based upon clinical signs, laboratory test results, exposure history, response to treatment, and final outcome. Fifty-seven cases were found where dogs had ingested naproxen ranging between 10 mg/kg to more than 500 mg/kg. Out of 57 cases, thirty-four dogs (60.00%) developed clinical signs. The commonly observed clinical signs included anorexia, drooling, vomiting and diarrhea (with or without blood), polydipsia, hematuria, ataxia, shock, depression, renal failure, and death. Increase in blood serum nitrogen, serum creatinine, some liver specific enzymes, and decreased hematocrit were some commonly reported alterations in clinical chemistry profiles. Four dogs died or were euthanized. The majority of the dogs recovered with treatment which included, induction of emesis, administration of activated charcoal, fluids, gastrointestinal protectants, and other supportive measures.

148 EFFECTS OF α -HEDERIN ON TCDD-MEDIATED *Cyp1a1* EXPRESSION IN THE MOUSE HEPATOMA HEPA 1c1c7 CELLS.

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Cultured mouse hepatoma cell line Hepa-1c1c7 cells were treated with α -Hederin to assess the role of α -Hederin in the process of Cyp1a-1 induction. Treatment of Hepa-1c1c7 cultures with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induced Cyp1a-1, as indicated by analysis of 7-ethoxyresorufin *O*-deethylation (EROD) activity and Cyp1a-1 protein. When α -Hederin and TCDD were both added to cultures, TCDD-inducible EROD activity was greatly suppressed by α -Hederin in a dose-dependent manner. TCDD-induced Cyp1a-1 protein and mRNA levels were markedly reduced in the concomitant treatment of TCDD and α -Hederin consistent with EROD activity. Electrophoretic mobility shift assay using nuclear extract of cells revealed that α -Hederin reduced transformation of the Ah receptor to a form capable of specifically binding to a oligonucleotide containing a dioxin-response element (DRE) sequence of the *Cyp1a-1* gene. These results suggest that the suppressive effect of α -Hederin on TCDD-induced *Cyp1a-1* gene expression in Hepa-1c1c7 cells might be antagonism of the DNA binding potential of nuclear Ah receptor. (Supported by KOSEF Grant 98-0503-008-1.)

149 MODIFICATION OF FUMONISIN B1 RESPONSE IN MICE CARRYING HUMAN TUMOR NECROSIS FACTOR ALPHA TRANSGENE.

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Our previous studies indicated the possible involvement of tumor necrosis factor alpha (TNF) in fumonisin B1 (FB)-induced toxic responses (J. Pharmacol. Exp. Ther. 285:317-324, 1998). To further investigate the role of TNF we employed transgenic mice expressing human TNF gene (TG) and their wild-type equivalent C57BL/6 (WT). It was hypothesized that TG animals would have enhanced response to FB. Repeated subcutaneous treatment of animals with 2.25 mg/kg/day of FB for 5 days caused minimal changes in body weight, organ weights, blood cell counts, and TNF levels (measured by immunoassay) in serum, liver or kidney, one day after the last injection. The mRNA for TNF in liver increased in TG but not WT after FB treatment. Liver and kidney lesions were found in TG after FB treatment. More extensive liver lesions were seen in FB-treated WT. In both FB-treated groups the liver pathology was directly correlated with sphinganine increase but inversely with TNF mRNA levels. Alterations of regional brain neurotransmitters and their metabolites by FB in both TG and WT were similar to those observed earlier; the increase in dopamine metabolite, homovanillic acid, was widespread in all regions of FB-treated TG. While the data do not support the original hypothesis, they provide evidence that hepatotoxic response to FB was modified in TG mice.

150 ALGAL PRODUCTS AS NATURALLY OCCURRING MODULATORS FOR THE MULTIDRUG RESISTANCE (MDR) TRANSPORTER, P-GLYCOPROTEIN.

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Extracts of various marine algae modulate activity of the multidrug resistance (MDR) transporter in the filter-feeding mussel, Mytilus californianus, and also in an MDR1/P-glycoprotein (P-gp) expressing human tumor cell line, MES-SA/Dx5. M. californianus has high titer of the MDR-transporter possibly to provide protection from products contained in its diet of seaweed particulates and phytoplankton. To test this hypothesis, extracts of various seaweeds and phytoplankton were examined for the presence of substrates by competition assays in mussel gills with a fluorescent P-gp substrate, rhodamine B. Methanol extracts of the seaweeds Macrocystis pyrifera, Egregia menziesii and Phyllospadix scouleri exhibited modulating activity in mussel gills as shown by increased accumulation of rhodamine and slowing of dye efflux. These data suggest that the mussel diet contains compounds that may be P-gp substrates, thus providing evidence that mdr genes may have evolved in response to dietary pressures. Recently, we have tested some of these algal extracts on the MDR overexpressing MES-SA/Dx5 human uterine sarcoma cell line for possible MDR modulating activity in tumor cells. Preliminary results suggest that methanol extracts of the seaweed Macrocystis pyrifera contain compounds that modulate P-gp-mediated drug transport in these human tumor cells when tested in competition assays using a radiolabelled MDR substrate, vinblastine. These findings indicate that marine algae contain compounds that are substrates for the MDR transporter in both marine organisms and in human tumor cells.

151 TOXIC PROPERTIES OF EXTRACTS OF CYANO-BACTERIAL MATS COLLECTED FROM ANTARCTIC MELTWATER PONDS.

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Cyanobacteria occur worldwide in temperate as well as extreme environmental conditions and are capable of producing toxic secondary metabolites. Even in Antarctica benthic and floating cyanobacterial mats colonize ponds originating from meltwater during the Antarctic summer. In samples taken in 1997 the toxic properties of Antarctic cyanobacteria were demonstrated (Hitzfeld et al. Toxicol. Sci. 42 (1-S), 288, 1998). In order to further characterize the toxins involved and to identify the toxin-producing species, 56 samples were collected on Bratina Island on the McMurdo Ice Shelf, Antarctica in January 1998. Taxonomy of the fresh samples was determined using light microscopy. Samples were freeze-dried and organic contents determined by furnace incineration. For toxicity assessment 32 samples were extracted with 70% methanol, rotary-evaporated and finally taken up in a defined volume of water. The protein-phosphatase-inhibition assay was used to demonstrate toxicity. Microcystin-LR was employed as a standard. HPLC was used for further characterization of the extracts. Cytotoxic properties of the extracts were investigated on isolated rainbow trout hepatocytes using the MTT assay. The results show that Oscillatoriales (*Phormidium* sp.) and the genera *Nodularia* sp. and *Nostoc* sp. constitute the most common strains in the cyanobacterial mats. Samples collected in 1997 and in 1998 show comparable species composition. Protein-phosphatase-inhibiting activity is shown by all samples although to a different degree. Sixteen of the 32

extracts had more than 50% and twelve extracts more than 70% inhibiting activity. A correlation between toxin content (protein-phosphatase inhibition) and species composition was detected. *Phormidium* sp. and *Nodularia* sp. were present in nearly all of the toxic samples. In contrast, cytotoxic properties could not consistently be detected in all samples.

152 EFFECT OF OZONATION IN DRINKING WATER TREATMENT ON THE REMOVAL OF CYANOBACTERIAL TOXINS.

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The presence of cyanobacterial toxins, such as microcystin, in drinking water supplies poses a serious health risk. Human exposure to drinking water containing toxins may result in chronic liver injury and possibly in the promotion of liver tumors. It is thus important to monitor cyanobacterial densities and toxin levels in water reservoirs and, in the event of a bloom, to remove these toxins by adequate water treatment procedures. Conventional water treatment (chlorination, flocculation, sand filtration) is ineffective in reducing cyanobacterial toxin levels to below acutely toxic concentrations; only activated charcoal can effectively remove the toxins. Public waterworks often take their raw water from lakes or rivers containing cyanobacteria. Previous studies have suggested that the best method to remove cyanobacterial toxins from drinking water is oxidation with ozone. In order to investigate the efficacy of ozone in the removal of cyanobacterial toxins *Microcystis aeruginosa* PCC 7806 and *Oscillatoria rubescens* (sample from Lake Zurich) were ozonated in a batch reactor with different O_3 concentrations ranging from 0,3-2 mg/l for 9 mins contact time and 60 mins reaction time (ozone off). The presence of toxins was detected by the protein phosphatase inhibition assay, HPLC and a primary hepatocyte assay. Ozone treated water still containing Microcystins was filtered with a system modeled on the Zurich Water Supply (fast filter, activated charcoal filter, slow filter). Ozonation by-products of cyanobacterial toxins were analyzed by HPLC to determine the presence of toxins. The results show that the residual toxicity of the cyanobacterial material depends on the cell number, the ozone concentration, the duration of ozonation and the temperature of the ozonated water. The level for a total detoxification of cyanobacteria (10^5 cells/ml) lies at 1mg ozone/ l water (7°C, 9 mins). Ozonation of higher cell numbers results in increased toxicity due to lysis of cyanobacterial cells and release of toxins. An ozone residual (0,05 mg/l) should therefore remain in order to completely destroy the toxins.

153 IMMUNOCHEMICAL DETECTION OF MICRO-CYSTIN-LR IN TISSUES AND CELLS OF RAINBOW TROUT.

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Microcystins (MC) are cyclic heptapeptide toxins produced by cyanobacteria such as *Microcystis aeruginosa*. MC have hepatotoxic and tumor-promoting activity. Intoxication with MC has lead to death of humans and animals such as livestock or fish. In order to determine the localization of microcystin in trout, an antibody was developed against MC-LR and tested for suitability of toxin detection in liver and other tissues and in isolated primary trout hepatocytes. Antibodies against MC-LR were produced in rabbits via conjugation of the toxin to keyhole limpet hemocyanin, antiserum was obtained and used directly for microcystin detection. Microcystin was probed in tissue, cell and subcellular homogenates after *in vivo* and *in vitro* exposure to the toxin by immunoblotting and immunocytochemistry. In order to identify putative protein adducts, Western blots were also stained with anti-human protein phosphatase 1 (PP1) as well as anti-human protein phosphatase 2A (PP2A). In Western blots of trout liver and hepatocyte homogenates the antibodies detected several microcystin-protein adducts with approximate molecular weights ranging from 34 to 38 kD. One of these adducts had an approximate molecular weight of 36 kD and this band was identified as PP2A by using a specific PP2A antibody. Immunoblotting of hepatocyte proteins showed that the activity was largely localized in the cytosol, while immunocytochemistry demonstrated cytosolic as well as nuclear staining.

154 MICROCYSTIN-LR TOXICODYNAMICS, INDUCED PATHOLOGY AND IMMUNOHISTOCHEMICAL LOCALIZATION IN LIVERS OF RAINBOW TROUT.

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The temporal pattern of toxin exposure and pathology, as well as the topical relationship between hepatotoxic injury and localization of microcystin-LR (MC), a potent hepatotoxin, tumor promoter and inhibitor of protein phosphatases 1 and -2A (PP), was investigated in livers of MC-gavaged rainbow trout (*Oncorhynchus mykiss*) yearlings via an immunohistochemical detection method using antibodies specific to MC (see Hitzfeld et al., SOT 1999). H&E-stains of liver sections were used to determine pathological changes (e.g. necrosis). Nuclear morphology of hepatocytes and TUNEL analysis were employed as endpoints to detect the advent of apoptotic cell death in hepatocytes. Trout were gavaged with lyophilized cyanobacteria (*Microcystis aeruginosa*, strain PCC 7806) at acutely toxic doses of 5700µg MC per kg bw, as described by Tencalla and Dietrich (Toxicon **35**, 583-595, 1997). Three of each, control and test animals were sacrificed after 1, 3, 12, 24, 48 and 72h and the livers frozen for determination of remaining PP-activity or fixed for immunostaining and histological analyses. Immunohistochemistry revealed a time dependent discernible increase in staining intensity throughout the liver, concurring with the kinetics of hepatic PP-inhibition. After 3h marked immunopositivity was observed in the cytoplasm, as well as the nuclei of hepatocytes which reached a constant level of staining intensity after 12h. Apoptotic cell death could be detected after 48h at the very earliest. These data suggest that accumulation of MC and subsequently changes in cellular morphology, PP-inhibition and hepatocyte necrosis represent the primary events in microcystin induced hepatotoxicity, while apoptotic cell death, as demonstrated here, is only secondary.

155 PROTECTION BY RED GINSENG TOTAL SAPONIN AGAINST ADRIAMYCIN-INDUCED CARDIOTOXICITY AND CISPLATIN-INDUCED NEPHROTOXICITY.

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The usefulness of adriamycin(ADR) and cisplatin(CP), potent anticancer agents, is limited by the those cardiotoxicity and nephrotoxicity, respectively. Subcellular changes leading to target organ(heart, kidney) toxicities are suggested to be mediated by a drug-induced increase in free radicals and lipid peroxidation. The present study was undertaken to examine whether this protection by red ginseng total saponin(RGTS) against adriamycin-induced cardiotoxicity and cisplatin-induced nephrotoxicity can be improved by co-administration with anticancer agents through *in vivo* experiments. Male SD rats were injected with (1) vehicle control, (2) ADR or CP, (3) RGTS, and (4) ADR or CP plus RGTS. ADR(cumulative dose, 10mg/kg, I.V.) was injected in four equal injection(I.V.) over a period of 10 days. CP(5mg/kg, I.V.) was injected as an I.V. single injection. These groups were sacrificed three days after the end of injection. ADR or CP-induced toxicities were characterized by antioxidant enzymes activities(GPx, SOD), glutathione(GSH) contents, lipid peroxidation in isolated organs, chemistry examination in blood and urine. The results of the study were as fellows. Co-administration of RGTS can provide partial protection against ADR-induced cardiotoxicity and CP-induced nephrotoxicity. This study suggests that the protection offered by RGTS against anticancer-induced target organ toxicity is partly related to antioxidant systems. This study suggests partial usefulness of RGTS therapy in ADR- and CP-induced target organ toxicity.

156 SAFETY AND EFFICACY OF DEGLYCOSYLATED A-CHAIN OF RICIN (DGCA-RICIN) VACCINE FOR THE PROTECT AGAINST AEROSOLIZED RICIN.

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Ricin, a highly toxic plant lectin, consists of two distinct ~ 30,000 MW polypeptides (A- & B-chain) held together by a single disulfide bond. Aerosolized ricin, at an inhaled dose of 10 to 20 µg/kg, causes severe lung injury and death in experimental animals. The A-chain separated from the B-chain has little or no toxicity. Partially deglycosylated A-chain has an increased biological half-life but retains an epitope that is highly antigenic.

A pilot lot of cGMP-produced DGCA-ricin was vialed, lyophilized, and stored at –20°C. This product was reconstituted with saline and used to determine its safety and efficacy when experimental animals were inoculated by intramuscular (im) injection of DGCA-ricin. Mice and rats inoculated with two or three im injections of 10-μg of DGCA-ricin developed neutralizing antibodies and at 90 days after the last inoculation all survived an aerosol challenge with 5 LD_{50} of ricin. In an acute dose preclinical study, 20 male and female BALB/c mice per group were injected with a single im dose of 100, 50, 10, or 0-μg of reconstituted DGCA-ricin. On day one and 15 after dosing, 10 male and female mice from each group were bled for blood samples and necropsied for tissue pathology. None of the mice died before necropsy and all gained weight. Except for a dose- related transient neutrophilia on day one after injection of DGCA-ricin, all of the other blood analyses were normal. The primary pathological lesion was a dose-related local inflammatory response at the sight of injection that had not yet completely resolved by day 15. To evaluate possible low level toxicity, a repeat dose preclinical safety study was initiated in which 570 female mice were dosed im with 10-μg of DGCA-ricin vaccine at 0, 4, and 8 weeks. On day one, 15 and 90 after the third dose, 20 mice were bled for blood samples and necropsied for tissue pathology. None of the DGCA-ricin treated mice died before necropsy and all gained weight. Thus, from the above data, DGCA-ricin appears to be a safe and efficacious vaccine.

157 HEALTH HAZARD ASSESSMENT FOR THE CONSUMPTION OF FRUIT BRANDIES CONTAINING CYANIDE-BASED COMPOUNDS.

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Fruit brandies contain prussic acid (PA) and lactonitriles (LN), both cyanide (CN)-based compounds that are highly toxic. This assessment addresses the potential health hazard of short-term and of chronic consumption of fruit brandy products that contain PA and LN. Fifteen fruit brandies were analyzed for the content of PA and LN by gas chromatography. The estimate of exposure to CN from fruit brandy consumption was based on total CN levels derived from brandy PA and LN content and point estimates of brandy intake (USDA, CSFII, 1989-1992). Mean and 90-99th percentile CN exposures were estimated for acute, single-day and chronic exposures. Consumption of three of the brandy products sampled, 2 cherry and an apricot, is associated with CN exposure levels of toxicological concern. Consumption of one of the cherry brandies over a day at the 99th percentile level could involve CN exposure in the acute lethal dose range. Some mean and higher percentiles of acute and one-day estimates of brandy-derived CN exposure exceed the oral minimum risk level (MRL) of 3 mg CN/day for an intermediate duration of exposure (ATSDR, 1995). The estimated chronic exposure to the three brandies noted exceeds the chronic reference dose (RfD) of 1.2 mg CN/person/day (IRIS, EPA, 1985) at several intake percentiles (mean, 90-99th). Thus, the possibility exists that some brandy products with particularly high levels of PA and LN may represent a hazard to human health.

158 NOVEL TOXINS AND NEW STRUCTURAL VARIANTS OF ENZYMES FROM COLUBRID SNAKE VENOMS.

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Snake venom toxins have provided instrumental tools for developing a molecular understanding of ligand binding at ion channels, and metalloproteases common in venoms have served as models for inducing endothelial damage and hemorrhage. Whereas many venoms from front-fanged snakes are well-characterized, venoms from rear-fanged snakes of the family Colubridae (comprising nearly 700 species worldwide) represent a promising and largely unknown source of novel compounds. Recently we published a method which greatly facilitated extraction of these venoms, and we have screened numerous venoms from this diverse family for novel toxins and structural variants of described toxic proteins (using SDS-PAGE, HPLC, enzyme analyses, protein sequencing and bioassays). Partial N-terminal sequence has been obtained for several purified components and subjected to homology analysis on the BLAST and FASTA protein sequence databases. Based on *in vitro* and *in vivo* assays, one toxin is a phospholipase A_2 (14 kD) which appears to contain novel structural motifs, and a second toxin is a potent metalloprotease (~44 kD) with fibrinogenolytic, myonecrotic and hemorrhagic activities. The phospholipase shows considerable homology with other venom phospholipases, but the protease shows no homology to venom proteins. Two additional proteins (26 kD and 3.5 kD) show no homology to protein sequences in databases and appear to be novel venom components. Further work with venoms from this family likely will yield additional novel compounds.

159 COMPARATIVE TOXICITY OF FUMONISIN DERIVATIVES IN 28-DAY FEEDING STUDY USING FEMALE B6C3F$_1$ MICE.

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Fumonisin mycotoxins are produced by *Fusaria* fungi which grow on corn. Our goal was to determine the relative toxicity of several fumonisins and derivatives. Female B6C3F$_1$ mice were fed diets for 28-days containing 0, 14, 70, and 140 μmol/kg fumonisin B_1 (FB_1), fumonisin B_2, fumonisin B_3, fumonisin P_1, hydrolyzed-FB_1, N-acetyl-FB_1, or N-carboxymethyl-FB_1. Serum analytes and tissue sphinganine:sphingosine ratios were elevated only with 70 and 140 μmol/kg FB_1. Cellular changes included increased hepatocellular apoptosis, hypertrophy, Kupffer cell hyperplasia, and macrophage pigmentation at 70 and 140 μmol/kg FB_1. The other fumonisin derivatives did not alter serum analytes, organ weights, or hepatic structure. These results suggest that, among the compounds evaluated, FB1 causes the most severe acute toxicity.

160 INHIBITION AND STABILIZATION OF TOXIC PROTEINS: A MODEL SYSTEM IN SNAKE VENOMS.

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Rattlesnake venoms are complex biological products containing potentially autolytic components, and they provide a useful tool for the study of long-term maintenance of enzymes in a competent state, both *in vivo* and *in vitro*. In the venom gland of the snake, venom is secreted into a lumen and stored in an inactive and competent state for months. We examined the effects of temperature and storage conditions on *Crotalus molossus molossus* venom using SDS-PAGE, metalloprotease zymogram gels, a cricket LD_{50} toxicity assay and enzyme assays. The results demonstrated that the venom contained at least 24 distinct protein components, and electrophoretic, toxic and enzymatic activities were largely unaffected by dilution effects and storage conditions, which varied by as much as 117° C. Therefore, endogenous mechanisms must be present within the gland and venom stored in the lumen that inhibit autolysis and promote protein stability during long-term storage. Further investigation focuses on the elucidation of these mechanisms. A tripeptide component (present in the venom at millimolar concentrations) inhibited the main metalloprotease (Cvo V) isolated from *C. viridis oreganus* venom. We hypothesize that endogenous polypeptide components both inactivate and stabilize metalloproteases (and perhaps other enzyme components) present in the stored venom. Upon injection, this low affinity inhibitor will spontaneously dissociate, releasing an active toxic enzyme in tissues which induces hemorrhage and tissue necrosis.

161 ISOLATION OF CERAMIDE SYNTHASE: ENRICHMENT OF PROTEIN FRACTIONS FROM RAT LIVERS CONTAINING CERAMIDE SYNTHASE ACTIVITY.

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Ceramide synthase (CeSyn) catalyzes the synthesis of ceramides from sphinoid bases (sphinganine or sphingosine) and acyl-coenzyme A. CeSyn is the key enzyme inhibited by fumonisin B_1. CeSyn activity was determined through the incubation of ^{14}C-palmitoyl coenzyme A and sphinganine, product separation using TLC, and detection of the N-[^{14}C]palmitoyl-sphingosine using phosphoimagery. The soluble proteins from sonicated rat liver microsomes were separated using strong anion exchange chromatography. Fractions enriched for CeSyn activity eluted under low salt conditions. CeSyn was further isolated using size exclusion, hydroxyapatite, and weak anion exchange chromatography; and electroelution from non-denaturing polyacrylaminde gels. Purification of CeSyn from the electroeluted fractions is in progress.

162 METABOLIC ACTIVATION OF GENOTOXINS IN *FUSARIUM* EXTRACTS AND EVIDENCE THAT FUSARIN C IS NOT RESPONSIBLE FOR DNA ADDUCT FORMATION.

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Fusarin C (FC) is a metabolite produced by some *Fusarium* fungi that contaminate crops worldwide. The consumption of feed containing *Fusarium moniliforme* is tumorigenic in rats. The incubation of *Fusarium* extracts with DNA in the presence of liver subfractions (S9) results in the formation of a unique DNA adduct detected by the ^{32}P-postlabeling assay. FC was purified from *Fusarium* cultures and characterized by NMR and HPLC/MS methods. FC did not produce the DNA adducts when incubated with S9 and DNA. Likewise, the formation of DNA adducts with extracts of different *Fusarium* cultures did not correlate with FC content. These and other data suggest neither FC, nor its metabolites, are responsible for the genotoxicity observed from *Fusarium* fungi.

163 INHIBITION OF TCDD-INDUCIBLE TRANSCRIPTION OF HUMAN CYP1A1 AND CYP1A2 GENES BY POLYPHENOLIC COMPOUNDS OF GREEN TEA.

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Green tea contains several polyphenolic flavonoids known as catechins which possess chemoprotective properties. Members of the plant-derived flavonoid family, including tea catechins, have been shown to alter the activities of some carcinogen-metabolizing enzymes. We examined the effects of commercially available polyphenolic extracts of green tea, Polyphenon 60 and Polyphenon 100 (from Sigma), on the expression of human CYP1A mRNA. We demonstrated that pretreament of the human hepatoma cell line, HepG2, and also primary cultures of human hepatocytes with P60 and P100 inhibits: i) TCDD-inducible CYP1A1 and CYP1A2 gene transcription, ii) mRNA expression, and iii) nuclear uptake of the Arylhydrocarbon Receptor (AhR). These data indicate that green tea polyphenolics (GTPs) can alter CYP1A expression by inhibiting gene transcription, most likely through changes in AhR function. We have shown P60 or P100 alone do not induce CYP1A1 mRNA, and it seems most likely that GTPs are not AhR ligands. To investigate the possibility that the individual catechins found in P60 and P100 could transform the cytosolic AhR into a DNA binding species, cytosol from the mouse hepatoma cell line, Hepa-1c1c7, was isolated. Incubation of cytosol for two hours with 20nM TCDD induced the transformation of the AhR in vitro to an XRE binding species, while treatment with the individual catechin components (from Sigma, product numbers E4143, E3768, E3893) at 50 mM or 200 mM did not transform the AhR as judged by electrophoretic mobility shift assays. We are currently investigating the mechanism by which GTPs inhibit the TCDD induced expression of human CYP1A genes. (Supported by NIH Grants GM54477.)

164 EFFECT OF DIVICINE ON SULFHYDRYL STATUS, MEMBRANE SKELETAL PROTEINS AND MORPHOLOGY OF RAT ERYTHROCYTES.

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Divicine (2,6-diamino-5-hydroxy-4-pyrimidinone) has been implicated as a mediator of an acute hemolytic anemia, known as favism, in susceptible individuals who ingest fava beans. We have shown recently that divicine is a direct-acting hemolytic agent in the rat. In this study, the effect of hemolytic concentrations of divicine on rat erythrocyte sulfhydryl status, membrane skeletal proteins and morphology was examined. *In vitro* exposure of red cells to divicine (0.8-2.0 mM) induced a concentration-dependent depletion of GSH with a concomitant increase in the formation of GSH-protein mixed disulfides. SDS-PAGE analysis of skeletal protein indicated that divicine induced the formation of high molecular weight protein aggregates (>250 kDa), an apparent loss of protein bands 2.1 and 4.2, and a broadening of bands 1, 2 and 3. Scanning electron microscopy revealed the presence of non-spherocytic echinocytes. Treatment of red cells with dithiothreitol following divicine exposure reversed both the skeletal protein and morphological changes with concomitant release of hemoglobin, indicating that these changes were due to disulfide-linked adducts between hemoglobin and the skeletal proteins. The data indicate that divicine alters sulfhydryl status, skeletal proteins and morphology under conditions that result in premature sequestration of injured cells by the spleen. (Supported by NIH grant DK47423.)

165 CYTOTOXICITY AND GENOTOXICITY OF CAPSAICIN IN THE HUMAN NEUROBLASTOMA CELLS SHSY-5Y.

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Capsaicin, a natural product of *Capsaicum* species, induces excitation of nociceptive terminals involved in pain perception. Our previous studies have shown that capsaicin inhibits protein synthesis on cultured monkey kidney cells (Vero cells) and in primary culture of rat astrocytes. The effect of capsaicin on human neuroblastoma cells SHSY-5Y are now investigated. The cytotoxicity was assessed by incorporation of [^{3}H]L-leucine into cellular protein in the presence of capsaicin, the genotoxicity using the comet assay and the fragmentation assay by electrophoresis in agarose gel after incubation of neuroblastoma cells with capsaicin 25 to 100µM. The concentration required to inhibit 50% of the protein synthesis (IC_{50}) was found to be 60µM after incubation with the toxin during one cellular cycle (5 days) of SHSY-5Y. The results of the comet test and DNA fragmentation assay clearly suggest that capsaicin is able to induce DNA strand breaks already with concentrations in the range of 50µM. Several daily topic applications of preparations containing 0.075% of capsaicin could lead to blood capsaicin concentration of this order following transdermal passage (5% of the total quantity applied). Because DNA strand breaks may affect cellular functions, lead to cell death, and/or carcinogenesis, our data may have important implications for the possible health threats of capsaicin, specially in case of unreasonable use of capsaicin preparations in pathological situations.

166 EVALUATION OF CASTOR BEAN TOXICITY IN ANIMALS.

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Castor bean (*Ricinus communis*) contains the toxic lectin, ricin. Ricin is a highly toxic glycoprotein reported to cause hypotension, gastroenteritis, hemolysis, hemorrhage, depression, and death. All parts of the castor bean plant contain the ricin, however, the protein is most abundant in the seeds, and the pericarps are four times as toxic as the fresh leaves. Ingestion of even one castor bean can be lethal in a child. Human deaths are often attributable to hypovolemic shock. Ricin has been used as both a homocidal and suicidal agent in humans, and is considered one of the most toxic agents known. In spite of this, very few deaths are reported following castor bean ingestion in animals. From January 1987 to September 1998, the National Animal Poison Control Center received 103 incidents of castor bean ingestion in animals. Although most calls (89%) concerned canines that ingested castor beans, feline, caprine, bovine, and equine cases of castor bean ingestion were also reported. The most commonly reported clinical signs were vomiting (with or without blood) 61%, diarrhea (with or without blood) 43%, and depression 35%. Death or euthanasia occurred in only 8% of the cases. Other signs reported include abdominal pain/colic, tachycardia, liver enzyme elevation, tremors and seizures. Most cases recovered uneventfully with appropriate veterinary care. Treatment of castor bean toxicosis includes induction of emesis followed by oral activated charcoal if given soon after ingestion, gastro-intestinal protectants, fluid therapy, anticonvulsants when necessary, and monitoring of cardiac, renal and hepatic function.

167 INHIBITION OF NADP(H) QUINONE OXIDOREDUCTASE BY COUMARIN (1,2-BENZOPYRONE) IN HUMAN, RAT AND CHICK EMBRYO LIVER.

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Coumarin, a naturally occurring dietary compound and drug currently under investigation for the treatment of some cancers and lymphedema, inhibits certain cytochrome P450 enzymes. In studies comparing effects on ethoxyresorufin *O*-deethylase activity in liver S9 from Aroclor 1254-treated

rats and chick embryos, coumarin increased accumulation of the resorufin product with chick embryo liver S9. The increase was two-fold with 25 μM coumarin and three-fold at concentrations greater than 50 μM. The increase was associated with coumarin inhibition of cytosolic NAD(P)H quinone oxidoreductase (QR) for which resorufin is a substrate. Coumarin had no effect on resorufin accumulation with Aroclor 1254-treated rat liver S9. Using 40 μM 2,6-dichloroindophenol as the electron acceptor, dicoumarol-sensitive QR activity was inhibited 50% by coumarin at approximately 5 μM in liver cytosol from untreated chick embryos with total inhibition at concentrations above 50 μM coumarin. Coumarin inhibition of chick embryo QR activity also occurred with menadione as substrate. Rat and human liver QR were much less sensitive with 50% inhibition at approximately 150 μM. These findings demonstrate a species difference in coumarin inhibition of liver cytosolic QR with the chick embryo enzyme being more sensitive than human or rat QR. Since QR is involved in the metabolism of many xenobiotics and therapeutic drugs, coumarin may alter the disposition of these chemicals. (Supported by UTMB Preventive Medicine & Community Health Research Initiative Program.)

168 FLOW CYTOMETRIC ANALYSIS OF BONE MARROW COMPARTMENTS CHIMERIC FOR EITHER THE ARYL HYDROCARBON OR ESTROGEN RECEPTORα REVEAL CELLULAR TARGETS OF TCDD AND ESTROGEN.

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The hemopoietic and stromal compartments of bone marrow may each contain cellular targets that mediate receptor-dependent and/or receptor-independent responses associated with either the environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or the endogenous hormone estrogen. The identity of marrow target cell(s) that respond to TCDD and estrogen through activation of the aryl hydrocarbon receptor (AhR) or the estrogen receptor isoform Erα, respectively, to effect marrow immunotoxicities remains uncertain. We have determined that single dose administration of either 30 μg/kg TCDD or 5 mg/kg estrogen result in altered flow cytometric enumerations of lineage-negative, c-Kit$^+$ Sca-1$^+$ murine pluripotent hemopoietic stem cells (HSC). While increases in HSC were detected in response to TCDD treatment, similar effects were absent in TCDD-treated AhR knock-out mice. Estrogen, however, caused reductions in the number of HSC both in wild-type mice and in estrogen-treated, estrogen receptor knock-out (ERKO) mice. These data provide evidence of differential hemopoietic immunotoxicities in response to *in vivo* TCDD or estrogen treatment. To determine whether these compounds differentially targeted bone marrow hemopoietic and/or stromal cellular elements, HSC responses to TCDD or estrogen were studied, respectively, in marrow-chimeric mice bearing TCDD- or estrogen-responsive (AhR+/+ or ERα+/+) stromal elements and TCDD- or estrogen-unresponsive (AhR−/− or ERα−/−) hemopoietic elements. Our results showed that the TCDD-induced HSC alterations were dependent upon the presence of the AhR within hemopoietic cells and occurred in the absence of AhR-bearing bone marrow stromal cells. Furthermore, while estrogen treatment caused reductions in the number of HSC through mechanisms independent of ERα gene-mediated signaling, proportionately more significant and global reductions to hemopoietic cell numbers were mediated through ERα genetically-dependent mechanisms associated with hemopoietic cells.

169 THE ROLE OF THE ARYL HYDROCARBON RECEPTOR IN THE MATURATION OF CONVENTIONAL B LYMPHO-CYTES AND IN 2,3,7,8–TETRACHLORO–P–DIOXIN (TCDD)–INDUCED B LYMPHOCYTE ALTERATIONS.

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The ligand–activated aryl hydrocarbon receptor (AhR) is a cytosolic protein belonging to the basic–helix–loop–helix superfamily of DNA binding proteins. Although a biologic role for AhR has not been fully elucidated, it has been shown to mediate the immunotoxicity of exogenous compounds such as TCDD. Several investigators have shown its involvement in altering the functional response of activated B lymphocytes following TCDD administration; however, little research has been done on its possible function in the early development of these cells, and whether exogenous ligands have an impact on this process. The purpose of this study was to determine, 1), the role, if any, of this receptor in the maturation of conventional B lymphocytes, 2), if a role exists, the relative importance of its presence in hematopoietic and/or stromal components and, 3), whether TCDD alters this process. Bone marrow chimeras were produced which were positive (AhR+/+) or negative (AhR −/−) for AhR in either their stromal or hematopoietic elements, or both. Bone marrow cells were analyzed for alteration of B lymphocyte maturation stages in both naive and TCDD–treated (30 μg/kg body weight) animals. Our results showed that, 1), naive AhR+/+ animals had higher percentages of mature B cells versus the naive AhR−/− animals, 2), TCDD treatment of AhR+/+ animals produced a shift toward a more mature B cell, as opposed to having a minimal effect on AhR−/− animals, and 3), 5 < AhR is required in both hematopoietic and stromal elements for maintenance of normal B cell maturation and mediation of the TCDD induced maturation alteration. (Supported by NIEHS grants ESO5774, ESO7216, ESO1247 and ESO4862.)

170 POTENTIAL FACTORS FOR DECREASED PLATELET COUNTS IN MICE ADMINISTERED PHOSPHOROTHIOATE OLIGODEOXYNUCLEOTIDES.

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Sustained and significant decreases in platelet count have been noted in mice following dosing with phosphorothioate oligodeoxynucleotides (ODN). Interestingly, ODNs that induce the greatest decrease in platelet count also show the most significant immune stimulation, of which splenomegaly is a prominent aspect. To investigate the correlation between splenomegaly and decreased platelet count, mice were dosed with either a highly stimulatory (ISIS 12449) or non-stimulatory (ISIS 12450) ODN. In addition to spleen weight, number of megakaryocytes (production), % reticulated platelets (release), and platelet life-span (destruction) were examined. In ISIS 12449-treated mice, megakaryocyte numbers were unchanged in sternum marrow, and increased in spleen. The percentage of reticulated platelets (12%) and platelet life-span (5 days) were similar to controls. Spleen weight, however, increased with a maximum (> 2-fold) at 96 to 120 hrs after dosing. Platelet counts progressively decreased, with the nadir (40% of control) at 120 hrs. As spleen weight returned to baseline values, circulating platelet counts returned to normal levels. There was no decrease in platelet count or increase in spleen weight in ISIS 12450-treated mice. Overall, these data support sequestration within an enlarged spleen as a primary factor in the severity and prolongation of decreased platelet counts in rodents. Immune stimulation, i.e. splenomegaly, is not a principal effect in monkeys, however mild and transient decreases in platelet counts have been observed. Thus, species specific factors need to be considered in determining the mechanism(s) most relevant for assessing and managing potential effects in humans.

171 PHARMACOKINETICS AND PHARMACODYNAMICS OF ERYTHROPOIETIN IN ETHANOL TREATED MICE.

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Sponsor: L G Sultatos.

A number of clinical findings in patients implicate ethanol as a causal factor in the development of hematological abnormalities. However, it has been difficult to separate the direct effect of ethanol from the multiple factors existing in the alcoholic patient. Erythropoietin (EPO), normally produced in the kidney, is the major growth hormone that stimulates the proliferation, differentiation and maturation of red blood cells. The hematological observations following chronic ethanol may result from modification of EPO production, changes in EPO disposition and/or alterations in response of its target cells. The current study was undertaken to determine the effect of chronic ethanol administration on the response to recombinant human EPO and on the disposition of EPO. Male adult mice were allowed to drink only ethanol (30% v/v) for 80 days. On day 42 the mice were administered EPO (1.2 μg/30g bw) and the % hematocrit determined over the next 40 days. The area under of percent hematocrit vs time curve was 61.2 in control mice (water treated) compared to only 45.6 in ethanol treated mice. In two other groups of mice, similarly allowed to drink either ethanol or water, EPO was administered iv and its disposition assessed from blood samples withdrawn at various times. There was no difference found in the EPO serum levels in these mice. Since ethanol decreased the response to exogenously administered recombinant human EPO, it is not due to an effect on EPO production or release. Furthermore, since EPO's disposition was unaffected, it appears the decreased effect is a consequence of a qualitative or quantitative change in EPO-responsive cells.

172 HYDROQUINONE ALTERS THE PHOSPHORYLATION STATE OF THE TRANSCRIPTION FACTOR PU.1 IN HUMAN CD34+ HEMATOPOIETIC PROGENITOR CELLS (HPC).

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As part of ongoing studies to elucidate the mechanism of HQ-induced alterations in the regulation of myeloid differentiation, we examined the effects of HQ on PU.1-DNA binding activity in human CD34+/CD19- HPC. PU.1 plays a critical role in myeloid differentiation by regulating genes such as the M-CSF receptor, GM-CSF receptor α, G-CSF receptor, FcγR1, IL-1β, CD18, CD11b and PU.1 itself. Control and HQ-treated CD34+/CD19- HPC (>95%) were cultured in liquid media supplemented with growth factors and the pattern of PU.1-DNA binding was compared in Electrophoretic Mobility Shift Assays (EMSA) over a three day period. Following single exposure to HQ, EMSAs performed with whole cell lysates from treated CD34+/CD19- HPC exhibit a decrease in a faster migrating PU.1-DNA binding species and a subsequent increase in a slower migrating PU.1-DNA binding species. Additional experiments using whole cell lysates reveal that the HQ-induced alterations in PU.1-DNA binding activity are the result of alterations in the phosphorylation state of PU.1 and theses alterations appear to be independent of protein kinase C (PKC) activation. These results demonstrate that HQ-induces an alteration in the phosphorylation state of PU.1 in human CD34+/CD19- HPC and suggests that PU.1 may be an important molecular mediator in benzene-induced alterations in myeloid development.

173 ROLE OF NITROSOBENZENE IN THE SPLENIC TOXICITY OF ANILINE.

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To elucidate the mechanism(s) of selective toxicity of aniline to the spleen, studies were conducted with nitrosobenzene (NB), an N-oxidized metabolite of aniline. Male SD rats were given 0.025, 0.05, 0.1 or 0.2 mmol/kg/day of NB in 0.5 ml of 0.25% agar by gavage for four days; control rats received the vehicle only. Animals were euthanized at 24 hr following the last dose. NB exposure resulted in decreased erythrocyte counts, whereas methemoglobin content increased at 0.1 and 0.2 mmol/kg doses. Splenic weight-to-body weight ratios were greater by 55 and 81% at 0.1 and 0.2 mmol/kg NB doses, respectively. Total iron content in the spleen showed dose-dependent increases of 27, 90, 192 and 213% at 0.025, 0.05, 0.1 and 0.2 mmol/kg doses, respectively. Splenic lipid peroxidation also showed a dose-dependent response and was greater by 19, 56, 74 and 85% at the four doses, respectively. Furthermore, NB exposure resulted in greater protein oxidation (carbonyl content) in the spleen at 0.1 and 0.2 mmol/kg doses. These results suggest that NB is a splenotoxin and therefore, can contribute to the splenic toxicity of aniline. Results of this study along with our earlier findings with aniline further support that oxidative stress is a potential mechanism in the splenotoxicity of aniline.

174 COMPARISON OF IN VITRO MYELOTOXICITY OF A NOVEL ONCOLYTIC AGENT, LY355703, TOWARD RAT AND DOG BONE MARROW PROGENITORS.

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For several oncolytics in which bone marrow suppression is the dose-limiting toxicity, in vitro inhibition of colony forming unit granulocyte-macrophage progenitors (CFU-GM) has been shown to be predictive of concentrations which cause neutropenia. By comparing the CFU-GM dose response in several species, including humans, the rational selection of a starting dose and escalation intervals may be possible. This could benefit clinical trial patients by arriving at safe, but efficacious doses earlier in the trial. To evaluate this approach in animals, species sensitivities to a novel oncolytic agent, LY355703, were compared in CFU-GM assays following in vitro exposure to the compound. Bone marrow cells from six dogs and six rats (3 per sex) were exposed for 14 hours to target concentrations of LY355703 ranging from 1 to 80 ng/ml, then assayed in the CFU-GM assay using GM-CSF as the sole cytokine. LY355703 concentrations interpolated at 90% colony inhibition (IC_{90}) were used to evaluate the ability of the CFU-GM assay to predict in vivo plasma concentrations at the MTD. The IC_{90} value in the rat and dog was 25.0±6.6(sd) and 14.5±1.0(sd) ng/ml, respectively. Comparison of IC_{90} values to the C_{MAX} at MTDs in toxicity studies in the rat (59.0 ng/ml) and dog (7.2 ng/ml) indicated there was relatively good agreement between the in vitro IC_{90} values and plasma LY355703 concentrations which resulted in significant myelotoxicity in vivo. This suggests that similar determinations of IC_{90} values using human bone marrow cells may be useful for predicting myelotoxic plasma levels of LY355703 in humans.

175 DIFFERENTIAL EXPRESSION OF GLUTATHIONE S-TRANSFERASE ISOZYMES IN K562, HL60 AND U937 HUMAN LEUKEMIA CELL LINES.

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The K562, HL60 and U937 cell lines have been used extensively to examine the effects of a variety of differentiating agents which may function through increased formation of membrane lipid peroxidation (LPO) products. Because electrophilic products of LPO are substrates for glutathione S-transferases (GSTs), we have studied isozyme compositions and kinetic properties of GSTs in these cell lines which are characterized by distinct morphology and lineage specificity of differentiation. These studies showed GST isozyme compositions were nearly identical for both myeloid origin cell lines, HL60 and K562, with the -class isozyme predominating (64% and 63% of total GST protein, respectively), followed by the µ-class isozymes (32% and 33%, respectively). The -class GSTs (GST A1-1 and A2-2) were absent in the K562 or HL60 cells, but both cell lines contained a GST isozyme, pI 6.0 (4 % and 3%, respectively) which was recognized by antibodies against mGSTA4-4, a GST isozyme belonging to a subclass of -class GSTs with high activity towards the LPO product 4-hydroxynonenal (4-HNE). The -class GST was also predominant in U 937 cells (93%). Unlike the erythroleukemic (K562) and promyelocytic leukemia (HL60) cell lines, GST-µ was not present in monocyte/macrophage origin U937 cells, but these cells contained significant amounts of GSTA 1-1 and GSTA 2-2. The pI 6.0 isozyme was present in U937 cells at levels comparable to K562 and HL60 cells. The catalytic efficiency (kcat/Km) towards 4-HNE was higher for the pI 6.0 isozyme (622 mM-1 s-1) than the -class (17.9 mM -1 s -1) or the µ-class (42.6 mM-1 s-1) GST isozymes, suggesting an important role of this enzyme in detoxification of LPO products. Because individual GST isozymes differ remarkably in their affinity for electrophilic products of LPO, their levels of expression may regulate intracellular levels of LPO products and perhaps the lineage specificity of differentiation. (USPHS grant CA-27967 to YCA and CA63660 to SA.)

176 THE PRESENCE OF A MUSCARINIC RECEPTOR ON CANINE ERYTHROCYTE MEMBRANES.

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A uniform population of M1 muscarinic receptors exhibiting similar pharmacological responses to those in mouse brain homogenates is present on human erythrocyte (RBC) membranes, and there is negligible nonspecific binding (Gen.Pharmac.17,281-285). We undertook this study to determine whether canine RBC membranes contain a similar population of muscarinic receptors. RBC membranes were prepared from canine blood by washing, lysis and serial centrifugation. Protein concentration was determined by the Lowry method. Suspensions of RBC membranes were incubated for various times at 25° C with various amounts of *l*-quinuclidinyl [*phenyl*-4-^{3}H] benzilate (^{3}H-QNB), with and without pre-incubation for 20 min with oxotremorine. RBC membranes were isolated on glass fiber filters by vacuum filtration. Radioactivity of filters and washings was measured and specific binding was calculated by subtracting binding in the presence of oxotremorine (nonspecific binding) from total binding. Using various concentrations of ^{3}H-QNB/membrane protein, association/dissociation curves over time and a Scatchard plot at 40 min were constructed. Muscarinic receptors were present on canine RBC ghosts at a concentration of 0.61 nmol/mg protein and had a K_D of 62.5 nmol. Saturation at 0.45 nmol ^{3}H-QNB/μg protein was reached in a plateau between 20 and 40 min, at which ligand depletion was 2.6%. Practical problems with this model were encountered; adherence of additional molecules of radioligand to receptor-ligand complexes occurred at only twice the radioligand concentration required to ensure saturation. This and other properties, including a mean nonspecific binding of 80% and the need for extensive dilution of RBC membrane suspensions to obtain practical quantities of muscarinic receptors, lead us to conclude that the canine RBC membrane is not a practical model to study

muscarinic receptors and their interactions with cholinergic agonists or antagonists.

177 COMPARITIVE EFFECTS OF PHENYLHYDRAZINE (PHZ) AND PHLEBOTOMY ON PERIPHERAL BLOOD, BONE MAR ROW AND ERYTHROPOIETIN LEVELS IN WISTAR RATS.

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This study was conducted to better characterize the response of rats to blood loss and hemolysis and to incorporate automated methods into the routine evaluations of those responses. Serial phlebotomies of 1.5 -2.0 mL blood per day for 5 days, or intraperitoneal injections of 50 mg/kg PHZ for 3 days, were used to cause anemia associated with blood loss or hemolysis, respectively. Comparable decreases in red blood counts and hematocrits (30-47%) were achieved in both groups. In the routine complete blood count (CBC), hemoglobin, hematocrit:hemoglobin ratio, and erythrocyte indices, could be used to discriminate between the two treatments. Free plasma hemoglogin in PHZ-treated animals resulted in marked elevations of mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) with a 2:1 hematocrit:hemoglobin ratio rather than the anticipated 3:1 ratio. Although both groups of animals had elevated white blood cell counts, PHZ-treated animals also had monocytosis and basophilia. Reticulocyte counts were more sensitive than erythropoietin (EPO) concentrations in predicting erythroid changes. Mean reticulocyte values were approximately 20% in serially phlebotomized animals and greater than 97% in PHZ-treated rats. Plasma EPO levels were 4-10 fold higher than EPO levels in urine, kidney, or liver. Flow cytometric differentials of rat bone marrow using 2'7'-dichlorofluorescin successfully predicted erythroid hyperplasia in both experimental groups. This method also provided information on absolute differential counts and non-nucleated erythrocyte number shifts not detectable either by manual examination of bone marrow smears or by histologic examinations of femur sections.

178 EFFECT OF GENDER ON THE HEMATOTOXICITY OF 2-BUTOXYETHANOL (BE) IN F344 RATS.

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BE is an extensively produced chemical with a wide range of uses. Administration of BE to rodents causes acute hemolytic anemia, and metabolic activation of BE to butoxyacetic acid (BAA) is required for the development of this effect. Recent studies showed that female rats treated with BE exhibited a variety of histopathologic lesions that were absent in males and many of these lesions were attributed to the hemolytic effects of BE. Current studies were designed to compare the hematotoxicity of BE in 3 month old male and female F344 rats. Rats were treated with 250 mg BE/kg (po). At 4, 8, or 24 hr after dosing, rats were anesthetized and EDTA-anticoagulated blood was collected via cardiac puncture. Using the Technicon H1 hematology analyzer, blood parameters were measured and included: red blood cell (RBC) counts, hemoglobin (Hgb), hematocrits (Hct), mean cell volumes (MCV). As expected, BE resulted in a time-dependent swelling of erythrocytes as evident by an early increase in Hct and MCV in both males and females. Subsequently, RBC, Hgb, and Hct declined as hemolysis progressed. However, the onset of BE-induced hemolysis was faster and the severity was significantly greater in females vs. males. In vitro incubation of BAA with blood showed no difference in the sensitivity of RBCs obtained from male and female rats. These data suggest that female rats are more sensitive to BE-induced hemolysis than males and it is likely that gender-related differences in the kinetics of BE and BAA may account for this observation.

179 MOLECULAR AND CELLULAR MECHANISMS OF ANTIOXIDANT ACTION.

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Antioxidant enzymes and small molecule antioxidants play key roles in cellular defense against reactive oxidants associated with environmental chemicals. Recent work provides new insights into the complex interplay of oxidants and antioxidants and places antioxidant chemistry into a cellular perspective. This symposium will address the chemistry of small molecule antioxidants and the enzymatic linkage of cellular redox metabolism to antioxidant function. The small molecule antioxidants vitamin E and ubiquinol provide mutually supportive antioxidant protection through redox cycles that can be coupled to electron transport pathways. The mitochondrial electron transport protein cytochrome c acts as a redox switch in the control of cellular redox status and may provide a link between mitochondrial redox status and apoptosis. Polymorphisims of NAD(P)H-quinone oxidoreductase (DT-diaphorase) may dictate susceptibility to oxidant stress-related toxicity and new work suggests links the enzyme to regulation of vitamin E antioxidant actions. Other molecular species may be key players in cellular antioxidant function. Emerging evidence suggests that nitric oxide acts both as an important cellular antioxidant and as a prooxidant. Rapidly growing interest in chemoprotective effects of plant derived polyphenols focuses new interest in antioxidant mechanisms and chemistry of green tea catechins, which exert notable chemoprotective actions against chemical carcinogenesis.

180 ANTIOXIDANT LABYRINTHS: VITAMIN E/COENZYME Q TRAILS.

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Free radical challenge results in either physiological adaptive changes (including elevated antioxidant protection) or progresses into execution of cell death mechanisms. Recently, antioxidant recycling has been established as one of the important pathways of antioxidant enhancement. In particular, recycling of vitamin E through ascorbate/thiol-cascades has been shown to operate as an important source of antioxidant equivalents in membranes. Reduced coenzyme Q (CoQ) represents another potentially essential vitamin E recycling mechanism as it links regeneration of vitamin E to major intracellular electron transport pathways. Reduced CoQ, ubiquinol, functions as an important lipid-soluble antioxidant. Our previous work demonstrated that in addition to its direct antioxidant activity, ubiquinol is capable of reducing vitamin E phenoxyl radical i.e., recycle vitamin E. Semireduced CoQ, ubisemiquinone are not realized in the presence of vitamin E. This is due to the ability of ubisemiquinone to reduce vitamin E phenoxyl radicals. As a result, when both CoQ and vitamin E, are present superoxide is consumed in CoQ-mediated vitamin E recycling. We have demonstrated that superoxide-driven recycling of vitamin E is mediated by ubisemiquinone in simple chemical models (an aprotic solvent, DMSO), as well as in enzymatic model systems (human recombinant NADPH-cytochrome P450 reductase and plasma membrane NADH-CoQ reductase). We conclude that vitamin E and CoQ are mutually beneficial as antioxidants because their combination provides for effective scavenging of radicals through redox coupling with the major electron transport pathways.

181 OXIDANTS AND ANTIOXIDANTS IN CELL GROWTH AND APOPTOSIS SIGNALING.

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Improved understanding of the mechanisms of redox signaling and the factors controlling cellular redox is providing new insight into oxidant induced toxicity. Of particular importance, effects on normal redox signaling provide a mechanism whereby oxidants can cause toxicity and antioxidants can protect against toxicity through subtle changes that can occur without irreversible oxidative damage to macromolecules. Under normal conditions, mitochondria are a major source of reactive oxygen species (ROS) that determines the redox poise of the cell. Apoptosis occurs in association with a substantial oxidation of cytosolic redox state. In staurosporine-induced apoptosis, oxidation occurs due to enhanced superoxide generation triggered by the release of cytochrome c (cyt c). Mitochondrial loss of cyt c results in a switch in electron flow from the normal 4-electron reduction of O(to water, to a pathway in which only 1 electron is transferred to O2. This switch is blocked by Bcl-2, indicating that regulation of distribution of redox active components provides an underlying control of redox signaling and phenotype control. (° cells, which lack a mitochondrial genome and do not consume oxygen, maintain cyt c-dependent activation of apoptosis and redox signaling. Antioxidants inhibit or delay apoptosis in several systems but do not interfere with cyt c-dependent activation of caspase 3. Thus, the data indicates that oxidants can exert toxicity and antioxidants can modulate this toxicity by altering redox signaling pathways that function to control cell phenotype, proliferation and apoptosis.

182 DT-DIAPHORASE (NQO1) AS AN ANTIOXIDANT ENZYME. RELEVANCE OF POLYMORPHISMS IN NQO1 FOR CHEMOPROTECTION.

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NQO1 is an obligate two electron reductase that catalyzes the reduction of a wide variety of substrates including quinones. Since reduction of quinones to hydroquinones generally results in the production of less reactive species, NQO1 is considered to be a detoxification enzyme. Many xenobiotic quinones are substrates for NQO1 but endogenous substrates have been more difficult to identify. Recently, NQO1 has been shown to catalyze reduction of ubiquinone and (-tocopherolquinone (TQ) to their antioxidant hydroquinone forms. TQ is formed via free radical attack on (-tocopherol and has no intrinsic antioxidant activity while its hydroquinone derivative is a potent antioxidant. This data suggests that one of the physiological functions of NQO1 may be to regenerate antioxidant forms of (-tocopherol after free radical attack. We have characterized a polymorphism in NQO1, a single point mutation coding for a proline to serine change in the protein. Cells carrying the homozygous mutation have no detectable NQO1 activity and genotype-phenotype relationships have been confirmed in human tissues. The prevalence of the NQO1 polymorphism varies from 4-22% as a function of ethnic group. A lack of NQO1 activity due to the polymorphism may result in a decreased capacity to protect against oxidative damage and has potential implications for chemoprotection. Individuals carrying the NQO1 polymorphism are more susceptible to hematotoxicity after occupational exposure to benzene and the role of the NQO1 polymorphism in susceptibility to various cancers is also under investigation. (Supported by CA 51210 and EPA R825281.)

183 THE ANTIOXIDANT ACTIVITIES OF NITRIC OXIDE.

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Nitric oxide (NO) is a hydrophobic free radical whose synthesis is catalyzed in vivo by the action of nitric oxide synthase. NO is multifunctional, and in some cases its actions are paradoxical. This is exemplified in studies of oxidative stress where NO has been demonstrated to have both pro- and antioxidant activities. In general, the pro-oxidant activities are associated with high flux NO formation or with co-generation of a secondary species (e.g. superoxide, hydrogen peroxide). The antioxidant activity tends to be associated with low flux NO. In addition, the terms 'pro-oxidant' and 'antioxidant' are relative to the biomolecule under examination. If lipid peroxidation is used as an index of oxidative stress then the following picture emerges. i) NO does not oxidize unsaturated lipid when generated slowly. ii) Rapid generation of NO or bolus additions of NO can oxidize lipid due to the formation of higher oxides of nitrogen. iii) Co-generation of NO and superoxide oxidizes lipid due to peroxynitrite formation. iv) Nitric oxide inhibits lipid oxidation when initiated by additional oxidants (e.g. copper ions/hydroperoxide, azo-initiators, iron/hydrogen peroxide, SOD/hydrogen peroxide, ferryl heme complexes, peroxynitrite). The mechanism of inhibition appears to be either the scavenging of the initiating agent or the scavenging of the propagatory lipid peroxyl radical. NO, on a mole-per-mole basis, is about a 105-times more potent peroxyl radical scavenger than (-tocopherol. The elevation of endothelial superoxide formation observed in hypercholesterolemia and hypertension is likely to remove the protective and regulatory effects of vascular NO and replace them with the pro-oxidant effects of peroxynitrite. Peroxynitrite has been implicated in several vascular pathologies.

184 ANTIOXIDANT CHEMISTRY OF GREEN TEA CATECHINS.

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Extracts of green tea are effective inhibitors of chemical and physical carcinogenesis. These activities are thought to be due in large part to antioxidant actions of catechins (flavan-3-ols) including (-)-epigallocatechin gallate (EGCG) and (-)-epigallocatechin (EGC). We have found that EGCG reacts with peroxyl radicals in aqueous acetonitrile to yield a novel seven-membered B-ring anhydride and a symmetrical B-ring oxidized dimmer as major antioxidant reaction products. The B-ring of EGCG appeared to be the exclusive site of antioxidant reactions. Analogous products were formed in antioxidant reactions of EGC, which lacks a 3'-galloyl moiety. Surprisingly, the model compound methyl gallate, which is similar to the 3'-galloyl moiety in EGCG was largely unreactive under the same conditions. The identification of product structures provides potential markers for EGCG antioxidant reactions. Although reaction with peroxyl radicals certainly is among the most probable antioxidant reactions of polyphenolic compounds in biological systems, reactions with the other oxidants singlet oxygen, peroxynitrite and hydroxyl radicals also may occur. The identification of oxidant-specific products together with the application of sensitive and specific analytical methods will provide analytical approaches to evaluating the antioxidant actions of catechins in biological systems. (Supported by NIH grants CA 75599 and ES06694.)

185 BIOTECHNOLOGY PRODUCTS: NOVEL COMPOUNDS AND CHALLENGING TOXICOLOGY.

D K Monteith. *Isis Pharmaceuticals, Carlsbad, CA, USA.*

Biotechnology is a significant industry with over 300 compounds for the treatment of human disease in development by over 100 companies. Inherent in the design of these products is species specificity, and thus, testing in animals may indicate adverse effects unique to the animal model. Also, animal models will not typically identify potential exaggerated pharmacologic effects. Thus, selection of animal models is critical to the appropriate evaluation of effects relevant to humans. Typically, these compounds are evaluated in non-human primates because of the potential homology with humans. In addition, other novel animal models or testing strategies are adopted so that safety may be characterized to allow the ethical administration of these agents to patients. Toxicology testing strategies and regulatory issues for the evaluation of recombinant proteins, cytokines, gene therapy, and oligonucleotides will be presented for a few selected compounds. The information in this session should be of interest to individuals in the pharmaceutical and regulatory sectors. In addition, the methodology associated with the use of these agents will be useful to basic researchers.

186 ANTISENSE OLIGONUCLEOTIDES: TOXICOLOGY FROM MICE TO HUMANS.

D K Monteith, R Geary, S P Henry, M Templin, and A A Levin. *Isis Pharmaceuticals, Carlsbad, CA, USA.*

Antisense therapeutics are modified oligodeoxynucleotides (ODN) that have species and target specificity derived from the genetic code and Watson-Crick base-pairing. These antisense therapeutics demonstrate broad tissue distribution in animal models and cellular uptake. Toxicities and pharmacokinetics are independent of sequence (i.e. effects observed regardless of molecular target). Currently, most antisense ODNs in development contain a sulfur substitution for an oxygen in the nucleotide linkage resulting in a phosphorothioate (P=S) backbone. In general, toxicities of these compounds correlate well with pharmacokinetic or tissue distribution parameters. The preclinical testing strategy typically involves mice and primates. In mice, both the human and murine specific homologues are evaluated to assess general toxicities, as well as target specific effects of suppression of protein translation (i.e., exaggerated pharmacology). In many instances, the pharmacology models evaluate the efficacy of an animal specific antisense ODN. However, human tissue xenograft models with immune deficient mice can be used to evaluate the efficacy and toxicity of a human antisense ODN in an animal model. In clinical studies, P=S ODNs induce only minimal side effects as is predicted by the animal studies and the large margin of safety established in preclinical evaluations. Specific examples of preclinical testing strategies for antisense oligonucleotides targeting cancer and inflammation will be presented.

187 GENE THERAPY: CHALLENGES IN THE DESIGN AND INTERPRETATION OF TOXICOLOGY STUDIES.

M E I Leibbrandt. *Department of Toxicology, Chiron, Emeryville, CA, USA.*

The ability to deliver genes through retroviral, DNA and other vectors provides exciting opportunities for the management of disease while demanding creative approaches to safety evaluation of these novel agents. For example, gene therapies require assays which assess biodistribution of the gene or vector, activity of the final product, and its efficacy in animal models. Because

gene therapy can require targeting to and uptake by appropriate cell types, transport to the nucleus, integration into native DNA, mRNA expression and translation, protein processing and functional activity, there are numerous steps at which successful therapy may fail or inadvertent toxicity develop. Toxicity may be associated with the inappropriate expression of the gene product or with the vector particle itself. Laboratory methods such as PCR and in situ hybridization assays are standard tools for the safety evaluation of gene therapies. A retroviral vector gene therapy for hemophilia A will be used to illustrate unique issues in the design and interpretation of nucleic acid-based toxicology studies.

188 CONSIDERATION OF BIOLOGY IN THE DESIGN OF TOXICOLOGY STUDIES TO SUPPORT CLINICAL DEVELOPMENT OF NEUROTROPHIC FACTORS.

H Davis, D Miller, A Standeven, C LeBel. *Amgen, Thousand Oaks, CA, USA.*

Amgen is developing neurotrophic factors (GDNF, NT-3 and BDNF) for Parkinson's Disease, peripheral neuropathies (PN) and amyotrophic lateral sclerosis (ALS), respectively. Intracerebroventricular (ICV) GDNF improved behavioral performance in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-lesioned (MPTP) monkeys. The ICV route was chosen to target dopaminergic neurons in the brain, to avoid the blood brain barrier and to avoid potential systemic side effects from GDNF receptors outside of the CNS. Monkeys treated monthly with ICV injections of GDNF had transient vomiting and weight loss at 75, 500 and 3,000 10 g/month. GDNF caused inflammation in the CNS and Schwann cell proliferation secondary to axonal sprouting in the pia mater and dorsal nerve roots, but no systemic histopathology. NT-3 was efficacious in pyridoxine-, cisplatin-, and streptozotocin-induced PN in rats. Chronic studies were performed in the rat and monkey with neuropathological findings limited to the rat. Rats received NT-3 at doses of 2, 10, or 30 mg/kg/day subcutaneously for 3/6 months. Reductions in body weight and food consumption occurred in all NT-3 groups. Myelinated fiber degeneration in sciatic nerves and neurofilament swellings in dorsal root ganglion neurons occurred at 10 and 30 mg/kg/day. BDNF promotes survival of developing and adult motoneurons. BDNF was administered intrathecally (IT) for 28 days in dogs and monkeys. Dogs were more sensitive to IT BDNF and were chosen for chronic IT studies. Dogs were treated by continuous IT infusion of 100, 300 or 1,000 10 g/day for 3/6 months. Prolonged IT catherization caused gross and histological changes. BDNF was well tolerated but caused hyperactivity and hindlimb effects including twitching and tremors at 1000 10 g/kg. Understanding the molecular biology and pharmacology of GDNF, BDNF, and NT-3 was fundamental to the design and interpretation of data from these toxicology studies.

189 ISSUES AND STRATEGIES IN THE DEVELOPMENT OF RECOMBINANT HUMAN INTERLEUKIN-4 (rhuIL-4).

M W Leach. *Schering-Plough Research Institute, Lafayette, NJ, USA.*

Interleukin-4 (IL-4) is a potent immunoregulatory cytokine. Initial preclinical biology studies showed activity against tumors, and thus the initial clinical indication was cancer. As rhuIL-4 was biologically active only in primates, safety studies were conducted primarily in the cynomolgus monkey. Single and repeated dose toxicology studies, with daily administration for up to six months, were conducted. Genotoxicity and developmental toxicity studies were also performed. Mortality, cardiotoxicity (inflammatory cell infiltrates and necrosis), and hepatotoxicity (inflammatory cell infiltrates and necrosis) were the most significant findings observed during the preclinical toxicology studies. Although these preclinical toxicology findings were significant, initial clinical trials were conducted in patients with terminal cancer where the benefit/risk ratio was high. Based on the preclinical toxicology findings, these patients were required to undergo extensive monitoring, including endomyocardial biopsies. Administration of rhuIL-4 was generally safe and well tolerated in human patients. The significant toxicity observed in monkeys was not generally seen, allowing initiation of clinical trials in non-life threatening conditions. To further determine whether the mortality, cardiac, and hepatic changes in the monkey were related to a response to the heterologous rhuIL-4 cytokine, recombinant murine IL-4 was administered to three strains of mice (BALB/c, C57BL/6, CD-1) selected for their differing immune responses. Doses were selected to provide 1X and 10X multiples of the systemic exposure in monkeys that resulted in significant toxicologic changes in the monkeys. Administration of the homologous IL-4 cytokine to mice, even at extremely high systemic exposures, did not reproduce the most significant toxicologic changes observed in monkeys receiving the heterologous IL-4 cytokine. As humans receiving homologous (rhu) IL-4 also lack the significant findings seen in the monkey, the toxicologic changes in monkeys may be related to atypical immunologic responses to the heterologous IL-4 cytokine, and may not represent relevant risks to humans receiving homologous IL-4.

190 DEFINING RELEVANT ANIMAL SPECIES FOR TOXICITY EVALUATION OF BIOLOGIC THERAPEUTICS; CRITICAL GROUNDWORK FOR BASING PRECLINICAL TOXICITY PROGRAM STRATEGIES.

L E Black and M D Green. *Food and Drug Administration, CBER/OTRR, Rockville, MD, USA.*

Unlike the development programs for small molecule drugs which typically employ two or more species, one relevant animal species may be sufficient to characterize the toxicity of biologic therapeutics (BTs). However, the value of the data derived from a single species is dependent on establishing a scientific basis for extrapolating between the laboratory species and human patients. This talk will focus on BT toxicity programs where the availability or absence of this scientific basis aided or impeded clinical development. Numerous factors contribute to establishing the correlation between animal and human responses; among these are the species differences which may affect the presence of functional receptors, physiologic role of cytokines, endogenous inhibitory/regulatory pathways, and dispositional or pharmacokinetic factors. Interferons are a well known example of species-specific receptor binding. However, some cytokines, like IL-2, produce similar toxicity profiles across a number of species. In cases where cross-reactivity is broad, toxicity model selection may be guided by avoiding species in which the human drug product is highly immunogenic. Selection of a relevant animal model may involve in vitro binding and activity studies, in vivo response profiles, or transgenic models. When no useful animal species can be identified, toxicity studies with analogous products may be useful in evaluation of toxicity.

191 VALIDATION OF TOXICOLOGY TEST METHODS: IMMUNOTOXICOLOGY CASE STUDIES.

G F Gerberick and A E Munson[1]. *Procter & Gamble, Miami Valley Laboratories, Cincinnati, OH, USA; [1]NIOSH/HELD, Morgantown, WV, USA.*

In the US and Europe guidelines exist for evaluation and validation of new alternative test methods. These guidelines have been used as a framework for the assessment of novel methods which offer benefits in the context of the requirement for experimental animals with respect to Replacement, Reduction and/or Refinement. The objective of this Workshop is to use examples, primarily form the discipline of Immunotoxicology, to consider the strategies necessary to gain acceptance of novel test methods by regulatory authorities and to bring to the attention of the toxicology community the issues that need to be addressed in assay evaluation and validation. The current guidelines used for consideration of alternative methods will be reviewed with particular emphasis on those developed by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM). As a classic illustration of a validation exercise, the evaluation of a novel phototoxicity method, the 3T3 Neutral Red Uptake Phototoxicity Assay, will be discussed. In addition, a rather different approach used for the assessment of the local lymph node assay, a method for the identification of contact allergens, will be described and the current validation status of this method discussed. Finally, the validation needs of the methods currently available for the identification of potential immunotoxicants will be considered in the context of new and emerging regulatory requirements. The main goal of this workshop is to stimulate discussion between those who are applying modern science to the development of novel approaches to toxicity testing and those charged with evaluating those new methods to ensure the level and consistency of performance necessary for regulatory acceptance.

192 GUIDELINES FOR VALIDATION AND ACCEPTANCE OF TOXICOLOGY TESTING METHODS.

W S Stokes. *Environmental Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA.* Sponsor: G F Gerberick.

Innovative new alternative test methods offer the potential benefits of improved safety assessments, greater efficiency (less expense and time), and refinement, reduction, and replacement of animal use. Before new test methods are used to generate information to support regulatory decisions, validation studies must be conducted to determine their performance characteristics, limitations, and usefulness for specific proposed uses. These results are used to determine the acceptability of the method for regulatory risk assessment purposes. Criteria and guidelines for test methods to be considered adequately validated and acceptable for regulatory risk assessment purposes have been published in a recent report prepared by an ad hoc Federal interagency committee. An Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and an Interagency Center for the Evaluation of Alternative Toxicological Methods (Center) have been established by the U.S. government to work with federal agencies and test developers to coordinate the evaluation and adoption of new test methods. The Center and ICCVAM collaborate to provide guidance to agencies and developers on criteria and processes for test method validation and acceptance; coordinate technical peer reviews of proposed new test methods of interagency interest; facilitate information sharing among agencies; and serve as an interagency resource and communications link with test method developers. These guidelines and initiatives seek to encourage the development, validation, and acceptance of new methods that will provide enhanced protection of public health and the environment, and that will benefit animal welfare where scientifically feasible.

193 IN VITRO 3T3 NEUTRAL RED UPTAKE PHOTO-TOXICITY TEST (3T3 NRU PT): A COORDINATED VALIDATION PROCESS.

H Spielmann. *ZEBET (National Center for Validation and Evaluation of Alternative Methods to Animal Experiments) at the BgVV (Fed. Inst. for Health Protection of Consumers and Veterinary Medicine), Berlin, Germany.* Sponsor: G F Gerberick.

UV-absorbing chemicals are employed as ingredients in various cosmetic products. Until now an accepted protocol to test for photoirritation potential does not exist. In 1991 the EU Validation Centre ECVAM (Ispra, Italy) and COLIPA (Brussels), the European Cosmetic, Toiletry and Perfumery Association, agreed to establish a joint EU/COLIPA program on developing and validating in vitro phototoxicity tests. The first phase (1992-93) was designed as a prevalidation study to identify in vitro test procedures for a validation trial under blind conditions. In the second phase (1994-95) the most promising in vitro phototoxicity tests were validated with 30 carefully selected test chemicals in 11 laboratories in a blind trial, in which the 3T3 NRU PT test was carried in all laboratories. The purpose of the second phase was to confirm reliability and relevance of the in vitro tests to predict phototoxic effects and to identify photoirritant chemicals. Results obtained with the 3T3 NRU PT test in the second phase were reproducible and the correlation between in vitro and in vivo data was very high even for chemicals insoluble in water. Therefore, in 1997 ECVAM and in 1998 the EU Direction Generals III (industry) and IX (environment) (both Brussels, Belgium,) concluded from the formal validation study under blind conditions "that the 3T3 NRU PT test is well validated test that is ready to be used for regulatory purposes".

194 LOCAL LYMPH NODE ASSAY: AN APPROACH TO VALIDATION AND CURRENT REGULATORY STATUS.

I Kimber. *Zeneca Central Toxicology Laboratory, Macclesfield, England.*

The murine local lymph node assay (LLNA) is a method for the identification of chemicals which have the potential to cause skin sensitization and allergic contact dermatitis. Contact sensitizing activity is measured as a function of proliferative responses induced in draining lymph nodes by topical exposure of mice to the test chemical. The performance of the LLNA has been compared with that of guinea pig predictive tests (the guinea pig maximization test and the occluded patch test of Buehler) and with the human maximization test. In addition, the assay has been evaluated extensively in the context of both national and international inter-laboratory trials. These investigations have demonstrated that the LLNA provides a reliable means of assessing the skin sensitization potential of chemicals and yields equivalent results when performed in independent laboratories. Moreover, it is clear that the assay is sufficiently robust to embrace minor procedural modifications without performance being compromised. On this basis it is proposed that the LLNA is suitable for use as a 'stand alone' method for the evaluation of sensitizing activity and as such provides a realistic alternative to guinea pig methods, not only for the accurate identification of contact allergens, but also for confirmation of the absence of skin sensitizing potential. It is in this context that the regulatory status of the LLNA is currently being considered.

195 VALIDATION STATUS AND REGULATORY ACCEPTANCE OF LABORATORY ANIMAL-BASED TESTING METHODS TO ASSESS DRUG AND CHEMICALLY-INDUCED IMMUNOSUPPRESSION.

P T Thomas. *Covance Laboratories Inc., Madison, WI, USA.*

The impact of environmental chemical immune dysregulation is of particular importance to children and the unborn fetus. Furthermore, unintentional immunosuppression in non clinical safety studies can have a significant impact on the drug development process and on the design of human clinical trials. It is for these reasons that considerable attention has been paid in recent years to the predictability of laboratory animal-based immune function assays. In an early effort to determine the utility of incorporating functional assays of immunity and host challenge into standardized subchronic toxicology studies, the National Toxicology Program sponsored a multilaboratory validation exercise in which performance of over 15 functional tests and challenge assays was evaluated. Furthermore, to improve risk assessment, the degree to which immune function correlated with experimental infectivity or tumor challenge assays was determined. The results of evaluation of more than 50 compounds in carefully designed study protocols demonstrated that: (1) the degree of correlation between host challenge models and immune function assays was good; and (2) certain combinations of assays were reliable predictors of immunotoxicity in rodents when employed in screening protocols. The functional tests that comprise the screening batteries represent immunologically relevant endpoints that are common to both rodents and humans. Many of the compounds evaluated in this exercise, however, were known or potential carcinogens. Nevertheless, pharmacokinetic and toxicokinetic differences must be taken into account during interspecies extrapolation. The results of this and other studies will be discussed in the context of risk assessment and future validation needs will be identified.

196 ANIMAL MODELS OF CARDIOPULMONARY DISEASE: IMPACT OF AIR POLLUTION ON AT RISK POPULATIONS.

J T Zelikoff[1] and D L Morgan[2]. [1]*New York University School of Medicine, Tuxedo, NY, USA;* [2]*NIEHS, Research Triangle Park, NC, USA.*

The adverse effects of exposure to ambient air pollution are often manifested primarily in individuals within certain segments of the general population. One such group may be those with preexisting cardiopulmonary disease. For example, evidence from various disciplines strongly suggests that individuals with preexisting heart and respiratory conditions are more susceptible to ambient particulate matter. This may be due to differences in dosimetry, exposure patterns or the influence of various lesions upon biological response to inhaled pollutants. Studies using animal models which mimic human disease conditions are essential to obtain data for exposure and dose modeling in such susceptible subgroups, to examine the mechanisms by which such individuals may succumb to environmental insults and to understand host characteristics which place individuals at increased risk. Use of these models allows for studies which cannot be carried out in humans. However, an understanding of the similarities and differences between the animal model and the actual human disease state is essential in order to correctly interpret data obtained in such studies.

197 ANIMAL MODELS OF CARDIOPULMONARY DISEASE: ROLE IN AIR POLLUTION TOXICOLOGY.

R B Schlesinger. *Department of Environmental Medicine, NYU School of Medicine, Tuxedo, NY, USA.*

The adverse effects of exposure to ambient air pollution are often manifested primarily in individuals within certain segments of the general population. One such group may be those with preexisting cardiopulmonary dis-

ease. For example, evidence from various disciplines strongly suggests that individuals with preexisting heart and respiratory conditions are more susceptible to ambient particulate matter. This may be due to differences in dosimetry, exposure patterns or the influence of various lesions upon biological response to inhaled pollutants. Studies using animal models which mimic human disease conditions are essential to obtain data for exposure and dose modeling in such susceptible subgroups, to examine the mechanisms by which such individuals may succumb to environmental insults and to understand host characteristics which place individuals at increased risk. Use of these models allows for studies which cannot be carried out in humans. However, an understanding of the similarities and differences between the animal model and the actual human disease state is essential in order to correctly interpret data obtained in such studies.

198 MODELS OF ASTHMA/ALLERGY.

T Gordon. *NYU School of Medicine, Tuxedo, NY, USA.*

Considerable evidence has demonstrated that gaseous and particulate pollutants can alter the incidence or severity of asthma. Among the body's portals to the external environment, the mammalian respiratory system has the largest surface area. Correspondingly, a specialized and partially compartmentalized immune system has evolved for protection against inhaled microbes and particulate and gaseous agents. Several endogenous and exogenous factors could play a role in the acute exacerbation of asthma, which is characterized by reversible (typically) airway obstruction, inflammation, and airway hyperresponsiveness. Hence, an important issue to be considered in examining the effect of air pollution on allergic asthma is its effect on the localized immune system's response to inhaled antigen. This talk will discuss animal models that are appropriate for: 1) examining the potential of inhaled agents to produce or exacerbate asthma and 2) studying the mechanisms involved in pollutant induced allergic asthma. A number of investigators have studied air pollution induced alterations in immune effector cells in the lungs of mice, rats, and guinea pigs. Studies have also directly examined the effect of pollutants on specific allergic responses in these test animals. This talk will focus on the utility of each animal model and its relevancy to the human disease process.

199 MODELS OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD).

D L Costa. *Pulmonary Toxicology Branch, ETD/ NHEERL, USEPA, Research Triangle Park, NC, USA.*

Clinically, COPD encompasses an array of lung diseases that exhibit a pattern of chronic impairment of airflow, especially upon forced expiration. However, while asthma and fibrotic disease affect airway structure and function, the term COPD most often is reserved for chronic bronchitis and emphysema. These diseases alter airway function via distinct mechanisms, mucus obstruction and loss of structurally tethering, respectively. In the human, these conditions typically develop over decades and frequently coexist - often associated with smoking. In contrast, the animal models of these conditions are usually the result of an imposed injury or insult which relatively rapidly progresses to disease, and it is this pathophysiology which is often studied along with various investigations of therapeutic mitigation. Bronchitis models can be induced by protracted high level exposure to SO2 or subacute inhalation to LPS, both of which stimulate mucus secretion and goblet cell proliferation. This disease in animal models, however, is largely reversible. Emphysema, on the other hand, is typically irreversible (as in the human) induced by exposure of the lung to elastolytic enzymes which damage and promote abnormal remodeling of the alveoli with overinflation and loss of compliance. The elastolytic process is thought to be part of the human pathophysiology as well. Recently, genetic models that spontaneously develop emphysema have also gained interest. Collectively, these models have had limited applications in air pollution studies representing potential susceptible groups, but have yielded only marginal evidence of unusual sensitivity. However, recent attempts to combine these models to sustain an inflammatory component of disease appear to be both more representative of the human disease and more promising. (Does not reflect EPA policy.)

200 MODELS OF RESPIRATORY INFECTION.

J T Zelikoff. *New York University School of Medicine, Nelson Institute of Environmental Medicine, New York, NY, USA.*

The imposition of respiratory infections in otherwise healthy animals is often used in an attempt to reflect those disease processes in humans. A variety of infectious agents have been used to develop models of respiratory infections in rodents. Viral infectivity in rodents, achieved by a variety of methods including intranasal inoculation and/or intratracheal instillation with influenza, respiratory syncytial virus, and parainfluenza type 1, focus primarily upon pollutant induced changes in viral proliferation, clearance, and associated pulmonary injury, or pathology. Alternatively, bacterial infectivity models have long been used to demonstrate air pollution induced increases in bacterial infection and related mortality. Although not as common as viral infections in healthy individuals, respiratory bacterial infections can be quite pathogenic in humans. In general, infected rodent models develop symptoms similar to those in humans; however, the pathologic outcome in rodents usually tends to be relatively mild in comparison, possibly because of the absence of human predisposing factors. While bacteria such as Hemophilus influenzae, Pseudomonas aeruginosa, and Staphylococcus aureus have been used with the aim of assessing pollution induced changes in susceptibility to a subsequent infection, the majority of studies have employed Streptococcus species. Although most previously performed studies have involved pre-exposure to the pollutant followed by bacterial challenge, in light of more recent studies demonstrating the ability of particulate air pollutants to exacerbate ongoing infections, interest is increasing in examining the effects of pollutant exposure on previously infected hosts. (Supported by contracts from CIAR# 94 03 and HEI # 95.)

201 MODELS OF CARDIAC AND CARDIOPULMONARY VASCULAR DISEASE.

U P Kodavanti. *NHEERL, U. S. Environmental Protection Agency, Research Triangle Park, NC, USA.* Sponsor: D L Costa.

There is an intimate hemodynamic relationship between the heart and the lung, such that disease of either organ is likely to impair the function of the other. The pathogenesis of hypertension and cardiac disease leading to congestive heart failure typically involves an initial cardiac burden, adaptive remodeling of the vasculature, cardiac tissue damage, and ultimately heart failure. Just as systemic hypertension and congestive heart failure are associated with impaired pulmonary function, chronic obstructive pulmonary disease is a common cause of right cardiomegaly. A number of genetic, surgical and chemical-induced animal models are available to represent human cardiac and cardiopulmonary diseases, each with advantages and disadvantages in regards to their ability to mimic human pathobiology, their ready availability, or ease of induction. The extent of cardiac involvement is critical in defining susceptibility to air pollution-related mortality and morbidity in cardiopulmonary compromised humans: thus, animal models representing these diseases offer an opportunity to investigate plausible biologic mechanisms underlying their susceptibility. Recently, a monocrotaline-induced pulmonary vasculitis/injury model has shown particulate matter (PM)-induced alterations in cardiac function leading to mortality. On the contrary, a hamster model of congestive heart failure was shown not to be more susceptible to PM. We have used a genetically based systemic hypertension model to assess susceptibility to inhaled PM and noted that hypertensive rats exhibited increased vascular leakage and pulmonary hemorrhage, but not inflammation/inflammatory cytokine expression in the lung. Thus, the availability of cardiac and cardiopulmonary disease models opens an avenue to pursue not only the susceptibility issue but also to understand the overall systemic host responsiveness in disease pathogenesis. (This abstract does not necessarily reflect EPA policy.)

202 THE UTILITY OF DATA FROM ANIMAL MODELS OF CARDIOPULMONARY DISEASE FOR AIR POLLUTION RISK ASSESSMENT.

J L Mauderly. *Lovelace Respiratory Research Institute, Albuquerque, NM, USA.*

The Clean Air Act mandates that the National Ambient Air Quality Standards be set to protect health "with an adequate margin of safety". Congress noted that the standards should protect "particularly sensitive citizens". Examples of air quality standards strongly influenced by estimated

risks to individuals having cardiopulmonary disease include carbon monoxide (coronary vascular disease and angina), ozone (exercising asthmatics), and particulate matter (the elderly with cardiopulmonary disease). The precision with which we can judge risks to sensitive subpopulations from epidemiological data is limited, and human exposure studies of seriously compromised individuals are limited by ethical concerns. Validated animal models of human cardiopulmonary disorders are needed to bolster the mechanistic foundations for pollution health relationships, and may, with sufficient ancillary knowledge, aid in the development of quantitative estimates of risk. Toxicological data are reviewed in the setting of all standards, but few standards are based primarily on results from animals. Reliance on animal models requires validation that the model accurately mimics the human condition, or some important feature of the condition, knowledge of the comparative sensitivities of both normal and abnormal animals and humans, and a high level of confidence in dosimetric extrapolations.

203 POTENTIATION OF ENVIRONMENTAL TOXICANTS CONTRIBUTES TO FROG DEFORMITIES.

J G Burkhart[2], D J Fort[1], T L Propst[1], E L Stover[1], K Gallagher[2] and J C Helgen[3]. [1]*The Stover Group, Stillwater, OK;* [2]*NIEHS Research Triangle Park, NC;* [3]*MPCA, St. Paul, MN.*

Evaluation of water samples from reference sites and sites associated with the increased incidences of malformations in frogs has revealed that separate environmental factors isolated from sites with low rates of malformation were capable of potentiating the toxicity of water from affected sites when tested in *Xenopus*. C-18 concentration followed by methanol gradient elution of pond water from affected sites has produced fractions that induce abnormal embryo-larval development (organogenesis) and limb mal-development in *Xenopus*. Various organic compounds have been identified within those fractions. Similar treatment of water from reference sites produced fractions which did not induce toxicity. However, addition of these nontoxic methanol fractions isolated from the reference sites to unaltered pond water from the affected sites markedly increased the incidence rate and severity of deformities produced. Compounds have been identified in the potentiating fractions and little similarity exists with the chemicals identified in the affected sites. The primary composition of the potentiating fractions appears to be a series of sterols and phytoestrogen-like compounds. Because the potentiating fractions are also capable of interfering with advanced stages of metamorphosis, the potentiating factors appear to be interfering with normal endocrine activity, particularly in the thyroid axis. Overall, these results indicate that complex mixtures such as those found in the environment must be thoroughly evaluated for potential toxicant interactions.

204 SULFONYL UREA HERBICIDES DISRUPT NORMAL LIMB DEVELOPMENT AND TAIL RESORPTION IN *XENOPUS LAEVIS*.

D J Fort[1], T L Propst[1], R Rogers[1], E L Stover[1], D Rappaport[2]. [1]*The Stover Group, Stillwater, OK;* [2]*The Vermont Public Interest Research Group, Montpelier, VT.*

Two sulfonyl urea herbicides, sulfometuron methyl (CAS #074222-97-2) and nicosulfuron (CAS #111991-09-4), were evaluated for effects on limb development (day 0-30) and impact on metamorphosis in *Xenopus*. In these studies, the rate of tail resorption was monitored as a morphological biomarker of thyroid disruption. A 14-d test starting at stage 60 (ca. day 50) and continuing through stage 66 (ca. day 64) was performed. During the 14-day assay, the rate of tail resorption was monitored by video image capture and tail length digitization at stages 63-66. The NOAEC and LOAEC values for limb development following exposure either to sulfometuron methyl or nicosulfuron were 0.01 and 0.05 mg/L, respectively. Malformations induced by sulfometuron methyl included selective reduction deficiencies distal to the femur. Malformations induced by nicosulfuron were similar to those observed with sulfometuron methyl, but also included a general necrosis of the soft tissue (connective tissue) throughout the hind limb. For sulfometuron methyl, the NOAEC and LOAEC values for tail resorption inhibition were 0.001 and 0.01 mg/L, respectively. For nicosulfuron, the NOAEC and LOAEC values for tail resorption inhibition were 0.0001 and 0.001 mg/L, respectively. Addition of 100 μ g/L thyroxin partially reversed the inhibitory effects of both sulfonyl urea herbicides. Results from the studies clearly document the importance of evaluating longer-term developmental processes in amphibians to assess ecological hazard.

205 BIOMONITORING OF ACRYLAMIDE EXPOSURE BY IMMUNOASSAY.

J L Wong[1,2], Y T Zheng[1], G Gang[1], C H Tamburro[2], and F S He[3]. *Departments of* [1]*Chemistry and* [2]*Pharmacology & Toxicology, University of Louisville, Louisville, KY, and* [3]*Chinese Academy of Preventive Medicine, Louisville, KY.*

Acrylamide (AM) is a ubiquitous chemical used in the manufacture of suntan lotion, soaps, shampoo, cosmetics, in water treatment systems, etc. Concerns are raised by FDA, EPA, TSCA about its potential neuro, reproductive, and carcinogenic toxicity at presently detectable levels. A simple, sensitive and accurate immunoassay for biomonitoring acrylamide exposure via hemoglobin (Hb) adduct has been developed. This study's goal was to determine the relationship between the Hb-AM biomarker levels and health effects of AM by surveillance of acrylamide workers and suburban residents. A monoclonal antibody reagent is added to a microplate as the first layer of a sandwich immunoassay. Hemoglobin of subjects is added to the plate as a second layer of the sandwich with any Hb-AM present binding with the monoclonal antibody. An anti-human hemoglobin antibody coupled with multiple enzyme molecules is added as the third layer of the sandwich. This immunoassay was applied in the medical surveillance of exposed workers in Xinxiang, China factories specializing in polyacrylamides for oil field applications. In our preliminary double-blind study of 2 controls and 9 AM workers (3 with AM poisoning, 6 exposed but no immediate effect), the AM immunoassay identified the 3 workers with disease as having the highest AM level, and 2/6 exposed group who later went on to develop disease as having higher AM levels. Control had lowest levels of AM. Biomonitoring of diseased and exposed workers showed anticipated changes in AM levels over time. Screening survey of Louisville residents (USA volunteer controls) revealed lowest detectable AM levels without chemical disease. Biomonitoring of AM exposure by immunoassay provides safe, simple, and accurate means to document risk of exposure to a ubiquitous chemical used in daily activities both in the home and workplace.

206 AVIAN BIOMONITORING OF HEAVY METALS IN LITHUANIA USING IMMUNODETECTION FOR METALLOTHIONEIN.

B L Bukaveckas[1] and J M Steffen[1]. [1]*University of Louisville, Louisville, KY.* Sponsor: H E Hurst.

The newly independent state of Lithuania has a legacy of heavy metal contamination. The cause was primarily the widespread application of heavy metal rich mineral fertilizers. Secondarily, electroplating industry and military bases were coupled with no wastewater treatment facilities. Finally leaded gasoline is still in use in this country. We decided to use immunodetection of metallothionenin (Mt) for a pilot study of urban metal contamination using pigeons as the sentinel species. In the fall of 1996 we collected 20 pigeons (*Columba passerina*) from two cities in Lithuania, Klaipeda and Smyltina. Bird livers were homogenized in 1:4 weight to volume in ice cold Tris-HCl buffer pH 8.0, then centrifuged at 10,000g for 10 minutes. We measured hepatic Mt content of the supernatant using dotblots. Immunodetection was carried out using polyclonal Mt antibodies (Alpha Gamma Laboratories, Inc.). A standard Mt curve was generated on each membrane using mixed Mt I and Mt II. Mt ranged from 3.78e-4 to 3.10e-3 μ g/g per wet weight of liver tissue. This range is below what would have been able to be detected with standard Mt assays, and do not indicate acute metal intoxication of pigeons from these two study areas in Lithuania. However the study successfully demonstrated the usefulness of immunodetection of Mt in avian species for biomonitoring.

207 COMPARISON OF SOIL METALS ALONG BUSY THOROUGHFARES VS. RESIDENTIAL STREETS OF INNER AND OUTER NEW ORLEANS.

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A soil survey (SS-I) conducted in New Orleans in 1989-91 elucidated the environmental health impact of Pb accumulations in the city. This report describes a preliminary evaluation of soil survey (SS-II) begun in 1998. SS-II includes soil samples from busy thoroughfares along with soil samples collected along residential streets. This preliminary report (55 or 19.2% of 286 census tracts) compares soil metal results of busy thoroughfares versus residential street samples for communities in the inner city and outer city of New Orleans. All soil samples were air-dried, sieved (2 mm screen), shaker

extracted 5:1 (volume to weight) with 1 mol L^{-1} nitric acid for 2 hours at room temperature, centrifuged (1000 x g for 15 min.), and filtered (Qualitative P4 Filter Paper). Extracts were analyzed for Pb, Zn, Cd, Mn, and Ni using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Using categories established in Survey I, the data from busy thoroughfares and residential street samples were stratified into inner city and outer city groups. The non-parametric data are described as percentiles. The Wilcoxon Signed Rank Test is used to evaluate differences and similarities of each element in different parts of the city. In the inner city, significant differences do not appear between busy thoroughfares and residential streets. In outer city areas, significant differences do appear for Pb, Zn, Cd, and Ni between busy thoroughfares and residential streets. Except for Mn, significant differences appear between inner city and outer city busy thoroughfares and residential streets. In the inner city of New Orleans, Pb, Zn, Cd and Ni are more uniformly distributed and exhibit a significantly higher concentration than in the outer city census tracts. (Supported by ATSDR cooperative agreement # U50/ATU398948 to Xavier University.)

208 MODIFICATION OF TOLERANCE OF SENSITIVE FISH SPECIES TO BATH TREATMENT WITH HYDROGEN PEROXIDE.

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Hydrogen peroxide and its primary decomposition products, oxygen and water, are considered environmentally compatible. The apparent lack of adverse environmental impacts of hydrogen peroxide combined with its effectiveness as an antimicrobial agent makes this compound an excellent candidate for further evaluation in aquaculture. Hydrogen peroxide is currently being tested for its potential as a chemotherapeutant for a variety of external disease organisms of fish. Research in our laboratory has focused on delineating effective hydrogen peroxide dosing regimes for treating bacterial gill disease in rainbow trout and walleye. Results from target animal safety studies indicate that toxicity of H2O2 is dependent on species, age, dose and water temperature. Acute toxicity is evident from histopathologic inspection of gills (e.g. extensive epithelial lifting, necrosis) of fish treated with high doses of H2O2. The value of hydrogen peroxide as a therapeutic compound for use in walleye culture has been questioned due to the high sensitivity of walleyes to this compound at suggested treatment levels. Studies indicate that exposure of walleye to low levels of hydrogen peroxide would result in increased tolerance to subsequent exposure. Mechanisms involved in the modification of tolerance of fish to treatment with hydrogen peroxide (e.g. induction of antioxidant enzymes) are currently being investigated. This phenomenon of increased tolerance may be of value in the development of management strategies that will permit the use of hydrogen peroxide with walleyes and other sensitive species.

209 CALCIUM DEPENDENT ZN ACCUMULATION ON RAINBOW TROUT GILLS.

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This study measured and modeled the accumulation of zinc on the gills of rainbow trout because the gill is the site of toxic action. Zinc accumulation was lowest when fish were acclimated in hard water (Ca = 120 mg/L) and tested in hard water and highest when fish were acclimated in soft water (Ca = 8 mg/L) and tested in soft water. Intermediate Zn accumulation was observed when fish were either acclimated in soft and tested in hard water or acclimated in soft and tested in hard water. Total Zn concentrations in gills ranged from 20 to 55 µg/kg fish in the different treatments after 24 hours exposure to 100 µg ^{65}Zn/L. Using gill ligand constants for Ca and Zn, we modeled the gill-Zn concentration on the gill surface over time in the various exposure waters tested using the geochemical speciation program MINEQL+. The results of this study show that the concentration of calcium in water determines the toxicity and accumulation of zinc in fish through both changes in gill permeability and competition for binding sites.

210 USE OF H4IIE BIOASSAYS TO ESTIMATE THE CONTRIBUTION OF HALOGENATED ENVIRONMENTAL CHEMICALS TO TOXIC POTENCIES IN FISH EXTRACTS FROM THE LOWER FOX RIVER, WISCONSIN.

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The toxic equivalency (TEQ) approach to ecological risk assessment of environmental mixtures of halogenated aromatic hydrocarbons (HAHs) has been judiciously applied where aryl hydrocarbon (Ah) receptor-mediated toxicological responses have the potential for affecting reproduction in birds or mammals. The H4IIE rat hepatoma cell line has been used as a bioassay for estimating TEQs of environmental samples based on the responsiveness of this cell line to Ah receptor "active" compounds. Bioassay determination of TEQs has the advantage of accounting for the Ah activity of compounds that have not yet been identified analytically. H4IIE bioassays were used in the present study to estimate TEQs in fish caught from the lower Fox River, and together with analytically-derived TEQs, bioassay-derived TEQs were used to find the incremental contribution of certain classes of HAHs (e.g., polychlorinated biphenyls) to overall toxic potencies of fish extracts. TEQs were determined in H4IIE cell bioassays on the basis of the dose-dependent induction of CYP1A1-dependent ethoxyresorufin-*O*-deethylase (EROD) activity, and the responses observed with whole fish extracts were compared with the response produced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Fish extracts were prepared using methods that preserved to the greatest extent possible the range of Ah receptor active compounds which could reasonably be expected to be present in whole fish extracts from the study area. Analytically-derived TEQs were calculated on the basis of measured concentrations of individual HAHs and toxicity equivalency factors (TEFs) established previously in H4IIE cells. Comparisons were made for several fish species caught in various areas of the lower Fox River. Bioassay-determined TEQs were consistently higher than TEQs calculated with measured concentrations of coplanar and mono-*ortho*-coplanar PCBs, suggesting that the total TEQ in fish from the lower Fox River are the result of a mixture of compounds. Further studies will test reconstituted mixtures of coplanar PCBs to validate the comparisons among bioassay and analytically-derived TEQs.

211 MOLECULAR CHARACTERIZATION OF AN ARYL HYDROCARBON RECEPTOR IN A WHALE, THE BELUGA DELPHINAPTERUS LEUCAS).

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The aryl hydrocarbon receptor (AHR) is a cytosolic, ligand-activated transcription factor that mediates some, if not all, of the toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related polycyclic halogenated aromatic hydrocarbons (PHAH). Studies in mouse strains show that slight variations in the AHR sequence can drastically change their sensitivity to dioxin effects. Similarly, differences in AHR expression or function may contribute to species-specific differences in susceptibility to PHAH toxicity. Because of their high trophic level and physiological dependence on large lipid stores, odontocetes (toothed cetaceans) bioaccumulate some of the highest PHAH tissue concentrations reported. Despite this, very little is known about the effect of such burdens on cetacean health. To ascertain the potential for PHAH effect at the biochemical level, we cloned, sequenced, and expressed an AHR from beluga. The 3.2 kb cDNA encoded an 845 amino acid protein with a predicted size of 95.5 kDa. The amino acid sequence is 83, 78, and 71% identical to the human, rabbit, and mouse AHRs, respectively. Protein synthesized in a rabbit reticulocyte lysate system demonstrates specific, high affinity ^{3}H-TCDD binding in the velocity sedimentation assay. Western blotting with an anti-mouse AHR polyclonal antibody detected a band of expected size in cytosols prepared from beluga liver, lung, thymus, and spleen cytosols. No band was detected in heart, kidney, or testis. The lysate-expression system is an abundant source of functional beluga AHR protein that reduces the need for tissue samples that can be difficult to obtain. Using *in vitro* expressed proteins is a promising approach for addressing molecular and biochemical questions in endangered and protected species where logistical and ethical concerns preclude the testing of PHAH effects in live animals. (Supported by NIH E506272 and NOAA Sea Grant.)

212 CHRONIC DOSING STUDY TO ASSESS HEALTH AND REPRODUCTIVE EFFECTS OF TUNGSTEN-IRON AND TUNGSTEN-POLYMER SHOT ON GAME-FARM MALLARDS.

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In 1991, the United States banned the use of lead shot for waterfowl hunting because of its toxic effects on waterfowl and other wildlife species upon ingestion. Steel shot is used as a nontoxic alternative to lead, but there has been a continual effort to develop shot compositions that emulate the ballistic characteristics of lead. Shot composed of tungsten-iron and tungsten-polymer were given conditional approval for hunting by the U. S. Fish and Wildlife Service based on the results of a 30-day acute toxicity trial. The present study examined the health and reproductive effects of the 2 shot types on mallards over a 150-day period. Sixteen male and sixteen female adult mallards were dosed with 8#4 steel shot, 8#4 tungsten-iron shot, or 8#4 tungsten-polymer shot every 30 days over the 150-day period. An additional 6 males and 6 females received 8#4 lead shot. During the first 60 days of the trial, birds were fed a nutritionally-deficient diet (shelled-corn) and maintained in a cold environment. Birds were then switched to a commercial layer mash for the subsequent 90 days during which reproductive performance was examined. Tungsten-iron and tungsten-polymer shot had no deleterious effect on adult body weights, blood parameters, egg production, fertility, hatchability, and duckling survivability. Lead-dosed mallards had significantly decreased hematocrit, hemoglobin concentration and delta aminolevulinic dehydratase activity on day 7. The only pathological changes were observed in the liver and kidneys of lead-dosed birds. All lead-dosed birds died by day 25. The erosion rates of lead, steel, tungsten-iron and tungsten-polymer shot were 20, 55, 75 and 97% respectively. The results of this study indicated that game-farm mallards dosed with tungsten-iron and tungsten-polymer shot were not adversely affected during the 150-day trial.

213 IN VITRO TOXICITY EVALUATION OF SUMITHION USING CILIATED PROTOZOAN *PARAMECIUM CAUDATUM*.

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Experiments were designed to investigate the intrinsic toxicity of sumithion (Fenitrothion) o-o dimethyl o-4-nitro-3-methyl phenyl thiophosphate pesticide on ciliated protozoan Paramecium caudatum (Pc). The free living protozoa are valuable organisms for toxicological investigations as they possess at the same time the specific features of single cells and whole organism. Bioassay experiments were conducted to evaluate acute and chronic toxicity. Concentration of 10 ppm and above killed the Pc instantly . The calculated Lc 50 value of sumithion for three hours duration time was found to be 4.2 ppm for Pc. The cytotoxicity was evident from the progressive inhibition of growth in treated cultures of Pc and the growth inhibition was dose dependent. The role of contractile vacuole of Pc. As a visible marker of physiological responses to pesticide was investigated which provide additional information for assessing ecosystem viability and stress recovery. Variations in phagocytic activity was significant. The cytotoxicity of Pc was evident by macro nuclear aberrations when exposed to 4.2 and 1.0 ppm of pesticide. Treatment of organisms resulted in fragmentation, vacuolation, stretching of macronucleus and its degenaration. An intresting development concerning research in this field could be to investigate in detail the indicator value of ciliate species in toxicity evaluations.

214 MERCURY EXPOSURE, TOXICITY, AND MALARIA PREVALENCE AMONG BRAZILIAN GOLD MINERS.

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Introduction. The use of mercury to extract alluvial gold is widespread in Amazonia and other regions of Brazil, resulting in significant dermal and inhalation exposures to inorganic mercury. Malaria prevalence is also high in this region. Limited animal data suggest that mercury exposure may compromise the immune system, increasing host susceptibility to malaria. **Methods.** Cross-sectional information was collected for 135 persons at a gold mine in Para, Brazil. A clinical exam and medical histories were carried out, blood and urine specimens were obtained for mercury determinations, and current malaria infection status was assessed by thin smears. **Results.** Mean mercury levels in the blood (25.6 μ g/dl) and urine (21.5 μ g/dl) were elevated in this population, well above the World Health Organization reference value of 10 μ g/dl. Almost 20% of the study subjects were currently infected with malaria and over 50% reported a history of 5 or more lifetime malaria episodes. However, individuals with mercury levels of 15 μ g/dl or more in the blood and urine did not have a higher prevalence of current malaria infection or symptoms associated with mercury toxicity compared to those below 15 μ g/dl. Mean mercury levels were lower among those with current malaria infection compared to those who were not infected (blood: 22.1 versus 26.4 μ g/dl; urine: 20.9 versus 24.0 μ g/dl) but the differences were not statistically significant. Those who had been working less than one year at the gold mine had a significantly (p=0.03) higher prevalence of current malaria infection (24.6%) compared to those who worked for 1 or more years (9.6%). **Conclusions.** The results of this field investigation indicate that gold miners are at risk for elevated mercury exposures. In addition, in the lower Tapajos, gold miners are also at high risk for exposure to malaria. The higher prevalence of current malaria infection among those more recently employed at the gold mine may be due to malaria exposure in an immunologically naïve population. (Supported by Pan American Health Organization, W. Alton Jones Foundation, and the Heinz Family Foundation.)

215 EVALUATION OF REPRODUCTIVE ORGAN DEVELOPMENT IN CF-1 MICE AFTER PRENATAL EXPOSURE TO BISPHENOLA.

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The objective of this study was to investigate potential effects on male reproductive organs of low-dose exposure to bisphenol A (BPA) during prenatal development as previously reported by Nagel *et al.* [*EHP* 1997;105:70-76] and vom Saal *et al.* [*Tox. Ind. Health* 1998;14:239-260]. Groups of 28 females were given 0.2, 2.0, 20, or 200 μ g/kg/day of BPA in tocopherol-stripped corn oil, once daily by deposition in the mouth on Days 11 to 17 of gestation. An additional group of 28 females administered 0.2 μ g/kg/day of diethylstilbestrol (DES) and a group of 56 females administered corn oil vehicle were treated for the same time period. Following the exposure period, the dams were allowed to deliver and raise their pups. On Day 22 of lactation, the litters were weaned, and a maximum of 4 males/litter was retained until 90 days of age. No treatment-related effects were observed in adult females or on growth or survival of offspring from dams treated with any dose of BPA. There were no treatment-related effects of BPA on prostate weights, sperm count or daily sperm production, testis histopathology, or preputial gland, seminal vesicle, or epididymis weights in offspring at any dose of BPA. There were no effects on any parameter measured in dams or on the growth or sexual development of male offspring of dams exposed to DES during gestation. In conclusion, those findings previously reported by Nagel et al. (1997) and vom Saal et al. (1998) were not confirmed at doses equivalent to theirs and at an order of magnitude higher and lower. (The study was sponsored by The Society of the Plastics Industry, Inc. and the European Chemical Industry Council.)

216 EVALUATION OF REPRODUCTIVE ORGAN DEVELOPMENT IN THE MALE OFFSPRING OF FEMALE WISTAR RATS EXPOSED TO BISPHENOL A IN THE DRINKING WATER.

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The objective of this study was to evaluate potential effects of bisphenol A (BPA) on sexual development as previously reported by Sharpe *et al.* [*10th Int. Congress of Endocrinology*:1996; S23-4]. Groups of 28 female Han Wistar Albino rats were given drinking water that contained 0, 0.01, 0.1, 1.0 or 10 ppm BPA or 0.1 ppm DES, 7 days per week for 10 weeks. Treatment

of the females began at 10 weeks of age and continued throughout a 2-week premating period, 2 weeks of mating, 21-22 days of gestation and 22 days of lactation. After 2 weeks of exposure the females were mated to untreated males. Weanling males were given untreated drinking water and maintained until 90 days of age. No treatment-related effects on growth or reproductive endpoints were observed in adult females exposed to any concentration of BPA. Similarly, no treatment-related effects on the growth, survival, or reproductive parameters (including testes, prostate and preputial gland weight, sperm count, daily sperm production, or testes histopathology) of offspring from dams exposed to BPA during gestation and lactation were observed. DES administered in the drinking water at 0.1 ppm resulted in decreased body weight, body weight change and food consumption in adult females. An increase in the duration of gestation and a decrease in the number of pups delivered and number of live pups were also observed in animals exposed to DES. In conclusion, these results do not confirm the previous findings of Sharpe *et al.* and show that low doses of BPA had no effects on male sexual development in the rat. (The study was sponsored by The Society of the Plastics Industry, Inc. and the European Chemical Industry Council.)

217 THE INFLUENCE OF LIFETIME EXPOSURE TO DIETARY BISPHENOL A ON THE MALE RAT REPRODUCTIVE TRACT.

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Exposure to estrogenic compounds during critical periods of development have been implicated in the induction of abnormalities in the male reproductive tract and on fertility. Bisphenol A, the monomer used in the formation of several common resins and plastics, is one of the major environmental compounds shown to possess estrogenic activity. In this study, male Sprague Dawley CD rats were exposed to 20 and 200 ng bisphenol A per gram diet from conception until day 70 postpartum. Adult animals were found to consume 2.2 and 23.1 ug bisphenol A /kg body weight per day, respectively. Lifetime exposure to bisphenol A resulted in significantly reduced body weights from days 1–70 postpartum for all ages investigated. The number of male and female offspring, anogenital distances for both sexes, and age of testes descent were not significantly altered. The seminal vesicles and epididymis were increased in weight in a dose–dependent manner, and kidney and spleen weights were decreased for both concentrations. Dorsolateral and ventral prostates, and testes weights were not significantly altered compared to control animals. Sperm density and motility were decreased in a dose–dependent manner, although the decrease was not significant. Androgen receptor protein levels were increased significantly in the 200 ng/g concentrations of bisphenol A in the diet are subtle but significant.

218 LACK OF DEVELOPMENTAL/ REPRODUCTION EFFECTS WITH LOW CONCENTRATIONS OF BUTYL BENZYL PHTHALATE IN DRINKING WATER IN RATS*.

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There are conflicting reports on sexual development of male offspring from rats administered ultra low doses (1 ppm) of butyl benzyl phthalate (BBP) in drinking water during mating, gestation and lactating (Sharpe et al., Environ. Health Perspect. 103, 1136-1143, 1995; Ashby et al., Regul. Toxicol. Pharmacol. 26, 102-118, 1997). We investigated the effects of BBP using a dosing regimen identical to that used by Sharpe et al. (1995) with the following enhancements: higher number of dose levels and test animals per group, use of glass bottles for dosing solutions, complete analytical characterization of dosing solutions and evaluation of female pups through maturity. Groups of 28 female Wistar rats were given nominal concentrations of 0.1, 1.0 and 3.0 ppm BBP in the drinking water 2 weeks prior to mating, during mating, gestation, and lactation. At weaning, groups of approximately 80 male and female pups were placed on regular drinking water and food for a period of 90 days. During maturation, sexual development endpoints, preputial separation and vaginal opening, were followed. At necropsy, gonadal organ weights, and caudal and testicular sperm counts were obtained. Unlike the results of Sharpe et al. (1995), no treatment related changes in organ weights or epidydmidal or testicular sperm production counts were observed among male rats receiving BBP in utero and during lactation. No treatment-related changes were observed in the sexual development of females following administration of low doses of BBP. Our results on lack of low dose BBP testicular effects confirm the results reported by Ashby et al. (1997). *Sponsored by the European Coucil of Plasitcizers and Intermediates and the Phthalate Ester Panel of CMA.

219 LACK OF EFFECTS ON MALE REPRODUCTIVE PARAMETERS IN RATS BY PERINATAL DIETHYLSTILBESTROL (DES) EXPOSURE AT MATERNALLY TOXIC LEVELS IN DRINKING WATER*.

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A study by Sharpe et al. (EHP 1995;103:1136-1143) reported decreased daily sperm production and testes weights in male offspring of dams exposed to low doses of DES (0.1 ppm in drinking water), a well-characterized synthetic estrogen. In two independent studies groups of 28 female rats were given nominal concentrations of 0.1 ppm (study 1) and 0.05/0.01 ppm DES (study 2), respectively, in the drinking water 2 weeks prior to mating, and during mating, gestation and lactation. At weaning, groups of approx. 80 male pups were placed on tap water and food until an age of 3 months. The DES-dosed dams showed significant toxicity in terms of reduced food and water consumption, an increase in duration of gestation and a decreased number of live pups. In 3 month-old offspring of DES-dosed dams, however, no effects were seen on the relative weights of the reproductive organs, and no significant changes in epididymal or testicular sperm counts were observed. These results corroborate the latest findings reported by Sharpe et al. (EHP 1998;106:A220-221), and challenge the allegation of a special susceptibility of mammals to in utero exposure of low-dose exogenous estrogens. (*Studies sponsored by the European Council of Plasticizers and Intermediates, the Phthalate Ester Panel of CMA, and The Society of Plastics Industry, Inc.)

220 PRENATAL EXPOSURE TO ESTROGENIC ENDOCRINE DISRUPTING CHEMICALS (EDCs) AFFECTS SEXUAL DIFFERENTIATION OF THE RAT BRAIN.

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Sexual differentiation of the rat brain occurs in response to perinatal production of gonadal steroids and results in permanent changes to brain architecture and neuroendocrine function. The sexually dimorphic nucleus of the preoptic area (SDN-POA) in the hypothalamus is the most apparent morphological sex difference in the rat brain. The nucleus is 2.2-8 times larger in adult males than in adult females and this volumetric difference is entirely dependent on estrogen during a restricted perinatal period. Estrogen masculinizes the SDN-POA by preventing apoptotic cell death. We utilized this developmental event to examine the effects of EDCs. Pregnant female Sprague-Dawley rats were given (ip) chlordecone (5 mg/kg), bisphenol A (BPA; 5 mg/kg), or diethylstilbestrol (DES; 5μg/kg) on day 16 of gestation. Offspring were weighed and the anogenital distance (AGD) was measured on the day of birth (PN0) and on postnatal day (PN) 6. The volume of the SDN-POA was measured on PN6. Chlordecone and BPA treatment increased body weight in males and females on PN0 and PN6. DES increased body weight in males and females only on PN0. AGD was increased on PN0 and PN6 in males given DES or BPA and on PN 6 in males given chlordecone. As in earlier experiments, female offspring exposed prenatally to chlordecone were shown to have increased SDN-POA volume compared to controls. The present experiments indicate that these three EDCs have varying effects. Only chlordecone and DES have estrogen-like effects on the SDN-POA in females, while all three have estrogen-like effects on weight gain in pups and on AGD in males. (Supported by grants from the Heinz Family Foundation and a VA Merit Award to MMM and EKS.)

221 APOPTOSIS IN THE SEXUALLY DIMORPHIC NUCLEUS OF THE PREOPTIC AREA AND PUBERTAL DEVELOPMENT IN RATS EXPOSED TO BISPHENOL A DURING PRENATAL AND POSTNATAL DEVELOPMENT.

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Bisphenol A (BPA) is used to manufacture epoxy resins and polycarbonates. BPA can leach from these materials into food stuffs and be ingested in small quantities. BPA has weak estrogenic properties. CIIT researchers previously observed that maternal exposure to BPA at 320 mg/kg/day from gestation day (GD) 2 through postnatal day (PND) 10 increased the volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in the hypothalamus of female rat offspring. Apoptosis has been implicated in sexual dimorphism in this specific region of rat brains. In the present study, we examined whether BPA would decrease the incidence of apoptosis in the preoptic area of female offspring and affect pubertal development. The chemical was administered to pregnant Sprague-Dawley rats by daily gavage at 0, 3.2, 32, or 320 mg/kg/day from GD 11 through PND 21. F1 females were examined for the effects of BPA on the incidence of apoptosis in the SDN-POA and on puberty. Fresh-frozen brains collected from 10-day-old females exposed to 320 mg/kg/day perinatally were analyzed for apoptosis with an ApopTag® in situ apoptosis detection kit. Under the present experimental conditions, there was no significant difference in the percentage of apoptotic cells in the SDN-POA (n = 4; 4 female offspring from 4 different litters) between BPA exposed (0.51 ± 0.09%) and control animals (0.64 ± 0.18%). BPA treatment of the dams with 320 mg/kg/day did not alter the age or body weight at which vaginal opening or first ovulation in the F1 offspring occurred. These preliminary data indicate that other factors in addition to apoptosis may be involved in the previously observed nucleus-enlarging effects of BPA on the SDN-POA during the critical period of sexual differentiation of the rat brain. Studies are in progress to determine whether BPA affects normal reproductive functions following pubertal development.

222 TESTICULR DAMAGE IN MALE RATS FOLLOWING NEONATAL EXPOSURE TO NONYLPHENOLS (NPs).

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We have reported previously that exposure of neonatal male pups to NPs led to cryptorchidism, testicular atrophy and infertility. We further examined the effects of NPs on the histology and biochemistry of the affected testes. Newborn male pups were given NPs at 8.0 mg/Kg or vehicle only (controls) for 15 days. They were sacrificed at 31 days of age and also at maturity after several matings to establish if they were fertile. At 31 days of age, NP treatment resulted in lesser cellular differentiation of the seminiferous tubules (ST) and increase in intertubular space compared to controls. At maturity, NP-treated males showed varying degrees of changes in the affected testes. In the moderately affected ones, about 20-30% of their STs showed poorly differentiated germinal elements. Cell lineage was less organized. In most severe cases, all the STs from the affected testis showed complete lack of differentiation. The center of the STs was occupied by large vacuoles. These impairments in germinal element differentiation might be the primary cause for a number of the NP-treated males to have a lower epididymal sperm count and a lower % of sperms that are motile compared to control age-matched males. Examination of testes homogenates by zymogram analysis using SDS gelatin gels revealed 2 major forms (64-66kD and 50-52kD) of gelatinases. The 50-52kD form was greatly reduced or absent in the affected testis. Thus exposure of neonatal male pups to NPs might affect their testicular proteinases that might be associated with abnormal development (cryptorchidism) and differentiation of their germinal elements (lowering of sperm counts and reduction in the % of sperms that are motile).

223 TWO-GENERATION REPRODUCTION STUDY WITH *P*-TERT- OCTYLPHENOL (OP) IN RATS.

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OP is an important commercial intermediate used primarily for the production of OP ethoxylate surfactants. Recently, OP has been implicated as an "environmental estrogen" due to its weak binding to the estrogen receptor and related responses. To determine if OP presents a risk to reproductive health, a 2-generation reproduction study in CD rats was conducted, following U.S. EPA OPPTS Draft Guidelines (870.3800) with the addition of dorsal prostate weights in adults, and monitoring F2 females through vaginal opening and F2 males until age 111± 5 days to evaluate possible reproductive organ effects and measurement of sperm parameters. Dietary concentrations of 0, 0.2, 20, 200, and 2000 ppm were used. The high dose resulted in OP intake near or exceeding known liver metabolic capacity, and the 0.2 ppm dose was used to evaluate potential low dose effects. Treatment-related effects in adult F0, F1, and F2 animals were limited to reduced body weights and feed consumption at 2000 ppm. No effects on any reproductive parameters were observed in either generation. No effects on sperm measurements, estrous cyclicity, or reproductive organs were observed in adult animals. Pup body weights during lactation were reduced at 2000 ppm. Minimal organ weight changes in weanling animals were attributed to the high daily intake of OP at this age and related body weight effects. Minimal (≤ 1.5 days) delays in vaginal opening and preputial separation, attributed to body weight decreases, were observed in the F1 and F2 generations at 2000 ppm. Slight increases in anogenital distance (<0.1 mm) were observed in newborn F2 females at all OP doses without clear dose response and were not attributed to OP toxicity. The NOAEL for systemic and postnatal toxicity was 200 ppm and for reproductive toxicity was >2000 ppm. In conclusion, although OP has weak estrogenic activity in some screening assays, no estrogenic or reproductive effects occurred from dietary exposure to rats for two generations over a 10,000 fold dose range.

224 EFFECTS OF DIETARY GENISTEIN EXPOSURE DURING DEVELOPMENT ON SPRAGUE-DAWLEY RATS.

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Genistein (CAS #446-72-0) is a naturally occurring isoflavone which interacts with the estrogen receptor and with multiple other targets. Human exposure to genistein is predominantly through consumption of soy products, and genistein has been implicated as being of potential benefit in preventing cancers at multiple sites and many adverse effects associated with menopause. A dose range-finding study was conducted as a prelude to a multigeneration bioassay. Genistein was administered in a soy-free diet at 0, 5, 25, 100, 250, 625, and 1250 ppm to dams (5 per dose group) from gestation day 7. Litters were standardized to 4 pups per sex at birth. Exposure of pups continued through lactation and after weaning until postnatal day 50. Plasma levels in animals at sacrifice ranged from approximately 10 nM to near 10 μM. Body weight and food consumption showed a decreasing trend with a signifcant reduction at the highest dose. Relative liver, pituitary and preputial gland weights showed increasing trends, with significant effects at the highest dose. Relative ventral prostate weight showed a decreasing trend, with a signifcant effect at 1250 ppm. Femur length and length:body weight ratio, as well as eye opening and ear unfolding were also affected at 1250 ppm. In female pups, mammary gland ductal/alveolar hypertrophy and hyperplasia were observed at 625 and 1250 ppm, and similar effects were observed in males at 5 ppm. Estrous cycle morphologic abnormalities were observed at 625 and 1250 ppm, and abnormal ovarian antral follicles at 1250 ppm. Males showed decreased spermatogenesis at 1250 ppm. Genistein thus affected multiple estrogen-sensitive tissues under these conditions, with males showing effects at lower doses than females.

225 MODULATION OF STEROID RECEPTOR EXPRESSION IN THE PROSTATE AND TESTES OF RATS BY DIETARY GENISTEIN.

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Steroid receptors play critical roles in growth, development, and maintenance of the male reproductive tract, including prostate and testes. Genistein is a naturally occurring isoflavanoid that is primarily found in soybeans. It is known that genistein exerts its estrogenic/antiestrogenic effects primarily through binding to estrogen receptors α and β (ERα and β), and thereby may affect the growth and development of prostate and testes. The current study was undertaken to examine if exposure to dietary genistein through an extended period that included development modulates the expression of selected steroid receptors such as ERα & β, and androgen receptor (AR). Genistein was fed to pregnant rats from gestation day 7 (GD7) at 0, 25, 250,

or 1250 ppm. Exposure of pups continued through lactation and after weaning until postnatal day 63 (PND63). At sacrifice (PND63), ventral prostate (VP), dorsal prostate (DP), and testes were removed from male pups (n=5) for histopathological evaluation and measurement of selected hormone steroid receptors levels by Western blot. Serum testosterone (T) levels were measured by radioimmunoassay. No treatment related histopathological lesions were observed in prostate or testis at this age. Serum T levels were elevated in dose-dependent manner (except at 25 ppm). Western blot analysis indicated that protein levels of AR increased in DP at 25 and 1250 ppm. In testes, AR protein levels increased at 250 ppm and decreased at 1250 ppm. Protein expression of ERα exhibited a significant increase in the DP at 25 ppm and in the VP at 250 and 1250 ppm. In contrast, ERα levels in the testes were reduced at all doses in comparison to the controls. ERβ protein levels were significantly decreased in DP at all doses, increased in VP at 250 and 1250 ppm, and were unchanged in the testes compared to the controls. The overall findings of this study suggest that genistein acts in a dose- and tissue-dependent manner in modulating the expression of AR, ERα, and ERβ which may have important implications in male reproductive tract function and susceptibility to or prevention of prostatic or testicular carcinogenesis.

226 EFFECTS OF DIETARY GENISTEIN ON STEROID AND GROWTH FACTOR RECEPTORS INVOLVED IN MAMMARY GLAND GROWTH AND DEVELOPMENT.

B S Blaydes[1], A Dalu[1], J R Latendresse[2], and K B Delclos[1]. [1]*National Center for Toxicological Research and* [2]*Pathology Associates International, Jefferson, AR, USA.*

Growth and development of the mammary gland, including neoplastic growth, are dependent on complex interaction among steroids and peptide growth factors. Previous studies have shown that the soy isoflavone genistein binds to estrogen receptors α and β (ERα and β) and affects mammary gland growth and development. Genistein was fed to pregnant rats from gestation day 7 (GD7) at 0, 25, 250, or 1250 ppm. Exposure of pups continued through lactation and after weaning until postnatal day 77 (PND77). At sacrifice (PND77), mammary gland from female pups (n=8) were removed for histopathological evaluation and measurement of selected steroid and growth factor receptors protein levels by Western blot. Serum sex hormones (17β-estradiol (E_2), progesterone (P), and prolactin (PLN)) were also measured. In the 1250 ppm dose group, hyperplasia and hypertrophy of primitive lobules was observed. In comparison to controls, serum levels of E_2 were significantly reduced at 25 and 250 ppm. P levels were elevated at 25 ppm, but reduced at 250 and 1250 ppm. PLN was significantly increased at 1250 ppm. ERα, epidermal growth factor, and erbB-2 were significantly increased over control level at all doses. ERβ and progesterone levels were unchanged. These data indicate that dietary genistein exposure during organogenesis (GD7-PND77) influences levels of hormones, and hormone and growth factor receptor that are critical to mammary gland growth and differentiation in a manner that suggests possible enhanced susceptibility to mammary carcinogenesis.

227 ANALYSIS OF PUBLIC HEALTH IMPACTS ASSOCIATED WITH REMOVAL OF LEAD PAINT FROM NYC BRIDGES.

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The New York City Department of Transportation (NYCDOT) is responsible for 770 bridges in NYC. To maintain structural integrity, NYCDOT must remove weathered paint that no longer protects against corrosion; much of the existing paint contains lead. To assist NYCDOT in developing health-protective paint removal procedures, potential public health impacts associated with exposure to lead particles generated during abrasive blasting and other paint removal techniques were analyzed. Six "surrogate" bridges, representing the range of environments and bridge structures in NYC, were evaluated. Emissions were modeled reflecting normal daily releases and catastrophic containment failures. Street dust and exterior soil lead levels were calculated using modeled deposition of bridge repair emissions and background dust fall, taking into account dust removal *via* precipitation washout. Based on air, dust, and soil concentrations, the IEUBK Model and the Bowers *et al.* Model were used to evaluate steady state blood lead levels (PbB) in children and adults, respectively, and the O'Flaherty Model was used to evaluate transient exposures. Predicted maximum incremental increases to geometric mean PbB, assuming maximum worst case release scenarios without mitigation, were approximately 1 ug/dL for children and substantially less for adults. Assuming a maximum worst case release scenario, a child may experience a one-day maximum PbB above typical levels of concern, but this level would decline quickly. PbB increments would be far less for residents living more than a few hundred feet from a bridge. With implementation of inspection and oversight procedures, precautionary work practices, and immediate clean-up of any release, the predicted impact on steady state PbB would be negligible. Overall, this analysis indicated that the proposed containment and mitigation measures are adequately protective of human health.

228 ISSUES RELATED TO MODELING OF AGE-DEPENDENT ABSORPTION AND BIOKINETICS OF LEAD.

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The U.S. EPA utilizes two models to assess lead risks at Superfund sites: the IEUBK Model for Lead in Children, applicable at residential sites to children <7 yrs of age, and the Interim Adult Lead Methodology (IALM), applicable at non-residential sites, where adults, rather than children, are the primary receptors. The IALM uses a simplified biokinetic slope factor (BKSF) to relate the blood lead concentration (PbB) to absorbed Pb. Default values used in the model include: BKSF=0.4 μg/dL per μg/day, and absorption fraction for soil-borne Pb (AFsoil) = 0.12. An issue commonly encountered in site risk assessments is: How representative are the model defaults (BKSF, AF) for increasingly younger age groups (e.g., adolescents, teenagers)? Although an empirically-based answer to this question is currently beyond us, it is useful to consider how these two variables are being represented in models of lead biokinetics. Current models, including the forementioned, and models developed by O'Flaherty and by Leggett, predict (or assume) similar BKSFs in children and adults; thus, the models predict that higher Pb uptakes per kg bw in children compared to adults can result in similar PbBs in children and adults. This implies that other biokinetic variables are represented differently in child and adult models. Current models also assume a 2 to 5-fold higher AF in children compared to adults. Although this is supported by data in young children and adults, it is uncertain at what age the child converts to the less efficient absorption status of the adult. Relevant new data on age-dependency of AF may shed some light on this issue, however, from a risk assessment perspective, it remains unclear how far can we *press the envelope* in applying the EPA adult methodology and IEUBK models to the ages between childhood and adulthood. (Conclusions do not necessarily reflect the opinions or policies of U.S. EPA.)

229 USING BLOOD LEAD STUDIES TO ESTIMATE SOIL INGESTION RATES IN CHILDREN.

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Tracer studies have long been the only approach available to estimate soil ingestion rates. These studies suffer from serious, well known drawbacks, including brief duration, detection limit problems, and exogenous sources of the tracers. When a problem seems intractable, it can be helpful to address the problem from a completely different angle, sometimes called "triangulation." Using blood lead data has been attempted before in the US and elsewhere; new drawbacks arose, including bioavailability issues, presence of lead-based paint and other non-soil lead sources, statistical uncertainty, and measurement error. We have taken three data sets from Rocky Mountain mining communities in which careful environmental measurements have been made and paired with blood lead levels in resident children. Using regression analysis, the IEUBK model assumptions, in vivo and in vitro bioavailability tests (performed by EPA), elimination of children exposed to lead-based paint where possible, and a Monte-Carlo based approach to account for measurement error, we have produced estimates of mean soil ingestion ranging from 10 to about 80 mg/day depending on the base study and level of uncertainty. The estimates depend on the IEUBK model, however, and errors in that model will produce errors in the soil ingestion estimates. This approach is useful in broadening our understanding of soil ingestion rates to use in risk assessments for all chemicals in soils, not just lead.

230 CALCULATION OF SOIL PRGS FOR LEAD FOR INTERMITTENT ADULT EXPOSURE.

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Historical mining operations in the eastern end of the Oquirrh Mountains in Salt Lake County, Utah, have produced large amounts of mine wastes and tailings, and migration of these materials has contaminated significant areas of soils. Certain individuals, mainly adults (e.g., industrial workers, agricultural workers, or recreational visitors), may be exposed intermittently to lead and other metals in these wastes and soils. To assesses potential lead exposure, a physiologically-based kinetic model for lead (O' Flaherty 1997) was evaluated to determine whether it could be used to develop PRGs for intermittent exposure. However, attempts to use an electronic version of the model to assess exposures that vary on a daily basis did not yield useful output because of run-time computer errors. Further investigation, however, supports the use of US EPA's adult lead methodology for assessing intermittent adult exposure. The US EPA provided information that suggests a linear response in pseudo-steady state blood lead levels in adults subchronically exposed to lead intermittently from one to three times per week. Monte Carlo simulation indicates that exposure frequency is the most sensitive input, and soil ingestion rate is the next most sensitive input to the US EPA's algorithms. Using site-specific inputs for the gastrointestinal absorption fraction (about 0.06 based on measurements in young swine) and exposure frequency, the US EPA Region VIII developed a range of possible risk-based PRGs for lead in soil using the adult blood lead algorithms. Depending on exposure assumptions, these PRGs range from less than 2,000 mg/kg to over 10,000 mg/kg. The PRGs are intended to protect the fetus in an exposed pregnant female from elevated blood lead levels.

231 AN INTEGRATED MODEL OF BLOOD LEAD AND BONE LEAD DURING AND AFTER PREGNANCY IN LATINA IMMIGRANTS.

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Single equation models, such as the multiple regression analyses previously used to examine the relationship between maternal bone lead and post-pregnancy maternal blood lead, cannot adequately describe relationships among the various lead variables when the same factors simultaneously affect both blood lead and bone lead. We used a system of simultaneous equations known as structural equation modeling to construct a single model of the effects of bone lead on maternal blood lead in the third trimester of pregnancy and in the first months after delivery. Blood lead was measured by atomic absorption spectrometry with graphite furnace. Bone lead was measured at mid-tibia (cortical bone) and calcaneus (heel: trabecular bone) by K shell x-ray fluorescence at the time of postnatal blood lead measurement. We restricted our analysis to immigrant Latina women in South Central Los Angeles (N>300). Geometric mean (range) prenatal blood lead was 2.8 μg/dL (0.4-81), calcaneus and tibia mean (range) lead concentration were 8.5 μg/g (-30.1 - 66.4) and 6.6 μg/g (-33.7 - 62.2). Blood lead during pregnancy was the strongest predictor of blood lead after pregnancy, as expected. Calcaneus lead had significant and independent positive effects on both prenatal and postnatal blood lead, although the effect on prenatal blood lead was about 25% larger than its effect on postnatal blood lead. Although tibia and calcaneus lead were significantly correlated, tibia lead was positively associated only with prenatal, not postnatal blood lead. Increasing length of time resident in the US was associated with simultaneous decrease in both prenatal blood lead and calcaneus lead in a logarithmic fashion. After accounting for the effects of bone lead on prenatal blood lead, inadequate dietary calcium during pregnancy was related only to increased prenatal blood lead. These results suggest increased risk of higher blood lead during and after pregnancy in immigrant Latinas may be due to lead stored in bones before immigration.

232 A LEAD MODEL (ECOTOX) FOR FULL-AGE RANGE HUMAN EXPOSURE BUILT FROM HUMAN DATA.

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A biokinetic risk assessment full-age range model has been built from data on bio-indicators (including tooth lead and blood lead up to 60-80æg/dl) collected in Belgian children and adults with known exposure to Pb. Input in the exposure model of time-related factors (exposure and nutritional)and of contamination (food, water, air, dust,paint..) results in lead uptake estimates.The human body is represented by blood - and fluids in rapid exchange with it, soft tissues and several compartments for hard tissues (various types of bone and teeth). Adjustment to observed data points out that lead transfers rates between compartments are age-related. First order kinetics fit data up to the highest blood lead. An "impact" law relates blood Pb and lead contamination or uptake. This relation is applied to data in the literature for validation exercises. Calculated PbBlood deduced from chronic environmental exposure differ from observed blood lead by less than 5% . Curvilinear relations (for example PbBlood vs PbAir) are explained by reference to exposure factors rather than by the order of lead kinetics. The model also explains the occurrence of peaks and plateaus in blood lead. Among other capabilities, the model also assesses the release of Pb from hard tissues vs storage, or others lead fluxes in- and out the body compartments, for any exposure history and at any time before reaching the steady state (SS). Response time (R), defined for each compartment, is the time required to reach a given % of the value that would be reached at SS. The time to reach critical values is estimated, and is shorter in children than in adults. It introduces new variables of interest for dose-effects studies,such as the dose to target tissue or compartment. This, and the higher transfer rates in early childhood have important implications for assessing risks in groups more vulnerable to Pb such as children.

233 SPATIAL DISTRIBUTION OF EEG THETA ACTIVITY AS A FUNCTION OF LIFE-TIME LEAD EXPOSURE IN NINE-YEAR OLD CHILDREN.

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Relative power of EEG theta activity (4-8 Hz) reaches a maximum in the first years of life, and generally declines at most scalp locations with increasing age. Children with attention deficit disorder show higher relative power of theta than age-matched controls. To determine how lead exposure might alter the relative power of theta in children participating in the Mexico City Prospective Lead Study, we measured eyes-closed, resting EEG for 60 seconds at 18 scalp electrode locations placed according to the 10-20 system. Informed consents were obtained. Spectral analysis of digitized data with a fast Fourier transform yielded power of theta relative to total EEG power. Lead exposure histories were calculated as mean blood lead levels for 12-36 week maternal concentrations (every 8 weeks during pregnancy) for prenatal exposure, and mean 18 month blood lead levels (every 6 months) from 6 to 96 months for postnatal exposure. Mapping of relative theta power across the scalp as a function of developmental stage of lead exposure was evaluated by spatially weighted multiple regression using Monte Carlo sampling, adjusting for chronological age at EEG testing and presence or absence of fetal suffering at delivery. Although most blood lead indices showed a significant positive effect of lead on theta at many electrode derivations, the most significant ($p<.001$) spatial gradient of lead effect on theta was with averaged blood lead between 54 and 72 months of age (blood lead range 5.0-26.2 μg/dL). The regression coefficient of theta on blood lead increased significantly in a frontal to occipital gradient, while there was no significant spatial gradient of theta on chronological age or on fetal suffering. EEGs of children with low to moderate blood lead levels resemble those of children with attention deficit disorder and the effect of lead is strongest in posterior (visual) cerebral areas.

234 REDUCTION OF ELECTRORETINOGRAM AMPLITUDE IN THE DARK-ADAPTED EYE OF CHILDREN ASSOCIATED WITH PRENATAL AND POSTNATAL BLOOD LEAD LEVEL.

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Early, low-level lead exposure has been associated with alterations in rod-mediated retinal function in animal preparations from rat to monkey. The electroretinogram (ERG) provides information regarding retinal function across species including humans. We recorded ERGs from children in the Mexico City Prospective Lead Study aged 7-10 years to determine the effect of prenatal lead exposure on retinal function. Informed consents were obtained. Subjects were dark-adapted for 40 minutes, Jet contact lenses were placed, and ERGs evoked by 5 consecutive white light flashes in a ganzfeld were averaged at each of 9 ascending flash intensities varying over a four common log luminance range. Interflash interval and intertrial interval increased over the series to reduce adaptation effects. Peak amplitudes of averaged a-wave and b-wave were measured by cursor placement for each stimulus intensity and a-wave - b-wave difference was calculated. Maternal blood lead level at 36 weeks gestation (range 1.0-32.5 μg/dL) was the only blood lead measure significantly associated with a reduction in ERG amplitude in multiple regressions adjusted for chronological age, and serum vitamin A and zinc levels. The association was apparent for each luminance level at which an a-wave and b-wave amplitude could be measured (3.6 - 1.0 log unit flash filtration). Across the range of blood lead levels the reduction in ERG a-wave - b-wave amplitude was 25%. The significant lead effect is temporally coincident with the rapid retinal development that takes place in the third trimester of pregnancy. Detection of the effect as late as middle or late childhood suggests that the effect of prenatal lead exposure on retinal response may be permanent.

235 BIPHASIC EFFECT OF PRENATAL BLOOD LEAD LEVEL ON BRAINSTEM AUDITORY EVOKED RESPONSE IN CHILDREN AT FIVE YEARS.

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Most, but not all, studies examining the effect of lead exposure on brainstem auditory evoked response (BAER) in children find significant associations between prior blood lead level and intervals measured between various peaks of the response. An earlier publication from the Mexico City Prospective Lead Study reported that increasing maternal blood lead level at 20 weeks gestation was most strongly related to increasing Peak III - Peak V interval in infants of about one month of age. We measured BAERs in children between 57 and 78 months from the same study. Biphasic clicks at 80 dB HL were monaurally delivered to each ear (contralateral ear masked with noise at 30 dB HL). We averaged 2048 responses from vertex electrodes referenced to linked mastoid to two separate click sequences presented at 11/sec. Latencies to Peaks I, III, and V were measured by cursor placement, and interpeak intervals were calculated. Maternal blood lead level at 20 weeks gestation was related to Peak III - Peak V interval, and Peak I - Peak V interval in a quadratic orthogonal polynomial regression, adjusting for chronological age and head circumference at recording. Peak intervals decreased significantly ($p=.004$) with blood lead levels between 1 and 8 μg/dL, then increased significantly ($p=.004$) with blood lead levels from 8 to 33 μg/dL. The quadratic regression was a significantly better fit to the data ($p=.009$) than was a linear regression. Brainstem auditory structures in humans undergo rapid development at mid-pregnancy. Though the biphasic nature of the lead effect on BAER may have several interpretations, the persistence of the effect of mid-pregnancy lead level on BAER beyond five years of age suggests a long-lasting effect of prenatal lead exposure on auditory brain response.

236 EFFECTS OF IN VIVO VERSUS IN VITRO Pb ON BRAIN PROTEIN KINASE C (PKC) ACTIVITY.

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Previously, we reported that Pb increased PKC activity in the brain of Pb-exposed rats (Toxic. Sci. 1998. 42:198.). Addition of an equivalent amount of Pb acetate in vitro to brain homogenates from those rats resulted in a greater than expected increase in PKC activity in both membrane and cytosol fractions. This lead to the hypothesis that in vivo (endogenous) Pb was less biologically available compared to Pb added in vitro, possibly as a result of differences in Pb complexation. Here we investigated differences in the subcellular partitioning in rodent brain of Pb delivered in vivo (i.e., oral exposure to the animal) versus in vitro (Pb added to brain homogenate), using a sensitive stable Pb isotope tracer method that allowed us to distinguish between in vivo Pb versus in vitro Pb. Animals were administered Pb-acetate from birth to 34 days age, sacrificed, and brain immediately removed for PKC analyses, as previously reported. Here, tracer ^{204}Pb (exogenous Pb) was added to brain homogenates from those animals in amounts comparable to the Pb present in the tissue. Homogenates were separated into pellet and cytosolic fractions by centrifugation at 40,000x g, and the cytosol further partitioned into <3,000 MW and >3,000 MW fractions using Amicon centrifugation. All fractions were analyzed for in vivo Pb and in vitro ^{204}Pb tracer using trace metal clean techniques and ICP-MS. The ratio of in vitro/in vivo Pb in the starting homogenate (0.82) compared to the cytosol (1.04) and pellet (0.18) indicate that a substantially greater proportion of in vitro Pb partitioned in the cytosol veers pellet, as compared to in vivo Pb. No differences in partitioning between in vitro versus in vivo Pb were seen within the cytosolic fraction (i.e., < or > 3000MW). These results are consistent with our previous observations that Pb added in vitro had a greater effect on increasing brain PKC activity than the Pb present in vivo. They also may in part explain existing discrepancies in the effects of Pb on PKC reported from studies using in vivo versus in vitro models. (Supported by NIEHS Grant #ES07535.)

237 EFFECT OF PRENATAL LEAD EXPOSURE ON COCAINE CONDITIONED PLACE PREFERENCE IN RATS.

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Prenatal lead exposure in children and animal models is associated with neurodevelopmental delays, learning deficits, and behavioral problems. In rodents, pre- and postnatal lead exposure is known to affect several neurotransmitter pathways in the CNS, specifically the release of dopamine, dopamine transporter proteins, and certain dopamine receptors (D2). These mechanisms have been associated with changes in arousal, activity, and learning. In addition, rodents exposed to lead perinatally show altered responses to several drugs that act on dopaminergic pathways (d-amphetamine, apomorphine, methylphenidate, haloperidol). We tested the interactions between prenatal lead exposure and later response to cocaine. Rats were exposed through pregnancy to lead via drinking water (25 ppm). This dose did not affect gestation, litter size, sex ratio, postnatal survival, or body weight in offspring. Pups were cross fostered to avoid postnatal exposure via lactation. As adults the F_1 offspring were trained on a conditioned place preference task, using 3, 6, or 12 mg/kg cocaine (i.p.). Prenatal lead exposure altered performance on this task. Male pups were less able to detect cocaine at the lowest dose, while females had an enhanced ability to detect cocaine. The results are consistent with the involvement of dopamine transporters and receptors in lead neurotoxicity. (Supported by a grant from the Heinz Family Foundation (EKS).)

238 EXPRESSION OF HEAT SHOCK PROTEIN 27, 60, AND 70 IN BLADDER FROM CONTROLS AND PATIENTS WITH INTERSTITIAL CYSTITIS.

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Interstitial cystitis (IC) is a chronic inflammatory bladder disease of unknown etiology. Several causes of IC have been proposed including toxic chemicals, autoimmune reactivity, hypersensitive response, defects in

urothelial cell permeability barrier and infection. A major intrinsic cellular mechanism for protection against stress is provided by the heat shock proteins (hsp) which are induced by a wide range of stimuli. This study examines the expression of hsp 27, 60 and 70 in normal human bladder and in bladder specimens obtained from patients with IC. Immunohistochemical methods were utilized to determine the localization of hsps in paraffin-embedded bladder specimens. Western blotting and reverse transcriptase polymerase chain reaction were used to confirm gene expression in normal bladder tissue samples. In normal bladder, the expression of hsp 27 was high in the transitional epithelium whereas the expression of hsp 60 was moderate. Heat shock protein 70 was present only in basally located transitional epithelial cells. The expression of hsp 27 was not altered significantly in the transitional epithelium of IC patients, though its intensity increased in smooth muscle cells and vessel walls of IC bladder specimens. In patients with IC, there was reduced expression of hsp 70 in basal transitional epithelial cells. The expression of hsp 60 was absent in the urothelial cells or smooth muscle cells from patients with IC. These results suggest that hsp 70 and 60 are down regulated in the bladder or patients with IC.

239 THE SUPPRESSIVE EFFECTS OF CORTEX MORI ON NO, TNF-α AND IL-1 PRODUCTION BY MACROPHAGE.

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Cortex Mori (*Morus loba L.*), the root bark of mulberry tree has been used as an autiphlogistic, diuretic and expectorant in herbal medicine. Recently, a few papers reported that phenolic extract of Cortex Mori had the hypotensive, hypolglycemic, antiviral and anticancer effects, and hot water extract of Cortex Mori had inhibitory effect on the degranulation and histamine release from activated mast cells. These previous studies suggest a possibility that CM has an antidotal activity against inflammation which was mediated mainly by macrophage-secreting inflammatory factors. This study was performed to evaluate the influences of Cortex Mori on carrageenan-induced edema *in vivo* and release of inflammatory mediators such as NO, TNF and IL-1 by macrophages stimulated with LPS or IFN-γ *in vitro*. Subcutaneous njections of carrageenan into the mouse paw rapidly induced local edema by increasing vascular permeability, but single intraperitoneal injection of CM extract at 30 minutes before carrageenan suppressed the development of edema. NO- and TNF production from macrophage stimulated by LPS or INF-γ were significantly suppressed, especially TNF secretion by up to 3-4 folds. LPS stimulated IL-1 production was also inhibited, but not significantly. Cell viability assay verified that the inhibition was not due to general cell toxicity. These results suggest that reduction of NO, TNF and IL-1 production may be one of the means by which Cortex Mori prevent inflammation associated diseases.

240 DECREASED RESPONSE TO ENDOTOXIN CHALLENGE BY PRE-TREATMENT WITH ERGOTAMINE.

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The objective of this experiment was to investigate whether the ergot alkaloid, ergotamine (ET), an alkaloid used to model fescue toxicosis in cattle, increases the response of cattle to endotoxin (LPS) challenge. Steers (n=16) were divided into the following treatment groups: control (C), ergotamine (ET), endotoxin (LPS), and ET+LPS. ET and ET+LPS groups received a single bolus i.v. injection of ET (40 μ g/kg/BW), whereas C and LPS steers received a single bolus injection of sterile vehicle. Thirty minutes after ET/vehicle administration, a single bolus i.v. injection of LPS (0.2 μ g/kg/BW) was given. Blood was collected at various time points for 48 h post LPS. Endotoxin alone increased the circulating levels of tumor necrosis factor alpha (TNF-α), cortisol, the acute-phase protein haptoglobin (Hp), thromboxane B_2 (TXB_2), and rectal temperature (RT), and decreased plasma glucose and insulin-like growth factor-1 (IGF-1). Importantly, haptoglobin, TNF-α , and TXB_2 increases were blunted by pretreatment with ET compared to LPS. The combination ET+LPS resulted in hyperglycemia followed by profound hypoglycemia. Ergotamine alone, increased RT, plasma urea nitrogen, packed cell volume and glucose and decreased serum prolactin (PRL). Therefore, administration of LPS to steers resulted in a typical metabolic and endocrine response. The combination of ET+LPS attenuated major effects of LPS alone. Thus, acute administration of ET appeared to be anti-inflammatory as it decreased the inflammatory response to LPS.

241 PLASMA INTERLEUKIN-10 AND NITRATE/NITRITE CONCENTRATIONS IN THE HUMAN ENDOTOXIN MODEL.

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I.v. administration of *E.coli* lipopolysaccharide (LPS, U.S. Standard Reference Endotoxin, CC-RE-Lot 2) to healthy volunteers has been used as a safe reproducible model of sepsis. To further characterize this model, studies were performed to evaluate patterns of plasma interleukin-10 (IL-10) and products of nitric oxide metabolism. **Methods**: To elicit the inflammatory response, two doses of LPS (20 EU/kg) were administered on two consecutive days to 15 men, and clinical responses (temperature/pulse) were monitored and compared to responses after a saline injection. At 4 time points after saline and 10 time points after the LPS injections, plasma IL-10 and NO_2/NO_3 concentrations were assayed. TNF-α and IL-6 were also assessed. **Results:** Expected clinical signs after each LPS injection were observed with mean temperature rise of 3.1 ±1.0 °F and pulse rise of 30 ± 9 bpm. Peaks of IL-10 concentration were found at 3.5 hr after each dose of LPS (176 ± 36 and 198 ± 30 pg/ml respectively). Baseline IL-10 after saline ranged from 32 to 60 pg/ ml. Unlike TNF-α and IL-6, the IL-10 peak after the second LPS dose was higher than after the first dose. Plasma NO_2/NO_3 profiles showed approximately 2-fold elevations after LPS with a delayed peak of 74 ± 12 μM at 12 hr after the first dose and an earlier 3 hr peak of 75 ± 13 μM after the second dose. NO_2/NO_3 concentrations after the saline injection ranged from 24 to 40μM. **Conclusions:** These data are the first to characterize the increases in plasma IL-10 and NO_2/NO_3 in the human endotoxin model of sepsis.

242 PERSISTENT PULMONARY INFLAMMATION AFTER INTRATRACHEAL INSTILLATION OF ABRASIVE BLASTING AGENTS.

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Use of silica sand in abrasive blasting is associated with pulmonary disability due to silicosis. However, the pulmonary toxicity of substitutes for silica sand in abrasive blasting remains incompletely investigated. Therefore, Sprague Dawley rats received a single intratracheal instillation (IT) dosage of 2.5 or 10 mg of the respirable fractions of abrasive blasting agents collected from the air during blasting of a steel bar. Four weeks post-IT, the right lung lobe was lavaged to isolate bronchoalveolar lavage cells and acellular bronchoalveolar lavage fluid (BALF); the left lung was processed for histopathology. Polymorphonuclear leukocyte (PMN) cell yield and alveolar macrophage (AM) chemiluminescence (CL) were significantly increased above control levels in rats instilled with coal slag (2.5 and 10 mg), silica sand (10 mg), staurolite (10 mg), and treated sand (10 mg). Rats which received garnet (10 mg) also had increased PMN numbers versus control but AM CL was not increased. Lactate dehydrogenase (LDH) was significantly elevated in the BALF of rats instilled with coal slag (2.5 and 10 mg), garnet (2.5 and 10 mg), silica sand (2.5 and 10 mg), staurolite (10.0 mg), and treated sand (10 mg), whereas BALF albumin was not increased in any of the exposed rats versus control. Hydroxyproline was significantly elevated over saline controls only in the rats receiving coal slag (10 mg). However, trichrome stained lung sections showed focal to multifocal, minimal fibrosis in 6 of 6 silica exposed rats (10 mg) and 3 of 6 coal slag exposed rats (2.5 and 10 mg). Only specular hematite (iron oxide) failed to increase some measure of pulmonary toxicity.

243 EFFECTS OF OZONE EXPOSURE ON CYTOKINE EXPRESSION IN HUMAN NASAL EPITHELIAL CELLS.

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Ozone is an oxidant pollutant gas that has been shown to induce adverse respiratory effects in humans, including airway inflammation. Although the mechanisms are poorly understood, airway epithelium is thought to play an active role in ozone-induced inflammation, since it is one of the first cellular targets to come into contact with inhaled ozone and/or its free radical

products. The present study investigated the mechanism of this inflammatory response. Cultures of human nasal epithelial (HNE) cells were initiated from excised nasal turbinates and exposed to 120, 240, or 500 ppb ozone for 3 hours. Basal media was then collected for tumor necrosis factor alpha (TNF-α) quantification by enzyme-linked immunosorbent assay. Mean TNF-α concentration in cultures exposed to 120 ppb ozone (10.34 ± 1.32 pg/ml) was not significantly different than in those exposed to air (11.01 ± 1.10 pg/ml). Exposure to 240 and 500 ppb ozone significantly increased mean TNF-α expression relative to controls by 75% (from 34.25 ± 4.39 pg/ml to 59.77 ± 8.72 pg/ml) and 28% (from 30.54 ± 4.61 pg/ml to 39.25 ± 7.42 pg/ml), respectively. These findings support the active role of HNE in ozone-induced airway inflammation and warrant further studies that delineate the interaction between ozone and inflammatory signal transduction molecules. Examples include possible ozone-induced activation of NF-κB, a transcription factor known to upregulate TNF-α expression. (Supported by NIH grant ES07033.)

244 AP-1 BINDING ACTIVITY IS ALTERED IN LPS-TREATED TNF ALPHA (p55/p75) DOUBLE RECEPTOR KNOCK-OUT MICE.

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It is well established that pro-inflammatory mediators of LPS induction of the acute phase response (APR) include interleukins (IL), interferons, and tumor necrosis factor alpha (TNF). Using TNF p55/p75 double receptor knockout mice (DKO), the role of TNF on transcription factor activation during the APR was examined. DKO and C57BL6 mice were administered LPS (2 mg/kg, i.p.) or saline and euthanatized 2 or 24 hours following LPS administration. Sera and liver tissue were then collected and analyzed for cytokine levels (sera) and transcription factor activation (liver). Serum cytokine levels were determined using direct ELISA. Serum levels of IL-1, IL-6, and TNF supported induction of the APR. Hepatic nuclear extracts were isolated and DNA binding to STAT3, $NF_{\kappa}B$, and AP-1 elements was analyzed using EMSA. The binding activity for STAT3 and $NF_{\kappa}B$, was increased with LPS treatment, but did not differ between DKO and C57BL6 mice at either time point. However, AP-1 binding activity was decreased in the DKO mice at 2 and 24 hours. The dampened AP-1 binding activity in the DKO mice may be explained by disruption of the TNF/SAPK/MAPK signalling pathway. The ultimate step in this pathway is the phosporylation of c-Jun; part of the dimeric complex of AP-1. These results support the role of TNF in the signalling pathways necessary for AP-1 activation following LPS administration.

245 JP-8 JET FUEL INDUCED RELEASE OF PRO-INFLAMMATORY CYTOKINES IL-1β AND IL-6 FROM RAT ALVEOLAR TYPE II CELLS IN CO-CULUTRE WITH RAT ALVEOLAR MACROPHAGES.

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Jet propulsion-8 (JP-8) jet fuel has been shown to induce lung injury after inhalation, however the mechanism of injury still remains unknown. Previous work conducted in our laboratory shows that aerosolized JP-8 jet fuel causes increased lung permeability and changes in lung compliance and resistance in an animal model. The purpose of this study focused on the interaction of the alveolar type II cell and the resident immune cell in the lung, the pulmonary alveolar macrophage (PAM). RLE-6TN cells (RLE cells), a rat alveolar type II epithelial cell line, were grown to confluence, dosed with JP-8 jet fuel at concentrations of 0.25, 0.3, 0.4, 0.5, 0.6, and 0.8 ug/ml, alone or in co-cultures with PAM isolated from F344 rats. At time points of 1, 2, 6, 12, and 24 hours cell culture supernatant was removed and assayed, by ELISA, for the presence of IL-β and IL-6. Results from these experiments show that JP-8 induces the release of IL-β and IL-6 in a time dependent manner, however the levels of cytokines released were not dose dependent in the cultures of RLE-6TN cells alone. In co-cultures of RLE cells and PAM the levels of cytokines released were increased significantly ($p<0.05$) versus the RLE cultures alone. The pattern of release of cytokines was similar to that seen in the cultured RLE cells. From this study it appears that injury to the alveolar epithelium may be caused by immune activation of the alveolar type II cells and this injury may be exacerbated by the presence and activation of PAM in the alveolar space. The release of these pro-inflammatory and chemotactic cytokines may lead to increased airway inflammation and recruitment of various immune cells to the lung which could exacerbate injury. (This work supported by AFOSR 49260-97-0217 and DoD AASERT program.)

246 INSTILLED SOLUBLE VANADIUM EVOKES PULMONARY INFLAMMATION BUT DOES NOT ELICIT PRO-INFLAMMATORY CHEMOKINE mRNA IN THE HEART.

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Inhalation of vanadium-rich fly-ash (~ 500 μ g/m³) results in increases in pulmonary inflammation and increased expression of pro-inflammatory chemokine levels in cardiac macrophages (Killingsworth et al, *Inhal Tox* **9**:541). Intratracheal instillation of vanadium compounds causes significant pulmonary inflammation and enhanced pulmonary chemokine mRNA levels (Pierce et al, *Tox Appl Pharm* **138**:1). HYPOTHESIS: Cardiac chemokine induction is due to soluble metal ions transported from the pulmonary region. METHODS: Rats were lightly anesthetized and intratracheally instilled with 210 μ g/kg sodium metavanadate or PBS. Following sacrifice at 24, 48, and 168 hr post-instillation (PI), bronchoalveolar lavage (BAL) was performed and cardiac tissue prepared for RNA extraction and Northern analysis. RESULTS: As previously observed, significant metavanadate-induced pulmonary inflammation was observed in BAL samples at 24 and 48 hr PI marked by significantly increased neutrophil and eosinophil levels ($p < 0.05$) and significant changes in inflammatory biochemical parameters in acellular BAL fluid; inflammation was resolved by 168 hr. Northern analysis for the pro-inflammatory chemokines macrophage inflammatory protein (MIP)-1α and MIP-2 showed only trace amounts of mRNA in heart tissue from vanadium-exposed animals at 24 and 48 hr PI. CONCLUSION: These results indicate that soluble vanadium induces pulmonary, but not cardiac, inflammation following instillation. This suggests that inflammation-related cardiac dysfunction is not caused by soluble vanadium transported following pulmonary exposure. (Supported by ES00002, ES08129, HL05947, and HL54958.)

247 DEVELOPMENT OF A TAQMAN® BASED QUANTITATIVE RT-PCR ASSAY FOR CANINE CYTOKINE INDUCTION.

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The inflammatory response consists of multiple components, including induction of several cytokine mRNA's. Accurate quantitation of cytokine levels in specific tissues can aid in understanding the extent and source of inflammatory mediators. Using a fluorescence-based ABI Prism® 7700 Sequence Detection System (TaqMan®), a quantitative RT-PCR assay has been developed to measure specific cytokine gene induction in canine tissues and cultured cells. Using the Primer Express software developed by Perkin-Elmer, primer/probe sets were designed for canine IL6, IL8, IL10, TNFα, and GMCSF. Each primer/probe set was specifically designed to span intron-exon junctions to eliminate detection of genomic DNA. GAPDH and 18s primer/probes were used as internal standards in co-amplification reactions. RNA expression vectors containing cloned RT-PCR product from canine RNA were used to generate RNA and DNA standards for absolute quantitation. LPS treatment was used to validate the assays. In canine liver, all the cytokines tested showed a dramatic induction relative to control 1 hour after oral gavage with LPS with the exception of GMCSF and IL 10. LPS also induced cytokines in canine large vessel endothelial cells. The TaqMan RT-PCR method of detecting and quantifying RNA has multiple advantages over endpoint RT-PCR, Northern blot and Rnase protection analysis. The use of specific primers and fluorescent probe results in an assay that is extremely sensitive and specific, does not require gel-based analysis, is very reproducible, and gives a linear response over 6 orders of magnitude without the need for dilutions.

248 A MODEL OF SUBCUTANEOUS ABSCESS FORMATION IN NON-HUMAN PRIMATES.

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Many anti-inflammatory agents affect the trafficking, adhesion and activation of cells of the immune system. Treatment with anti-inflammatory drugs

or biologics can impair the body's ability to respond to or prevent bacterial infections as demonstrated in a rabbit model of subcutaneous abscess formation (J. of Immunol, 151:4982-88, 1993). A non-human primate model of subcutaneous abscess formation was characterized and employed as a means of assessing the potential side effects of primate specific anti-inflammatory agents. Cynomolgus monkeys (*Macaca fascicularis*, n=4) were shaved to expose the dorsal intrascapular skin. Duplicate challenge (1.0 mL/site) of gram-positive bacteria, *Staphylococcus aureus* (*S. aureus*), were injected subcutaneously. Challenge doses ranged from 10^5 to 10^{10} colony forming units (cfu). Animals were then treated with an antibiotic (Cefazolin 25 mg/kg, IM) to prevent bacteremia. The effect of bacterial challenge was assessed by abscess incidence, body weight, body temperature, clinical observations, hematological and serum chemistry parameters. Morphometric analysis of the abscess surface area was also determined by photographic projection/planimetry. In the absence of anti-inflammatory drugs, subcutaneous inoculation with *S. aureus* resulted in an abscess incidence of approximately 25% at 10^8 cfu/mL, and 100% at 10^9 and 10^{10} cfu/mL. Bacterial challenge induced transient fever, leukopenia, neutropenia, lymphopenia and mild monocytosis but had no effect on body weight. Abscesses of variable size formed in those animals inoculated with *S. aureus*. The surface area (mm^2) was 433.89 at 10^8, 393.07 ± 52.91 at 10^9 and 534.26 ± 194.88 at 10^{10}. The demonstrated consistency of abscess formation formed by carefully titrated doses of bacteria provides a reliable and reproducible endpoint for evaluation of the safety of anti-inflammatory compounds.

249 REAPPLICATION OF A NOVEL NONINVASIVE TAPE S AMPLING METHOD FOR ASSESSING THE RELATIONSHIP OF GINGIVAL INFLAMMATION AND BLEEDING TO GINGIVAL EPITHELIUM CYTOKINE CONCENTRATIONS.

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We previously reported development of a simple noninvasive method to assess inflammatory changes in human skin using a dermal tape (Sebutape™) to adsorb molecular mediators (Fund. & Applied Tox. 42:1:388, 1998). In the present human clinical study, we reapplied this tape adsorption method to collect cytokines from gingival epithelium, as opposed to the more commonly sampled gingival crevicular fluid. The primary objective of this study was to examine the relationship between changes in cytokine levels and clinical inflammation. In this clinical study, 10 subjects participated in a 14 day Experimental Gingivitis (EG) model, where 5 days following a dental prophylaxis, subjects refrained from all oral hygiene measures for 14 days. Clinical measures including both Loe-Silness Gingival Index (GI) and a bleeding index derived from the GI were made at baseline and following 14 days of EG. Sebutape samples of each subject's gingival surface were collected from the right posterior buccal quadrant at baseline (prior to EG) and day 14 (post EG). The tapes were extracted in buffered saline and the extracts analyzed for both IL-1α and IL-1β using commercial immunoassay kits. Over a 14 day EG period, statistically significant ($p < 0.05$) increases in GI, gingival bleeding, and IL-1α were observed (tested by matched pair t-test and Wilcoxan signed ranks test). A directional increase in IL-1β was also observed. These data confirm the utility of using dermal tape for sampling of epithelium cytokine levels from the gingiva and demonstrate a positive relationship between changes in IL-1α and IL-1β concentrations and changes in gingival inflammation.

250 CHARACTERIZATION OF SULFUR MUSTARD-INDUCED DERMAL PROINFLAMMATORY MEDIATOR RESPONSE.

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Sulfur mustard, [bis(2-chloroethyl) sulfide; HD] is an alkylating agent that produces incapacitating dermal injuries to exposed individuals. Although the mechanism(s) of dermal toxicity are not fully understood, the pathologic changes occurring within 24 hr of exposure include erythema, edema, epidermal necrosis, and vesication. Recent studies suggest that cytokines/chemokines may be useful biomarkers of cutaneous injury. Studies were undertaken to characterize proinflammatory mediator expression in the skin of animals exposed to HD vapor. Male euthymic hairless mice, housed under AAALAC guidelines, were used in this study. The dorsal surface of anesthetized animals was exposed to HD vapor for 6 min. At approximately 2 hr, 6 hr, and 24 hr following exposure, skin biopsies from euthanatized animals were taken, snap frozen in liquid nitrogen, and stored < -70 C. Samples were processed and analyzed for proinflammatory mediator messenger ribonucleic acid (mRNA) with a ribonuclease protection assay. Results demonstrated an elevation of mRNA coding for interleukin-1 beta, monocyte chemoattractant protein (MCP-1), and macrophage inflammatory proteins (MIP-1 alpha and MIP-2) in exposed sites relative to sham control tissue. In general, mediator message was elevated by 2 hr and increased through 24 hr. These data contribute to the understanding of the early events associated with HD-induced skin injury. (Supported by USAMRDC Contract 17-89-C-9050.)

251 THE EFFECT OF TCDD EXPOSURE ON SERUM AMYLOID A (SAA) INDUCTION IN RESPONSE TO ENDOTOXIN OR SHEEP RED BLOOD CELL STIMULATION IN C57BL/6 MICE.

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2,3,7,8 Tetrachlorodibenzo-p-dioxin (TCDD), a highly toxic environmental contaminant, is suppressive to both humoral and cell-mediated immune responses in mice. In addition, TCDD enhances inflammatory responses to endotoxin and sheep red blood cells (SRBC). Inflammatory responses are accompanied by increased production of various acute phase proteins (APPs) including serum amyloid A (SAA) by hepatocytes. SAA, a major APP in mice, is an apolipoprotein and is known to be induced by proinflammatory cytokines such as interleukin-1 and TNF. Chronic production of SAA has been shown to suppress immune responses. This series of experiments was conducted to address the hypothesis that TCDD exposure in C57Bl/6 mice modulates the APP response to inflammatory stimuli that may contribute to immune suppression. In order to determine the effect of TCDD on APP, mice were gavaged with vehicle or 15 μg/kg TCDD and injected ip one day later with endotoxin (*E. coli* 055:B5) or SRBC. SAA levels were measured by ELISA while plasma TNF levels were measured using a L929 bioassay. Plasma SAA levels were greatly increased as early as 5 hours after endotoxin treatment with peak levels occurring at 24 hours and declining by 48 hours. When TCDD was given prior to endotoxin injection, an increased level of SAA was observed at 24 hours while the kinetics of the overall response was similar to vehicle treated mice. Plasma TNF levels were maximally elevated 1.5 hours after stimulation with endotoxin and TCDD did not influence these TNF levels. In response to SRBC sensitization, vehicle-treated animals exhibited basal levels of SAA and TCDD exposure did not affect SAA levels in plasma or in the peritoneal cavity. TNF was not detected in plasma of SRBC-sensitized mice. We conclude that TCDD exposure up-regulates SAA production in endotoxin-challenged mice but not in SRBC-sensitized mice. However, this TCDD-induced SAA modulation in endotoxin-treated mice does not appear to be mediated by altered TNF production. (Supported by NIEHS Grant ES03966.)

252 ROLE OF PROLIFERATION IN THE *IN VIVO* RESPONSE OF RAT LIVER TO FUMONISIN B.

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Fumonisin mycotoxins are produced by several species of *Fusaria* fungi which are common contaminants of maize, and are the responsible agents for several fatal animal diseases. Fumonisin B_1 (FB_1) is the predominant analog found, and has been shown to be a tumor promoter and possible carcinogen. The mechanism by which fumonisins cause toxicity is believed to be through their ability to disrupt sphingolipid metabolism by inhibition of ceramide synthase. It has previously been noted in our lab and others that although liver is a target organ of fumonisins in intact animals, hepatocytes and liver slices *in vitro* are resistant to cytotoxicity when exposed to fumonisins, even for periods up to 72-96 hr. Several hepatoma cell lines, on the other hand, were sensitive to the effects of fumonisins. We hypothesized that a requisite for fumonisin cytotoxicity is cell proliferation, and used partial hepatectomy (PH) as a model to determine whether fumonisins produced greater toxic response in PH-treated rats than in sham-operated controls. Rats were dosed (ip) with FB_1 24 hr after sham operation or PH, and killed 24 hr later. The dose related increase in free sphingoid bases (a biomarker of ceramide synthase inhibition) caused by FB_1 was enhanced in the PH-treated rats. Serum cholesterol and enzymes (ALP and ALT) were higher in PH-treated rats dosed with FB_1 than those given PH without FB_1 or sham operated, FB_1-dosed rats. Multiple doses of FB_1 after surgery elevated the number of apoptotic hepatocytes observed in both sham and PH-treated rats to about the same degree, suggesting that apoptosis is not associated with the enhanced

cytotoxicity of FB_1 in regenerating liver. The results suggest that proliferating cells are more sensitive to the toxic effects of fumonisins. Whether this enhanced cytotoxicity is related to the ability of fumonisins to disrupt sphingolipid metabolism is yet to be determined.

253 FUSARIC ACID DID NOT MODIFY THE HEPATIC OR RENAL TOXICITY OF FUMONISIN-PRODUCING *FUSARIUM MONILIFORME.*

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Fumonisins B (FB) and fusaric acid (FA) are mycotoxins produced by *Fusarium moniliforme* and other *Fusarium* spp. They are commonly found in corn and may naturally co-occurr. FB cause the animal diseases associated with *F. moniliforme*, promote liver cancer in rats, and are suspected human carcinogens. FA inhibits beta-hydroxylases and is a hypotensive agent. When given together, fumonisin B_1 (FB_1) and FA were synergistically embryotoxic to chicks *in ovo* and it has been hypothesized that FA may exacerbate the effects of co-occurring mycotoxins *in vivo*. To investigate this possibility using a 3 x 4 exposure scheme, male rats (n=5/group) were fed 3.4 (four groups), 18.4 (four groups), or 437 ppm (four groups) FB (FB_1 + FB_2 + FB_3) provided by *F. moniliforme* culture material to which, at each FB concentration, 0, 20, 100, or 400 ppm FA were added. An FA control group was fed 400 ppm FA only and untreated controls were given neither FB nor FA. Toxicity was evaluated over four weeks by observation, body weight, food consumption and selected clinical chemistry, organ weight, kidney sphingolipid profile, and histopathology endpoints. All FB diets were nephrotoxic and the four high dose FB diets were hepatotoxic: microscopic lesions and other findings were consistent with the known, organ-specific effects of FB. In addition, relative heart weights of the four groups fed the high dose FB diets were decreased. FA was without effect. No differences between the untreated and FA controls were found and no significant differences were found in the type or severity of effects among the four groups fed 3.4, the four groups fed 18.4, or the four groups fed 437 ppm FB. Thus, simultaneous dietary exposure to high levels of FA, up to 400 ppm, did not modify the subchronic toxicity to rats of FB provided by *F. moniliforme* culture material.

254 THE MYCOTOXIN FUMONISIN B_1 ALTERS SPHINGO-LIPID BIOSYNTHESIS AND INHIBITS BACTERIAL PHAGOCYTOSIS.

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Fumonisins, fungal metabolites produced by *Fusarium moniliforme*, contaminate corn world-wide and interfere with sphingolipid biosynthesis by inhibiting ceramide synthase. Based on our previous finding that pulmonary clearance of intravenous *Pseudomonas aeruginosa* was decreased in fumonisin-treated pigs, we hypothesized that macrophage phagocytosis would be inhibited by fumonisins. A murine monocyte/macrophage (RAW 264) cell line was incubated with 0, 2.5, 25, 100, 500, or 1000 μM of purified fumonisin B_1 (FB_1) for 24 or 41 hr, respectively. After FB_1 exposure, RAW cells were incubated with FITC-labeled Salmonella for 0, 3, 7, 15, and 60 min and analyzed by flow cytometry. There was a significant dose-dependent decrease in phagocytosis for cells treated with 25, 100, 500, and 1000 μM FB_1 for $\geq$3 min as compared to controls. Similar results were obtained *in vivo* in pigs fed FB_1-containing culture material at 15 mg FB_1/kg BW for 3 days. Phagocytosis by pulmonary alveolar macrophages (PAMs) from treated pigs was significantly decreased when incubated with FITC-labeled Salmonella for 10 and 30 min. Sphinganine (Sa) and sphingosine (So) concentrations were increased in RAW cells exposed to FB_1 (Sa:So maximum of 38.67) as compared to control (0.06), and in PAMs from treated pigs (Sa:So 2.74) as compared to controls (0.34). Thus, FB_1 inhibited macrophage phagocytosis. Interference with sphingolipid biosynthesis by FB_1 may be causally related to this inhibition. Therefore, FB_1 can cause immune suppression which may increase susceptibility to infectious diseases.

255 THE EFFECTS OF FUSARIUM TOXINS IN LOW DOSE ON HOST DEFENSE AGAINST INFECTIOUS DISEASE.

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Fusarium toxins are potent immunosuppressive mycotoxins which frequently contaminate common agricultural producs, such as corn and wheat. The immunosuppressive effects of these mycotoxins include decreased host resistance to infectous diseases. However in previous studies, animals have been exposed toa high doses and the effect of low doses of these mycotoxins on host resistance against bacterial infectious diseases is still unknown. We studied the effect of relatively low doses of six fusarium toxins (fumonisin B1(FB1), deoxynivalenol (DON), diacetoxyscirpenol (DAS), T-2 toxin (T-2), fusarenon-X (FX) and nivalenol (NIV)),on the host resistance against Salmonella infection using mice. Mice given daily each fusarium toxin in drinking water were infected orally with Salmonella enteritidis 14 days after the commencement of exposure to these toxins. It was found that DON was the most effective among these fusarium toxins in decreasing the resistance against salmonellosis. At 24 microgram/Kg/day dietary exposure, DON reduced serum anti-Salmonella IgM titer and delated type hypersensitivity reaction , both of which are regarded as defense mechanisms against Salmonella infection. These results suggest that dietary exposure to low doses of DON enhance the susceptibility to oral infection to Salmonella through toxic effects on cellular and humoral immunity.

256 SUBCUTANEOUS FUMONISIN ADMINISTRATION DISRUPTS SPHINGOLIPID METABOLISM IN THE DIGESTIVE EPITHELIA IN MICE.

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Fumonisins are mycotoxins produced by *Fusarium moniliforme*. Fumonisins are commonly found in corn and corn products. They are known to be potent inhibitors of ceramide synthase (sphinganine [sphingosine] N-acyltransferase). Fumonisin inhibition of ceramide synthase causes a rapid increase in free sphinganine, the immediate sphingoid base precursor in the *de novo* biosynthesis of dihydroceramide and ceramide. High levels of free sphinganine in tissues and biological fluids are used as a biomarker for fumonisin exposure. The liver and kidney are target organs in all species tested thus far. Because fumonisins are poorly absorbed and rapidly excreted, the digestive epithelia of farm animals consuming low quality corn-base feeds can be exposed to high concentrations of fumonisins. Thus, the gastrointestinal tract may also be a target of fumonisins. The purpose of this study was to determine if a single subcutaneous (s.c.) injection of fumonisin B1 (25 mg/kg body wt) could cause an increase in free sphinganine in epithelial cells harvested from the small intestines of eight week old BALB/c mice (n=3/time point) over a 24 h period. It was hypothesized that because fumonisins are excreted in the bile following i.p. or i.v. administration, fumonisin administered s.c. should be excreted in the gut via biliary excretion and this should be detectable by an elevation of free sphinganine in the digestive epithelial cells of the small intestines. The results of this preliminary study indicate that there is a significant ($p \leq 0.05$) time-dependent increase in free sphinganine that peaks at 4 to 8 h and declines to near the control level by 24 h post dosing. The increase in free sphinganine suggests that s.c. administered fumonisin B1 is rapidly excreted into the small intestines. However, the rapid return to control levels is unexpected since ceramide synthase inhibition in cultured epithelial cells is quite persistent. Nonetheless, s.c. administration may prove to be a useful tool for studying the effects of fumonisin on the digestive epithelia *in vivo*.

257 PATULIN EXHIBITS MULTIPLE ELECTROPHILIC PROPERTIES AND COVALENTLY CROSSLINKS PROTEINS *IN VITRO.*

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The mycotoxin patulin is thought to exert its cytotoxic and chromosome damaging effects by covalent modification of cellular nucleophilic sites such as thiol groups of proteins and glutathione. In the course of elucidating chemical structures of patulin adducts with simple thiol and amine model compounds *in vitro*, we observed a high preference for adduct types containing two and three molecules of nucleophile per molecule of patulin. As similar reactions at the protein level could lead to covalent intra- or inter-

molecular crosslink formation which might severely disturb the biological functions of proteins, we have investigated the ability of patulin to induce protein-protein crosslinks. By means of sodium dodecylsulfate - polyacrylamide gel electrophoresis, the formation of patulin-induced protein-protein crosslinks was clearly demonstrated *in vitro* for bovine serum albumin containing one thiol group per molecule, but also for the thiol-free hen egg lysozyme. Characterization of the crosslink sites was carried out by modulation of the thiol status (N-ethylmaleimide, 2-iminothiolane), comparison with various positive controls (phenylenedimaleimide, glutaraldehyde, dimethylsuberimidate), and protein fluorescence incorporation studies using dansyl-labelled amino acid derivatives and glu-N-dansyl-glutathione. The amino acids responsible for patulin-induced crosslink formation were shown to be preferably cysteine, but also lysine, histidine, and α-amino-groups. Patulin can act both as a homobifunctional as well as a heterobifunctional crosslinking reagent: the formation of an initial thiol adduct appears to activate patulin for a subsequent reaction with either thiol groups or amino groups. These results raise the question whether patulin can also react with DNA, possibly leading to protein-DNA or DNA-DNA crosslink. (Supported by Fonds der Chemischen Industrie and Deutsche Forschungsgemeinschaft (Me 574/14-1).)

258 APPLICATION OF IMMOBILIZED ACTIVATED CARBON FOR THE SORPTION OF PATULIN FROM AQUEOUS SOLUTION.

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Patulin, a heterocyclic lactone produced by various species of *Penicillium* and *Aspergillus* fungi, is often detected in apple juices and ciders. The cytotoxic and mutagenic properties of patulin warrant its removal from juices consumed largely by infants and children. Previous research has shown the effectiveness of activated carbon (AC) for reducing patulin in aqueous solutions, apple juices and ciders. In this study, ultra-fine activated carbon (< 45 μm particle size) was tightly bonded onto granular quartz (212-300 μm particle size) to achieve a sorbent with high surface area, enhanced flow characteristics, and increased bulk density. The immobilized carbon (IMAC) was packed in glass columns and tested for its ability to sorb patulin from aqueous solution. Columns containing 1.0 g, 0.5 g and 0.25 g of IMAC were continuously loaded with a patulin solution (10 μg/ml; pH 3.5) and eluted at a flow rate of 1 ml/min. Eluate from the columns was collected in 2 ml aliquots with an automated fraction collector, then analyzed with UV spectrophotometry for patulin content. Results indicated that 50% breakthrough capacities for patulin on 1.0 g, 0.5 g and 0.25 g IMAC columns were 137.5, 38.5, and 19.9 μg, respectively. The effectiveness of IMAC columns to sorb patulin and prevent toxicity was further confirmed *in vitro* using adult hydra in culture. Hydra were sensitive to the effects of patulin, with a minimum affective concentration equal to 0.7 μg/ml; following filtration with IMAC, toxicity was prevented until breakthrough capacities were achieved. The results clearly demonstrate a correlation between patulin levels and toxicity, and indicate that IMAC may provide an effective and practical strategy for the clarification of apple juices and ciders. (Supported by USDA 9703230.)

259 BIOCHEMICAL FACTORS INVOLVED IN AGE-RELATED SENSITIVITY TO AFLATOXIN B_1 IN POULTRY.

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Poultry are the most susceptible food animal species to the toxic effects of the mycotoxin aflatoxin B_1 (AFB_1). The extreme sensitivity of turkeys to AFB_1 is due to a combination of efficient AFB_1 activation by cytochrome P450s (CYPs) 1A2 and 3A4 and deficient detoxification by glutathione *S*-transferases (GSTs). In addition, younger poultry are more sensitive to AFB_1 than older ones. To determine the underlying factors involved in age-related sensitivity, AFB_1 activation and AFB_1-relevant phase II enzyme activities were assessed in liver microsomes and cytosol prepared from 9, 21, 31, 41, and 65 day old turkeys. Younger turkeys were more efficient activators of AFB_1, as measured by a lower apparent Km and higher Vmax toward the microsomal formation of the AFB_1-8,9-epoxide, compared to older birds. General cytosolic GST activity was approximately two-fold greater among the 41 and 65 day old birds, as compared to the younger poults. However, immunoblots against a rat Yc2 antibody indicated that with increasing age, the amount of GST Yc2 immunoreactive protein decreased. There was an age-related increase in cytosolic aflatoxin aldehyde reductase (AFAR), an important AFB_1-detoxifying enzyme. Thus, the greater sensitivity of younger poultry to the toxic effects of AFB_1 may be explained by the combination of more efficient hepatic AFB_1 activation and less efficient AFB_1 detoxification compared to older birds. (Supported by USDA-NRI grants 970-3081 and 980-3754.)

260 ALTERATIONS IN CYTOCHROME P-450 EXPRESSING HUMAN LUNG CELLS AFTER AFB_1 EXPOSURE.

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Aflatoxin B_1 is a potent dietary hepatocarcinogen in animals and probably in humans. Inhalation of AFB_1-laden grain dusts has been associated with an increased incidence of lung cancer. Mutations in the tumor suppresser gene p53 have been seen in liver tumor tissue from people exposed to high dietary AFB_1 levels. To study the effects of AFB_1 on p53 levels in immortalized human lung cells, we exposed SV40 immortalized human bronchial epithelial cell types (BEAS-2B), transfected with the genes for either cytochrome P450 (CYP) 1A2 or 3A4, to AFB_1. These isozymes are thought to be responsible for AFB_1 activation in the liver and possibly lung. Immunoblotting revealed that untreated, non-transfected normal human bronchial epithelial cells (NHBE) did not express detectable amounts of p53. Untreated, SV40 immortalized (1A2 and 3A4 expressing) cells constitutively expressed p53. Exposure to 0.015μM-15μM AFB_1 for 30 min caused a dose-related decline in p53 expression in 1A2 cells, and to a lesser extent, 3A4 cells. The AFB_1-elicited decrease in p53 continued for at least 6 hrs after exposure to 1.5 μM AFB_1. At these doses 1A2 expressing cells were affected to a greater extent than 3A4 expressing cells. These data indicate that AFB_1 exposure significantly alters p53 protein levels in SV40 immortalized human lung cells. The fact that p53 was altered to a greater extent in 1A2-expressing cells agrees with our previous studies showing that this isozyme is more catalytically active toward AFB_1 activation than 3A4 at these concentrations.

261 DEVELOPMENT OF LIVER INJURY IN RATS TREATED WITH AFLATOXIN B_1 AND ENDOTOXIN, AND PROTECTION BY NEUTROPHIL DEPLETION.

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Exposure to small, nontoxic doses of bacterial endotoxin augments the toxicity of a variety of hepatotoxicants. This study was conducted to delineate the development of injury in a rat model of augmentation of aflatoxin B_1 (AB1) hepatotoxicity by endotoxin and to explore the role of neutrophils in the pathogenesis. Male, Sprague-Dawley rats (200-300 g) were treated with either 1 mg AB1/kg, ip, or its vehicle (2% DMSO/water) and 4 hours later with either endotoxin (E. coli LPS; 7.4 X 10^6 EU/kg, iv) or its saline vehicle. Liver injury was assessed 6, 12, 24 or 48 hr after AB1 administration from alterations in the concentration of bile acids and the activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and 5'-nucleotidase (5'-ND) in serum and from histologic examination of liver. At all times and for all markers, injury in rats treated with either AB1 or LPS was absent or modest. In the AB1/LPS group, injury was significant by 12 hr and maximal by 24 hr. Increased numbers of neutrophils were apparent in liver lesions from this group. To examine the role of neutrophils in the pathogenesis, an antibody to rat neutrophils was given prior to AB1/endotoxin treatment to reduce blood concentration of these cells. Neutrophil depletion protected against hepatocellular injury as evidenced by prevention of the increase in ALT and AST and attenuation of morphologic injury. By contrast, neutrophil depletion did not affect the increase in 5'-ND or bile acids. These results suggest that endotoxin potentiates AB1 hepatocellular injury by a mechanism which is dependent on neutrophils; however, cholestatic changes appear to be neutrophil-independent. (Supported by NIH grant ES04139.)

262 EFFECTS OF PHYTOCHEMICALS ON AFLATOXIN-INDUCED CYTOTOXICITY.

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Aflatoxin is a potent mycotoxin produced by strains of Asperigillus flavus

and parasitius. The cytotoxicity induced by aflatoxin B1 to fibroblasts and the toxic effects on human cancer cells have been examined. The cellular responses were measured by MTT assays. The results show that different cellular responses among cell lines were observed. The cancer cell displayed greater viability in an equivalent solution of the toxicant. Also, the chemoprotective effects of some common dietary vitamins such as vitamins C, A and E on the aflatoxin-induced cytotoxicity were studied. By comparing the cellular responses, all three vitamins show chemoprotective effects on the cytotoxicity. Vitamin C appeared to be more effective in protecting the cell from the toxicity. The cells show better viability (>30%) than the control in the presence of vitamin C. The study provides useful information on the toxic action of aflatoxin and allows us to get an understanding of the defense mechanism of cancer cells against the aflatoxin-induced toxicity.

263 QUALITATIVE STRUCTURE ACTIVITY RELATIONSHIP ANALYSIS OF TRICHOTHECENE MYCOTOXINS.

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Trichothecenes are a group of secondary fungal metabolites which are common contaminants of grain based food and feeds. The effect of trichothecene physical and chemical characteristics on toxicity was assessed using previously reported values generated from *in vivo* and cellular toxicity model systems. Three dimensional structures were generated using Allinger's molecular mechanisms force field analysis (Chem 3-D Software, CambridgeSoft, Cambridge, MA). Physico-chemical properties were calculated using Molecular Modeling Pro (WindowChem Software, Fairfield, CA). The software generated thirty four variables, although many were highly correlated. To reduce problems associated with colinearity, Pearson correlation, Gittin's condition number, and consideration of variable calculation were used to reduce the variables to Hansch Log P, Crippen's Log P, CNDO Log P, molecular connectivity index, solubility parameter (Sol), and hydrophilicity - lipophilicity balance (HLB). Seven data sets were analyzed using principal components analysis and canonical correlation analysis. Sol and HLB always weighted heavily as predictors of toxicity in canonical correlation. The same variables served as surrogates for toxicity, structuring the data set in principal component analysis. These results suggest that solubility is an important influence on trichothecene toxicity.

264 EFFECT OF PIROXICAM ON OCHRATOXIN A–INDUCED INHIBITION OF PROTEIN SYNTHESIS IN VERO CELLS.

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Ochratoxin A (OTA) is a mycotoxin produced by *Aspergillus ochraceus* as well as other moulds. It is a natural contaminant of mouldy food and feed. OTA is increasingly regarded as the main causal agent of Balkan Endemic Nephropathy (BEN). In addition to its nephrotoxic effects OTA is immunosuppressive, teratogenic, genotoxic and carcinogenic. It also interferes with blood coagulation and carbohydrates metabolism. OTA induces oxidative damage by enhancing lipid peroxidation and inhibits protein synthesis by competition with phenylalanine in the phenylalanine - tRNA aminoacylation reaction. An attempt to prevent its toxic effects, mainly the inhibition of protein synthesis, has been made using piroxicam, a non-steroidal anti-inflammatory drug (NSAID). Protein synthesis was assayed in monkey kidney cells (Vero cells) treated with increasing concentrations of OTA (10 - 100 μM). Vero cells lines were cultured in 24-well culture discs, in RPMI 1640 medium, supplemented with L-glutamine (2 mM) and penicillin (100 U) - streptomycin (100 mg / ml). Cells (10^5 cells/ml) were routinely incubated, for 24 h, in an humidified 5% CO_2 / 95 % air mixture, at 37°C. Protein synthesis was assessed by [^{3}H] - leucine (5.0 Ci / mmol) incorporation during a 2h - pulse. After 24h incubation with the toxin, protein synthesis was inhibited by OTA in a concentration dependant manner. The 50% inhibitory concentration (IC_{50}) was about 9μM. When cells were pretreated with piroxicam (10 - 100 μM) 4h before OTA intoxication (10 - 100 μM) the inhibition of protein synthesis induced by OTA was partially prevented (IC_{50} = 27 μM). This result could explain the protective effect of piroxicam on OTA - induced nephrotoxicity in rats (I. Baudrimont et al., 1995, Toxicology, 95, 147).

265 OCHRATOXIN A(OTA) REVERSIBLY INHIBITS PROLIFERATION IN THE LLCPK-1 CELL LINE.

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Ochratoxin A is a mycotoxin produced as a secondary metabolite by certain *Aspergillus* and *Penicillium* species. It is commonly found as a contaminant of both human and animal feedstuffs. All human blood samples tested to date have proved positive for OTA. Average daily intake in humans is estimated to be approximately 5 – 10ng/kg. OTA has been demonstrated to be immunotoxic in pigs (antiproliferative effect in T-cells) and teratogenic in several species including rat, mouse, hamster and chick, primarily giving rise to craniofacial abnormalities such as exencephaly, micropthalmia and facial clefts. Although little is known about its potential for human teratogenesis, OTA displays similarities in animal models to other well-characterized human teratogens such as valproate and thalidomide, especially with respect to dose-dependency and gestational sensitivity periods. A disruption in the normal cellular proliferation control could contribute to both the observed immunotoxicity and teratogenicity of OTA. Therefore, we investigated the effect of the toxin on proliferation rates in LLCPK-1 cells following acute exposure. Cells were exposed to increasing concentrations of OTA over 24 and 48hrs. Antiproliferative effects were determined by direct counting of crystal violet stained nuclei and confirmed using a Coulter counter. The concentrations producing 50% proliferative arrest were 5 and 1μM following 24 and 48h exposure respectively, with an IC_{80} of 10μM following 48 hour exposure to the agent. The antiproliferative effect was observed to be time dependent and reversible as cells which had been exposed to 10μM OTA for 24h resumed proliferation within 24h of drug removal and replacement of full fresh medium whereas cells exposed to the same concentration for 48h required 48h to re-enter the cell cycle. The nature of the proliferative arrest was examined using standard flow cytometric /ICP analysis of propidium iodide/Hoechst stained DNA and was found to be specifically located to the G2- + M-phase of the cell cycle suggesting e.g. a reversible inhibition of cell cycle specific protein synthesis.

266 TOXIC EFFECTS OF OCHRATOXIN A ON CATECHOLAMINE - RICH HUMAN NEUROBLASTOMA CELL LINE.

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Ochratoxin A (OTA) is a mycotoxin produced by mould spores such as *Aspergillus* and *Penicillium*. It is a natural contaminant of a wide variety of both human and animal foodstuffs. Via dietary intake, it passes into the blood of both human and animal and accumulates in several organs such as the kidney, being nephrotoxic to all animal species studied so far and the brain with selective toxicity in the ventral mesencephalon (VM) and the cerebellum. Structural analogue to phenylalanine (phe), OTA inhibits protein synthesis in cells but with higher sensitivity of neurones of the ventral mesencephalon (NVM). In order to confirm the regional selectivity and the sensitivity of NVM primary cultures, investigations were designed to study the mechanism of the cytotoxicity of OTA in cultures of NB69 cell line whose biochemical and pharmacological profiles previously described resemble VM neurone profiles. The NB69 cells, a human catecholamine neuroblastoma were then studied in the presence of OTA for MTT conversion, lactate dihydrogenase release (LDH), protein synthesis and lipid peroxidation evaluated by production of malondialdehyde (MDA). The protective effects of some substances (structural analogues of OTA and antioxidants such as SOD+catalse, vitamin E, epicatechin, trans-resveratrol) were also assayed. After a 24h incubation period, OTA (10-150 μM) decreased MTT conversion and increased of LDH release in a dose-dependent manner. In parallel levels of MDA produced were increased significantly and protein synthesis was inhibited (inhibitory concentration 50% (CI50% = 14μM). Interestingly the levels of dopamine were decreased from 3.56 ± 0.84 to 1.02 ± 0.34 ng/mg of protein with 28 μM of OTA. Analogues of OTA and antioxidants partially reversed the inhibition of protein synthesis and MDA production. Altogether these results indicate that OTA is also a neurotoxic in addition to its well-documented nephrotoxicity and that its effects are partially related to phe-dependent enzymes in addition to its oxidative pathway and protein synthesis inhibition.

267 ERYTHRITOL: AN INTERPRETIVE SUMMARY OF BIOCHEMICAL, METABOLIC, TOXICOLOGICAL AND CLINICAL DATA.

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A critical and comprehensive review of the safety information on erythritol was undertaken. Numerous toxicity and metabolic studies have been conducted on erythritol in rats, mice, and dogs. The toxicity studies consist of long-term feeding studies conducted to determine carcinogenic potential, intravenous and oral teratogenicity studies to determine the potential for effects on the fetus, oral studies in which erythritol was administered over 1 or 2 generations to determine the potential for reproductive effects, and studies in bacterial and mammalian systems to determine mutagenic potential. The majority of the safety studies conducted were feeding studies in which erythritol was mixed into the diet at concentrations as high as 20%. The metabolic studies in animals have shown that erythritol is almost completely absorbed, not metabolized systemically and is excreted unchanged in the urine. The safety studies have demonstrated that erythritol is well-tolerated and elicits no toxicological effects. The clinical program for erythritol involved a series of single-dose and repeat-dose, short-duration studies which have been used to investigate the human correlates to the physiological responses seen in the pre-clinical studies. The clinical studies showed erythritol to be well-tolerated and not to cause any toxicologically relevant effects, even following high-dose exposure. Erythritol administered orally to humans was rapidly absorbed from the gastrointestinal tract and quantitatively excreted in the urine without undergoing metabolic change. At high oral doses, urinary excretion accounted for approximately 90% of the administered dose with minimal amounts appearing in the feces. A comparison of the human and animal data indicated a high degree of similarity in the metabolism of erythritol and this finding supports the use of the animal species used to evaluate the safety of erythritol for human consumption. It can be concluded, based on the available studies that erythritol did not produce evidence of toxicity.

268 ORAL TWO-GENERATION REPRODUCTION STUDY IN RATS ON PHYTOSTEROL-ESTERS (PE)) - A NOVEL FUNCTIONAL FOOD.

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A new margarine has been developed by Unilever which contains the novel ingredient, phytosterol esters. PE are a natural component of vegetable oils which lowers blood cholesterol by blocking the absorption of cholesterol from the gut. As part of an extensive programme of safety evaluation studies we have conducted a two-generation reproduction study in rats, in which the possible effect of PE on male and female reproductive performance and on the growth and development of the offspring, was carried out. Wistar rats were fed diets containing various levels of PE (0, 1.6, 3.2 and 8.1% w/w) over two successive generations and a wide range of reproductive- and developmental parameters were determined. Histopathological examinations were performed on various organs of F1- and F2-weanlings and of F0- and F1-parental animals.

Daily clinical observations did not reveal any remarkable findings. In the highest-dose group, food intake and body weights were statistically significantly decreased. In both generations no effects of PE were observed on pup mortality calculated on litter basis, precoital time, mating index, male and female fertility, female fecundity, gestation index, duration of gestation and number of females with stillborn pups and post-implantation loss, pup development, sexual maturation and estrus cycle length. Apart from an increased relative liver weight of the F0-males of the 1.6 and 3.2% PE groups no differences were observed in organ weights of adrenals, brain, epidymides, pituitary, prostate, seminal vesicles, ovaries and uterus. Furthermore, no histopathological changes were observed in these organs.

In conclusion, dietary administration of up to 8.1% PE during two generations had no effects on reproduction of parental F0- and F1-generation Wistar rats and the development of the F1- and F2-pups.

269 GENETIC TOXICITY *IN VITRO* OF BISPHENOL A-DIGLYCIDYL ETHER (BADGE) AND ITS HYDROLYSIS PRODUCT.

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BADGE is released from epoxy resins and organosoles, which are used for the interior lacquer-coating of food cans. Hydrolysis may occur at the epoxide rings leading to a diglycol, and, after metabolic hydroxylation, at the ether bonds leading to bisphenol A (BPA). The health implications of BADGE and its putative hydrolysis products are yet unclear. We have recently reported that BPA induces metaphase arrest and micronuclei with whole chromosomes in cultured Chinese hamster V79 cells, indicative of an aneuploidogenic potential (Mutat. Res. 390, 21, 1997). In the present study, the induction of micronuclei and gene mutations in V79 cells by BADGE and its epoxide hydrolysis product was investigated. BADGE was very stable in cell culture medium at pH 7.5 and 37°C: after 24 h, only unchanged BADGE was extracted and identified by HPLC. However, in the presence of rat liver microsomes or S-9 supernatant with or without NADPH-regenerating system at 37°C, BADGE disappeared completely within a few min, and the only product formed was the diglycol. At concentrations ranging from 10 - 50 μ M, BADGE in the absence of S-9 mix clearly induced micronuclei with acentric chromosomal fragments and gene mutations at the HPRT locus. Higher concentrations were cytotoxic. In contrast, the diglycol of BADGE was devoid of clastogenicity and mutagenicity even at concentrations up to 200 μ M. Contrary to BPA, neither BADGE nor its diglycol exhibited aneuploidogenic potential. The present study provides evidence that BADGE is a gene mutagen and very resistant against chemical hydrolysis, but is rapidly inactivated by enzymatic epoxide hydrolysis to a non-mutagenic metabolite. (Supported by Deutsche Forschungsgemeinschaft (Me 574/9-2).)

270 SUBCHRONIC ORAL TOXICITY OF EPIGALLOCATECHIN GALLATE (EGCG) IN RATS AND DOGS.

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Human consumption of green tea is inversely associated with cancer risk in several organ sites. EGCG is the most abundant polyphenol in green tea, and has significant chemopreventive activity in animal carcinogenesis models. Ninety-day oral toxicity studies were conducted to characterize the toxicity of EGCG, and to establish its No Adverse Effect Level (NOEL). Sprague-Dawley rats (20/sex/group) received oral doses of 0, 45, 150, or 500 mg EGCG/kg/day via gavage; beagle dogs (4/sex/group) received oral doses of 0, 30, 100, or 300 mg EGCG/kg/day via capsule. Toxicologic endpoints included survival, body weight, food consumption, clinical signs, clinical pathology, ophthalmology, electrocardiography (dogs only), organ weights, gross pathology, and microscopic pathology. In dogs, EGCG induced no gross toxicity, body weight effects, alterations in functional parameters, or gross or microscopic pathology. By contrast, early deaths in the high dose group and a dose-related suppression of body weight gain were seen in both sexes of rats. Intestinal dilatation was a common finding both in rats dying or euthanized *in extremis* during the dosing period, and in rats surviving until the terminal necropsy; this change may be secondary to alterations in digestive processes and/or gut microflora. Microscopic findings suggesting possible alterations in gastrointestinal function included pancreatic necrosis and hepatic (periacinar) degeneration and necrosis; modest elevations in ALP, AST, and ALT were seen in female rats in the high dose group. High dose rats of both sexes also demonstrated necrosis/atrophy of the thymus. In dogs, the NOEL of EGCG is ≥ 300 mg/kg/day. Histopathologic data suggests that the NOEL of EGCG is 150 mg/kg/day in both male and female rats. However, body and organ weights appear to be more sensitive indicators of EGCG toxicity in male rats. On the basis of reduced body weight gain and decreased absolute and relative thymus weights, the NOEL of EGCG in male rats is 45 mg/kg/day. (Supported by NCI-N01-CN-55146.)

271 SUBCHRONIC ORAL TOXICITY OF GREEN TEA POLYPHENOLS IN RATS AND DOGS.

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Consumption of green tea is inversely associated with human cancer risk in

several tissues; green tea contains a number of polyphenols whose biological activity is consistent with anticarcinogenic efficacy. These studies were designed to evaluate the subchronic oral toxicity of a green tea polyphenol (GTP) fraction [Polyphenon E; Mitsui Norin] whose major components are: epigallocatechin gallate (EGCG; 53.4%); epigallo-catechin (11.4%); epicatechin (9.1%); gallocatechin gallate (5.1%); and epicatechin gallate (4.9%). Sprague-Dawley rats (20/sex/group) received daily oral doses of 0, 90, 300, or 1000 mg GTP/kg via gavage; beagle dogs (4/sex/group) received daily doses of 0, 60, 200, or 600 mg GTP/kg via capsule. Toxicologic endpoints included survival, body weight, food consumption, clinical signs, clinical pathology, ophthalmology, electro-cardiography (dogs only), organ weights, and gross and microscopic pathology. When administered to dogs, GTP induced no gross toxicity, suppression of body weight gain, alterations in functional parameters, or gross or microscopic pathology. By contrast, early deaths were seen in both male and female rats receiving the high dose of GTP. Dose-related suppression of body weight gain, food consumption, and relative and absolute spleen and thymus weights were also seen in both sexes. As seen with purified EGCG, intestinal dilatation was a common finding in early deaths, moribund kills, and in rats surviving until the terminal necropsy; gross intestinal pathology may be secondary to alterations in digestive processes and/or gut microflora. Microscopic findings included pancreatic necrosis, hepatic degeneration/necrosis, and thymic necrosis/atrophy. The NOEL of GTP in dogs (both sexes) is ≥ 600 mg/kg/day; the NOEL of GTP in rats (both sexes) is 90 mg/kg/day. Mortality patterns in rats indicate that GTP is more toxic than would be predicted based on its EGCG content alone, suggesting that polyphenols other than EGCG play a role in its toxicity. (Supported by NCI-N01-CN-55146.)

272 ADRENERGIC AGONISTIC EFFECTS AND CYTO-TOXICITY OF *CHINESE EPHEDRA* (MA-HUANG) USED FOR WEIGHT REDUCTION.

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Ma-huang is a traditional Chinese medicinal herb derived from stems and branches of *Ephedra sinica* Stapf and other species of *Ephedra* plants. It contains 1 - 2% by weight of ephedrine alkaloids, of which ephedrine accounts for 40 - 90%. Through their β -adrenergic agonistic action, these alkaloids can induce thermogenesis and energy expenditure and hence sustain weight loss when ma-huang is taken on a long-term basis. However, these alkaloids produce myocardial stimulation through their β_1-agonistic effect. They also constrict peripheral blood vessels via their α -agonistic effect, causing an increase in blood pressure and heart rate. Recently, great volumes of ma-huang have been used as an active ingredient in some dietary supplements used for weight reduction, causing serious side effects and numerous cases of poisoning, some of which were fatal. In the present study, ma-huang extracts were prepared under various conditions for possible different potencies. For each extract, ephedrine content was analyzed by HPLC, biological activities were determined with mammalian cell lines stably expressing adrenergic receptors, and cytotoxicity to a hepatocarcinoma cell line (HepG2) was measured using MTT colorimetry. The results indicate that the potencies of adrenergic agonistic activity and cytotoxicity of ma-huang extracts are in correlation with their ephedrine contents. Grinding of herb was found to be a crucial condition for increased potency. (Supported by Hong Kong UGC PGS and Hong Kong ISF.)

273 HEAT RESISTANCE CHARACTERISTICS OF *ESCHERICHIA COLI* O157:H7 ISOLATED IN JAPAN.

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Thermal resistance of enterohemorrhagic *E. coli* O157:H7 was compared between the strains isolated from large outbreaks occurred in Japan in 1996. Difference in the shape of heat resistance curves upon pH at the heating was also studied. Four strains isolated in outbreaks and one from meat specimen in Japan and one obtained from ATCC were cultured individually in Tryptic Soy Broth (TSB) for 16 hr at 37°C. The cultures were diluted in TSB (pH 7.3) to give 1 x 106 cells per ml of cell suspension, and pH was adjusted to 4.0 or 5.0 with HCl when needed. Syringe vials (15 x 40 mm) were used instead of TDT ampules or plastic bags to heat the samples, in order to improve the handling efficiency and to enable to measure inner temperature during the heating process. Temperature of the contents was monitored using a type K thermocouple. Duplicate vials were removed at each prescribed time interval, transferred to an ice bath, and the content of the vials was spiral plated on Tryptic Soy Agar. When the bacterial suspension was heated at 55°C in the medium with pH 7.3, D-value varied from 2.46 to 5.34 within the strains isolated in Japan. Heat resistance curve has been understood as logarithmically declining against heating time. However, the pattern of the curve was dramatically altered and accordingly different D-value was given when the samples were heated in the medium with different pH. Our observations suggest that there exist various types of *E. coli* O157:H7 strains from the viewpoint of thermal resistance, and arrest attention to the effect of pH on the thermal resistance of pathogenic bacteria when heating duration is estimated for food processing.

274 STRATEGIES FOR ESTIMATING PROVISIONAL ACCEPTABLE RESIDUES (PAR) FOR EXTRALABEL DRUG USE IN FOOD ANIMALS.

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In 1996, the US Congress passed AMDUCA, which allows some veterinary drugs to be used off label. In order to implement this and protect the US consumer, tolerances, or safe concentrations are required before a withdrawal time can be adequately estimated for extralabel drug use. This paper presents several strategies that can be used to derive an equivalent safe concentration, referred to as a Provisional Acceptable Residue (PAR) for specific tissues, that may be used to estimate drug withdrawal times after extralabel use of several antibiotics and other drugs. Derivation of the proposed PAR ensures that daily intakes of drug residues in milk and/or edible tissues do not exceed Acceptable Daily Intakes (ADIs) specific for the US and also takes into account safety factors and food consumption values. Method A requires partitioning of 50% of the ADI to the target tissue and reserving the remainder for milk. Method B equally partitions the ADI into all edible tissues, including milk. Method C assigns 50% ADI to milk and equally partitions the remaining 50% ADI into edible tissues. These simulations showed that provided the safe concentration or its equivalent PAR is based on rigorous toxicology data (e.g., ADIs), the health of the most sensitive population may not be compromised. It is proposed that these PARs can be used for estimating withdrawal times after extralabel drug use and for purposes of trade harmonization.

275 BELL-SHAPED RELATIONSHIP BETWEEN MOLECULAR WEIGHT AND DOSE-RESPONSE TO WHITE MINERAL OILS IN FISCHER 344 (F344) RATS.

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Dietary exposure to certain white mineral oils (WO) causes inflammatory effects in the liver and lymph nodes of F344 rats, but not in other strains or species. The average molecular weight (MW) of previously tested oils ranged from ~300-500. The inflammatory effects were inversely related to MW and were not seen with heavier paraffinic WO (MW 485). This study extends the relationship between MW and dose-response to lighter (MW<300) WO. Four groups of female F344 rats (n=15/group) were fed a diet with 0, 0.02, 0.2, or 2.0% of a light white mineral oil (MW=270) for 90 days. No effects were seen in the 0.02% group. Treatment related changes included increased relative liver and mesenteric lymph node (MLN) weights at 0.2%, MLN inflammation (macrophage accumulations) at 0.2%, and liver histopathologic changes (microgranuloma formation) at 2% dose level. These effects had a lower incidence and were less severe than those seen previously with heavier oils (MW=320-420), suggesting a bell-shaped structure activity relationship may exist for WO-induced inflammatory effects in F344 rats. Analysis of WO residues in livers from these study animals showed that lower hydrocarbons (C_{15}-C_{21}) were underrepresented compared to the original test material. The relative absence of lower MW hydrocarbons in liver tissue is most probably due to increased metabolism rather than decreased absorption of the WO.

276 LOW MELT POINT PARAFFIN WAX (LMPW) CAUSES FORIEGN BODY RESPONSE IN FISCHER 344 (F344) AND SPRAGUE-DAWLEY (SD) RATS.

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Previous 90-day feeding studies of LMPW have shown differences in tissue response between F344 and SD rats. F344 histopathology included liver and mesenteric lymph node (MLN) microgranulomas, and cardiac mitral valve inflammation; whereas, effects in SD were limited to a low incidence of MLN effects. This study further assesses strain differences by phenotypically characterizing the inflammatory cell infiltrates in affected tissues. Samples of liver, MLN and cardiac mitral valves were taken from female F344 and SD rats fed 0, 0.2 or 2.0% LMPW for up to 90 days. Sections were stained for the presence of cells expressing CD3+ (T-cells), CD4+ (helper T-cells) CD8+ (suppressor/cytotoxic T-cells), CD45R+ (B-cells), ED2+ (resident macrophages), and lysozyme (activated macrophages). The microgranulomas observed in both the F344 (liver and MLN) and SD (MLN only), were consistent with a foreign body reaction as characterized by the presence of large epithelioid lysozyme+ macrophages and multinucleated giant cells (predominantly Langhan's type). Lymphocytes (mostly CD3+) surrounded the macrophages; CD4+ and CD8+ cells were present in about equal numbers. CD45R+ were a minor component of the lymphoid infiltrates. The minimal lymphoid infiltrates observed in the F344 mitral valve consisted predominantly of CD3+ cells with few CD45R+ cells and macrophages. Electron microscopic analysis of the liver indicated activated KCs in the F344 only, as well as intralysosomal crystalloids with angular shapes in KCs and hepatocytes from the F344 but not the SD. In conclusion, dietary LMPW induced a classic foreign body response in F344 (MLN and liver) and SD (MLN only) rats.

277 POTENTIAL PROBLEMATICAL GLYCOALKALOIDS IN PORK CHOPS.

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Sponsor: J Spoo.

Sensitivities or allergic responses to certain foods is documented in veterinary and human medicine. A woman with known sensitivity to members of the Solanaceae family (tomatoes, potatoes, nightshades) became ill after consuming part of a cooked pork chop purchased from a local butcher. She experienced severe gastric upset, trembling, weakness and near prostration. She recognized her symptoms as being identical to those she commonly experienced following consumption of potatoes or tomatoes. The next day when she tried the remains of the pork chop the clinical signs recurred, and she was taken to the hospital. The clinical signs are consistent with those produced by glycoalkaloid intoxication. When questioned, the butcher indicated the pigs had been raised locally. Local producers fed their pigs the potato remains and leavings from a nearby potato chip manufacturing company. Analysis of the pork chops by gradient reverse-phase HPLC confirmed the presence of the potato glycoalkaloids, solanine and chaconine, albeit at levels not normally associated with human intoxications. Implications of this finding are far reaching for sensitive individuals, who may inadvertently consume natural toxicants which they are normally able to avoid.

278 CHOLESTYRAMINE PROTECTION AGAINST OCHRATOXIN A (OTA) TOXICITY: ROLE OF OTA ADSORPTION BY THE RESIN AND BILE ACID ENTEROHEPATIC CIRCULATION.

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Ochratoxin A (OTA)may contaminate animal feed and food, leading to major health problems. The addition of cholestyramine (CHA), a bile acid binding resin, to OTA contaminated diets, reduced plasma levels of the toxin which was associated with enhanced fecal excretion. To elucidate the mechanism of action of CHA, we carried out *in vitro* experiments to determine whether the resin may adsorbe the toxin. Results showed that CHA adsorbes OTA and has greater affinity for OTA than for bile salts. However, the saturation of the resin was reached at higher levels with bile acids than with OTA. The binding of OTA to CHA was electrostatic in nature and reinforced by hydrophobic links. The results of the *in vivo* study showed that the toxin's plasma levels were significantly decreased in bile salt depleted rats compared to the control. The decreased OTA plasma levels in CHA treated animals may be explained by direct adsorption of the toxin and also by the alteration of bile salt biliary pool. (Supported by NSERC.)

279 EFFECT OF MENTHA PIPERITA AND MENTHOL ON CCL_4-INDUCED HEPATIC LIPID PEROXIDATION IN FEMALE RATS.

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Leaves of Mentha piperita (peppermint) are considered carminative and stomachic, and are used against pains and headache. Menthol, an active constituent of peppermint has been used as a counter irritant, an antiseptic and a stimulant. The effects of an aqueous extract of peppermint on CCl_4-induced lipid peroxidation, phosphorylase a activity and protein content in female rats were investigated. In vitro addition of menthol on hepatic lipid peroxidation was also studied. A 0.5 % peppermint extract was administered as an oral dose of 1 ml/kg twice/day for 21 days before and during CCl_4 treatment. This peppermint extract contained 1.24 mg/ml of peppermint solid per ml. The results demonstrate that peppermint administration markedly inhibited hepatic lipid peroxidation and produced a 32 % decrease in thiobarbituric acid reactive substances (TBARS) in livers of CCl_4- treated animals, but had no effect on phosphorylase a activity or protein content. In contrast, In vitro addition of menthol produced a dose-dependent increase in TBARS. Thus, administration of Mentha piperita provided significant protection against CCl_4-induced lipid peroxidation. However, decreased CCl_4-induced lipid peroxidation cannot be attributed directly to its active constituent menthol since addition of menthol to liver homogenates significantly increased TBARS. Therefore, other active ingredients in Mentha piperita may have provided protection against CCl_4- induced lipid peroxidation.

280 OXYGEN REACTIVE RADICALS PRODUCTION IN CELL CULTURE BY OKADAIC ACID AND THEIR IMPLICATION IN PROTEIN SYNTHESIS INHIBITION.

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Okadaic acid (OA), a diarrhetic shellfish toxin is a potent promoter of tumours in mouse skin and a specific inhibitor of protein phosphatases 1 and 2A. Recently it has been shown that OA inhibited protein synthesis in a cell-free system, with 50% inhibitory concentration of 6.3 x $10^{12}M^{-1}$ but the mechanism whereby this inhibition is mediated was still unclear. In this study, the effect of OA on protein synthesis in Vero cell was investigated. Protein synthesis was inhibited by OA alone in Vero cells in a concentration-dependent manner. Since OA also induced lipid peroxidation and likely oxygen reactive radicals, it was interesting to know whether these radicals impair the protein synthesis process. Therefore, SOD + catalase were added in the culture medium in the presence of OA and labelled leucine. These enzymes partially prevented the inhibition of protein synthesis induced by OA, indicating that the formation of high reactive free radicals could be one of the pathways this marine toxin induces its toxicity. Since this prevention was only partial, it was speculated that the production of oxygen reactive radical scavenged by SOD + catalase is not the main mechanism whereby OA induces its cytotoxicity. Vitamins E and C also prevent the lipid peroxidation induced by OA in cells, but failed to reduce the inhibition of protein synthesis to the same level, indicating that a more specific mechanism might be responsible for protein synthesis inhibition. However our results pointed to lipid peroxidation being a precocious phenomenon following the OA exposure, since a concentration wich enhanced MDA production was lower than that inducing significant cellular protein synthesis inhibition.

281 MECHANISM OF ASCIDIDEMIN TOXICITY TO CHO CELLS.

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Ascididemin, a marine pyrido[2,3,4-kl] acridine-based alkaloid, is cytotoxic

to tumor cell lines *in vitro* and *in vivo*. In this study ascididemin and two analogs are shown to produce DNA cleavage through the production of reactive oxygen species (ROS). A whole plasmid assay quantitated concentration dependent DNA cleavage for BC-109-1, BCMH-1-48 and BC-1-51 (ascididemin). This assay highlighted the enzyme independence of ascididemin DNA cleavage, the sole requirement for maximal cleavage being dithiothreitol (DTT). Reacting the compounds with NiCl or $FeSO_4^+$ failed to increase DNA cleavage. This argues against a Fenton-mediated mechanism of DNA cleavage. Likewise, chelating agents failed to provide protection. However, catalase and reduced glutathione, antioxidants, protected against DNA cleavage *in vitro* and protected Chinese hamster ovary (CHO) cells in culture from toxicity. This result is consistent with a mechanism based on production of ROS. Also consistent with this mechanism was the non-specific fragmentation of plasmid by ascididemin or its analogs visualized by PAGE. Cyclic voltammetry revealed less energy is necessary to reduce the three DNA cleaving analogs than the two inactive analogs. Treatment of mutant CHO cells highlighted a predilection of these drugs to induce single strand breaks in cells. Preclinical screening at NCI revealed the cytotoxic and antineoplastic features of ascididemin and BCMH-1-48. BC-109-1 is set to enter screening. It is therefore important to characterize the mechanism of DNA cleavage by these marine natural products, a mechanism likely responsible for their anticancer activity.

282 FUNCTIONAL CHARACTERIZATION OF ARYL HYDROCARBON RECEPTOR PHOSPHORYLATION MUTANTS IN THE MAJOR PHOSPHORYLATION REGION.

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The Aryl Hydrocarbon Receptor (AhR) is a ligand-dependent transcription factor that increases the transcription rate of many dioxin-responsive genes, including some drug-metabolizing enzymes and cytokines. Binding of a ligand, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) to AhR initiates the multi-step transformation process, including release of HSP90s, nuclear localization, dimerization with Ah Receptor Nuclear Translocator (ARNT), binding to the dioxin-responsive element (DRE), and recruitment of the basal transcription machinery proteins into the promoter region of the target genes. Indirect biochemical studies have shown that phosphorylation signal transduction pathway is extensively involved in this process. In addition, a 32P-radiolabeling study with mouse hepatoma cell line suggested that both AhR and ARNT are phosphoproteins and the major phosphorylation site in AhR is located within amino acids 368 - 398. This fragment has six hydroxylated amino acid residues that are potentially phosphorylated. In this study, all the six amino acid residues in the major phosphorylation fragment of AhR were changed to non-phosphorylatable amino acids by site-directed mutagenesis to determine the physiological role of the phosphorylation in this region. All testing mutants have similar DNA binding activity compared to that of wild-type (WT) AhR. However, the mutants which have alanines instead of threonine and serine showed lower TCDD-dependent transcription activity than WT AhR when they were expressed in the AhR-deficient cells along with DRE-reporter gene. These data suggest that phosphorylation on those residues might be important for the interaction of AhR with the crucial components of the cellular machinery that regulate the dioxin-responsive genes. (Funded in part by NIEHS Grant ES02515 and Center Grant ES01247.)

283 MODULATION OF BENZO[a]PYRENE TOXICITY BY 3'-METHOXY-4'-NITROFLAVONE.

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This laboratory has previously evaluated a number of flavone derivatives for aryl hydrocarbon receptor (AhR) agonist and antagonist potential using cell free and cell culture systems. The experiments presented here extend this line of investigation by testing the most potent AhR antagonist, 3'-methoxy-4'-nitroflavone (3'M4'NF), for in vivo activity. Male C57Bl/6J mice were treated with solvent, 60mg 3'M4'NF/kg, 150mg benzo[a]pyrene/kg, or concurrently with 60mg 3'M4'NF/kg and 150mg benzo[a]pyrene/kg. Fifty-four hours after benzo[a]pyrene (B[a]P) exposure, peripheral blood samples were collected. An index of genotoxicity was obtained by scoring the incidence of micronucleated reticulocytes. Results from this experiment demonstrate that 3'M4'NF is not genotoxic in this assay, but does significantly reduce the frequency of B[a]P-induced micronuclei. To isolate the effects that 3'M4'NF has on AhR-mediated gene transcription from possible modulations to constitutive P450 activities, a follow up study was conducted with wildtype C57Bl/6J mice and AhR knockout mice (C57Bl/6 background). These animals were treated with solvent, 3'M4'NF, B[a]P, or 3'M4'NF and B[a]P. In this experiment, peripheral blood samples were collected over a 72hr time frame, and animals were closely monitored for an extended period of time (120 hours post-B[a]P exposure) for overt signs of toxicity. Additionally, spleens were collected at time of sacrifice and histology was performed on these tissue samples. The peripheral blood micronucleus data again demonstrate that 3'M4'NF can antagonize the genotoxicity of B[a]P. Since attenuation of B[a]P genotoxicity occurred in both knockout and C57Bl wildtype animals, it is likely that AhR-dependent and independent mechanisms contribute to the protecting effect of 3'M4'NF. Even though this chemical was able to reduce bone marrow clastogenicity associated with B[a]P exposure, overt toxicity (death) and histological observations indicate that other toxicities are in fact enhanced. Further experiments will be needed to determine whether a 3'M4'NF dose level and/or treatment schedule can be developed which provides protection from B[a]P-induced DNA damage, while eliminating or minimizing the toxicity evident in splenic architecture. (This work was supported in part by NIEHS Training Grant ES07026 and Center Grant ES01247.)

284 ANALYSIS OF AH-RECEPTOR-MEDIATED SIGNAL TRANSDUCTION IN CELL CULTURE MODELS EXPOSED TO HYPOXIC CONDITIONS REVEALS LACK OF COMPETITION FOR THE ARNT PROTEIN.

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The aryl hydrocarbon nuclear translocator (ARNT) protein is a dimerization partner the AH receptor (AHR) and for hypoxia factor 1a (HIF-1a). Central questions with respect to ARNT are whether utilization of this protein by one pathway can affect another and whether sequestration of ARNT can account for some of the toxicity associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The current study focused exclusively on AHR-mediated changes in endogenous expression of CYP1A1 and the utilization of ARNT in cell culture models, placed under various hypoxic stimulation. AHR mediated signaling was evaluated by measuring the TCDD-mediated induction of the endogenous CYP1A1 mRNA and P4501A1 protein and the presence of AHR•ARNT heterodimers. The results show that (i) the concentration of ARNT protein tightly associated with nuclear structures following treatment of cells with hypoxia depends on the time course of exposure and the cell type being evaluated, (ii) hypoxia does not influence the concentration of ARNT but may affect the concentration of AHR, and (iii) hypoxia affects the induction of P4501A1 protein and CYP1A1 mRNA in most cells, but the effects ARE NOT related to reductions in AHR•ARNT complexes. The implications of these findings are that inhibition of AHR-mediated gene regulation under hypoxia may be cell specific and is the result of secondary effects related to the hypoxic condition of the cell and not functional interference of hypoxia and AHR-mediated signaling through competition for ARNT protein. (Supported by NIH grant #ES-08980.)

285 IDENTIFICATION OF A DOMAIN RESPONSIBLE FOR THE NEGATIVE FUNCTION OF rtARNTa IN AH RECEPTOR DEPENDENT SIGNALLING.

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Rainbow trout express two isoforms of ARNT, termed a and b, which have divergent functions in AHR-mediated signalling due to the presence of distinct COOH-terminal domains. rtARNTb functions positively in AHR-dependent signalling while rtARNTa has a negative impact on this pathway due to the failure of the rtARNTa• AHR heterodimer to bind DNA. Previous analyses revealed that the PST-rich COOH terminal domain of rtARNTa was responsible for repression of DNA binding. Studies were performed to determine whether domains existed within the PST-rich region that contributed to the negative function of this sequence. Computer analysis of the PST-rich COOH domain revealed that the last 20 amino acids are strongly hydrophobic. Truncation of the hydrophobic domain of rtARNTa restored the ability to complement P4501A1 induction in ARNT defective cells. Gel shift assays revealed that the gain in function was related to the recovery of DNA binding. Addition of the hydrophobic domain to mARNT or Gal4 resulted in proteins that were unable to bind DNA. These findings suggest that the hydrophobic domain and not the PST-rich region of rtARNTa is primarily responsible for repression of DNA binding. In addition, Gal4 analysis revealed that the truncated COOH terminal domains are capable of binding

DNA but lack strong transactivation function. Our previous studies revealed that transactivation of rtARNT is not needed for function in AHR-dependent signalling. Collectively, these results narrow the negative function of rtARNTa to the presence of a C-terminal hydrophobic domain and illustrates how changes in the C-terminal domain can affect protein function. (Supported by ES08980.)

286 ANALYSIS OF AH-RECEPTOR DOWN REGULATION IN CELL CULTURE LINES DERIVED FROM HUMAN TISSUES.

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Previous studies have documented that the AH-receptor (AHR) protein is rapidly degraded following ligand binding and that the concentration of AHR protein is a sensitive indicator of exposure to AHR agonists (typified by 2,3,7,8 tetrachlorodibenzo-p-dioxin; TCDD). To date, studies on AHR down regulation have utilized hepatic and non-hepatic cell culture lines as the *in vitro* model and rats and mice as the *in vivo* model of this process. Therefore, studies were initiated to investigate whether AHR protein was depleted form human cell culture lines that had been treated with TCDD. The cell culture lines used in this study included the HepG2 hepatoma line and the MCF-7 breast cancer line. Cells were treated with graded concentrations of TCDD for 16 hours and the level of AHR, AH-receptor nuclear translocator (ARNT), P4501A1 and ß-actin protein determined by quantitative Western blotting. The results showed that the AHR but not ARNT protein levels were depleted in both cell lines in a dose-dependent manner. A concentration of 500pM TCDD was sufficient to deplete 95% of the AHR in both cell lines. Of interest was the finding that the induction of P4501A1 and reduction in AHR protein showed distinct EC_{50} values in the HepG2 cell line. The EC_{50} for induction of P4501A1 was 8pM while the EC_{50} for AHR down regulation was approximately 80pM. Collectively, the results of these studies suggest that AHR down regulation occurs in humans and offers a model for evaluating the percentage of the AHR pool required for induction of P4501A1. (Supported by ES08980.)

287 OXIDATIVE STRESS IN FEMALE B6C3F1 MOUSE LIVER AND BRAIN FOLLOWING ACUTE AND SUBCHRONIC EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD).

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TCDD is a highly persistent trace environmental contaminant and is one of the most potent toxicants known to man. Our laboratory has reported an increase in the production of reactive oxygen species (ROS) in the brain of female B6C3F1 mice following subchronic exposure to TCDD at doses as low as 0.45 ng/kg/day. In this study, oxidative stress was characterized in the brain and liver following subchronic (0.15 to 150 ng/kg; 5 days/ wk for 13 weeks, *p.o.*) and acute exposure (0.001 to 100 µg/kg, *p.o.)* to TCDD in order to investigate the interaction between tissue concentration and time on the production of ROS. Seven days following acute administration of TCDD, mice were sacrificed and demonstrated an increase in liver superoxide anion production (SOAP), and thiobarbituric acid reactive substances (TBARS) at doses of 10 and 100 µg/kg. In addition, increases in glutathione, alpha tocopherol (AT), and a reduction of incorporation of O^{18} into macromolecule and lipid fractions in the liver suggests a "rebound" effect 7 days following TCDD treatment. The brain did not exhibit an increase in SOAP, TBARS, or O^{18} incorporation, but AT levels in the brain were increased following 10 and 100 µg/kg TCDD dosing. The preliminary data suggests that much higher tissue TCDD concentrations are required to elicit oxidative stress following acute dosing when compared to responses observed following subchronic TCDD dosing. Therefore, the mechanism of ROS production following TCDD exposure does not appear to be solely dependent upon the concentration of TCDD within the tissue. (This abstract does not necessarily represent EPA policy.) (BPS supported by EPA, CT 902908.)

288 INTERFERON γ ABROGATES CYPIA1 mRNA EXPRESSION IN MURINE SPLENOCYTES FOLLOWING TCDD EXPOSURE.

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These studies sought to begin to characterize the mechanism by which IFNγ attenuates the biochemical actions of TCDD in murine leukocytes. Cotreatment with TCDD and increasing doses of IFNγ in primary splenocytes and purified primary splenic B cells, produced a dose dependent decrease in the magnitude of TCDD-mediated CypIa1 mRNA induction. Interestingly, a gamma activated sequence (GAS) overlaps the DRE3, but not the DRE4 site, in the murine CypIA1 promoter which can be bound by STAT1α homodimers following activation of the IFNγ receptor by IFNγ. DNA binding studies employing either a DRE3 or DRE4 oligomer were undertaken in order to investigate the potential of competition for DNA binding between protein complexes activated following TCDD and IFNγ cotreatment. These studies demonstrated a loss of DNA binding to both DRE3 and DRE4 in the cotreated group (i.e. TCDD+ IFNγ) relative to the DNA binding demonstrated by nuclear proteins in the TCDD treated group when using either of the radiolabeled oligomers as a probe. These results suggest an interaction between the TCDD and IFNγ signal transduction pathways upstream of DNA binding. Our findings also negated the possibility that STAT1α homodimers were competing for DRE binding and therefore precluding the ability of TCDD to induce CypIA1 mRNA expression. Western analysis of AhR protein in the nuclear versus cytosolic fractions of mouse splenocytes demonstrated that the relative magnitude of nuclear and cytosolic AhR protein expression was comparable in the TCDD and cotreated groups, suggesting that there was no interference with AhR nuclear translocation. In summary, these findings suggest that the ability of IFNγ to attenuate TCDD-mediated CypIA1 induction in lymphoid cells is due to a disruption of AhR/ARNT DRE binding. The mechanism responsible for this phenomenon is presently unclear, but may be due to crosstalk between the signal transduction pathways activated by both of these agents. Moreover, the nature of this interaction does not appear to interfere with the ability of the AhR to translocate from the cytosol into the nucleus. (This work was supported in part by NIEHS Grants ES 07255 & ES 02520.)

289 CHARACTERIZATION OF THE AH RECEPTOR ASSOCIATED PROTEIN, ARA9.

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The unliganded aryl hydrocarbon receptor (AHR) is found in a complex with other proteins including the 90 kDa heat shock protein (Hsp90) and a 37 kDa protein we refer to as ARA9. We found that the three tetratricopeptide repeats found in the C-terminus of ARA9 are necessary and sufficient for interaction with the AHR complex. Conversely, the AHR's "repressor"/Hsp90 binding domain is required for interaction with ARA9. Because ARA9 closely resembles the 52-kDa FK506 binding protein (FKBP52), found in the unliganded glucocorticoid receptor (GR) complex, we compared the binding specificities of ARA9 and FKBP52 for AHR and GR. In coimmunoprecipitation experiments, ARA9 specifically associated with AHR-Hsp90 complex but not with GR-Hsp90 complexes. In addition, ARA9 showed a greater capacity than FKBP52 to associate with AHR-Hsp90 complexes. The biological importance of this interaction was demonstrated by the observation that in a yeast expression system ARA9 expression enhanced the response of AHR to the agonist β -napthoflavone, decreasing the EC_{50} by greater than 5-fold and increasing the maximal response 2.5 fold. In contrast, co-expression of FKBP52 had no effect on AHR signaling. In addition, although ARA9 contains a domain similar to that found in other FK506 binding proteins, ARA9 binding to ^{3}H-FK506 could not be detected. Finally, we have characterized the developmental and expression pattern of ARA9 in the developing mouse embryo and mapped the ARA9 locus to human chromosome 11q13.3.

290 TCDD AND OTHER AH-RECEPTOR AGONISTS ARE POTENT ANTI-ESTROGENS IN CULTURED CARP HEPATOCYTES.

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The yolk-protein vitellogenin (Vtg) is excreted by the liver of female, as well as male fish, in response to estrogenic compounds. An in vitro system was developed, using cultured hepatocytes from a genetically defined strain of carp (Cyprinus carpio), in which Vtg-production could be determined after exposure to 17β -estradiol or other estrogenic compounds. Vtg was measured by means of a competitive ELISA, using an anti-goldfish-Vtg antibody that also reacts specifically with carp-Vtg. Cyt. P4501A induction, measured as EROD, was used to assess Ah-receptor activation. The hepatocytes were co-exposed to 17β -estradiol and a concentration-range of anti-estrogens. 2,3,7,8-TCDD was shown to be approximately 10,000 times more potent than the model anti-estrogen tamoxifen. Anti-estrogenicity, measured asVtg-reduction, did not concur with EROD induction, but started at concentrations at which EROD was already maximally induced. Also other (weaker) Ah-receptor agonists were tested (PCB 126, β -naphtoflavone, benzo(a)pyrene, benzo(a)anthracene, PCB 118, hexachlorobenzene, MCDF and DIM. If these compounds caused Vtg-reduction, it was also at higher concentrations than the EROD induction. However, their relative anti-estrogenic potencies were approximately equal to their relative EROD inducing potencies; suggesting that the anti-estrogenicity is linked to the Ah-receptor. These results raise mechanistical questions, but also stress the potential importance of anti-estrogenicity in environmental endocrine disruption.

291 TWO AH RECEPTORS IN THE MARINE FISH, *FUNDULUS HETEROCLITUS*: TCDD BINDING AND TISSUE-SPECIFIC EXPRESSION.

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The aryl hydrocarbon receptor (AHR) is a bHLH-PAS family transcription factor through which halogenated aromatic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) cause altered gene expression and toxicity. We are examining the AHR signaling pathway in the estuarine fish *Fundulus heteroclitus*. *Fundulus* is abundant in both pristine and polluted estuaries and is capable of developing heritable xenobiotic resistance, and thus it represents a unique aquatic model for the study of AHR signaling. Unlike mammals, in which a single functional AHR has been reported to date, *Fundulus* has two distinct AHR genes, FhAHR1 and FhAHR2 (PNAS 94:13743). FhAHR1 and FhAHR2 cDNAs encode proteins of approximately 100 kDa. Both contain bHLH and PAS domains that share extensive sequence identity with mammalian AHRs; FhAHR1 is more closely related to mammalian AHRs than is FhAHR2. FhAHR1 and FhAHR2 cDNAs were expressed by *in vitro* transcription/translation and analyzed for ligand and DNA binding. Velocity sedimentation on sucrose gradients showed that both proteins specifically bind TCDD. FhAHR2 exhibits specific, TCDD-, and ARNT-dependent interactions with a mouse DRE sequence; the DNA-binding activity of FhAHR1 is as yet unresolved. The two *Fundulus* AHR genes display different tissue-specific patterns of expression; the FhAHR1 transcript is primarily expressed in brain, heart, and ovary, while the FhAHR2 transcript is equally abundant in many tissues. Sequence and phylogenetic analysis of AHRs in other fish demonstrates that AHR2 orthologs exist in several other species of bony and cartilaginous fish. The widespread distribution of AHR2 forms in fish suggests important functional roles for this protein. (Supported by NIEHS ES06272, ES07381, and ES05800.)

292 FUNCTIONAL DIVERSITY OF VERTEBRATE ARNT PROTEINS: ARNT2 IS THE PREDOMINANT FORM OF ARNT IN THE MARINE FISH, *FUNDULUS HETEROCLITUS*.

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The aryl hydrocarbon receptor nuclear translocator (ARNT) is a member of the bHLH/PAS protein superfamily. ARNT dimerizes with several PAS superfamily members, including the ligand-activated aryl hydrocarbon receptor (AHR), forming a complex that alters transcription by binding specific elements within the promoters of target genes. Two genes encode different forms of the protein in rodents: ARNT1, which is widely expressed, and ARNT2, which is limited to the brain and kidneys of adults and specific neural and branchial tissues of embryos. In an effort to characterize aryl hydrocarbon signaling mechanisms in *Fundulus heteroclitus*, a marine teleost that can develop heritable xenobiotic resistance, we have isolated a liver cDNA encoding an ARNT homolog. The protein exhibits AHR-dependent DNA binding capability typical of other vertebrate ARNTs. Unexpectedly, phylogenetic analysis reveals that the cDNA encodes an ARNT2. This is the only detectable ARNT sequence in *Fundulus* liver, gill, ovary, and brain, suggesting that ARNT2 is the predominant form of ARNT in this species. Also surprising is the relative lack of sequence identity with another fish ARNT protein, rainbow trout ARNTb, which we show forms a distinct branch outside the ARNT1 and ARNT2 clades in phylogenetic analyses. Functional diversity of ARNT proteins in fish may have significant implications for the assessment of aryl hydrocarbon effects on natural populations. The increasing use of fish models in toxicological studies underscores the importance of identifying taxon-specific roles of ARNT proteins and their potential dimeric partners in the PAS superfamily. (Supported by NIH: ES05800 ES07381, ES06272.)

293 ISOLATION AND EXPRESSION OF TWO RAINBOW TROUT ARYL HYDROCARBON RECEPTOR cDNAs WITH DISTINCT FUNCTIONS.

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Two rainbow trout (rt) aryl hydrocarbon receptors (AhRs) were cloned from a gonad cell line (RTG-2). Screening of two cDNA libraries produced two full length cDNAs, rtAhRα and rtAhRβ, which encode distinct AhR proteins of 1058 and 1059 amino acids. The two forms of rtAhR appear to be derived from separate genes. rtAhRα and rtAhRβ mRNAs are expressed in a tissue specific manner with highest expression of rtAhRβ in the heart. Exposure of RTG-2 cells to graded concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) caused a significant increase in rtAhRβ mRNA abundance whose time course and EC_{50} were essentially identical to that for CYP1A mRNA induction. Exposure of juvenile rainbow trout to an overtly toxic dose of TCDD caused a significant, 2-fold increase in rtAhRβ mRNA in liver. Both clones were expressed by *in vitro* transcription/translation producing 125 kDa proteins. Gel shift analysis demonstrated rtAhRα and rtAhRβ proteins dimerize with the aryl hydrocarbon nuclear translocator (rtARNTb) and recognize rainbow trout dioxin response elements (DREs). In COS-7 cells expressing rtAhRα or rtAhRβ in conjunction with rtARNTb, TCDD induced transcription of a reporter gene driven by the mouse CYP1A1 enhancer containing 4 DREs (pGudluc1.1). In COS-7 cells expressing rtAhRα and rtARNTb, TCDD induced transcription of a reporter gene driven by the rainbow trout CYP1A promoter and enhancer containing 2 DREs (prt1Aluc) approximately 9-fold. Unexpectedly, TCDD induced transcription of prt1Aluc only approximately 2-fold in COS-7 cells expressing rtAhRβ and rtARNTb. Determining the basis for the differential transcriptional activity of rtAhRα and rtAhRβ is currently underway. This study, in conjunction with the previous cloning of two rainbow trout ARNT transcripts, will now allow a detailed examination of the AhR pathway in the TCDD-sensitive species rainbow trout. (Supported by UW Sea Grant and NIEHS.)

294 TEMPORAL AND SPATIAL EXPRESSION OF zfAhR2, zfARNT2, AND zfP4501A mRNA IN DEVELOPING CONTROL AND TCDD-TREATED ZEBRAFISH.

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To understand the etiology of the tissue-specific signs of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity in developing zebrafish, the temporal and spatial expression of zebrafish aryl hydrocarbon receptor (zfAhR2) and aryl hydrocarbon nuclear traslocator (zfARNT2) mRNA was determined by *in situ* hybridization. To localize tissues with a functional AhR/ARNT pathway, expression of zebrafish cytochrome P4501A (zfP4501A) mRNA was also determined. Embryos within 4 hours of fertilization were exposed to vehicle or a LC_{100}egg concentration of TCDD for 1 hour. Control and TCDD-exposed embryos were sacrificed for *in situ* analysis at 12, 18, 24, 36, 48, 72, 96, 120, 144, and 168 hours post fertilization (hpf). Whole mount *in situ* hybridization was conducted on embryos up to

48 hpf using RNA probes specific for zfAhR2, zfARNT2, and zfP4501A. *In situ* hybridization of embryos older than 48 hpf was conducted on mounted tissue sections. zfAhR2 mRNA expression was first detected 12 hpf in both control and TCDD-treated embryos. zfAhR2 mRNA expression in the branchial arches, vasculature, and otic capsule was evident beginning 36 hpf. zfARNT2 mRNA was first detected at 12 hpf in control and treated embryos. zfARNT2 expression was localized in the fore-, mid-, and hindbrain at 18 hpf. Expression of zfARNT2 was also evident in the vasculature by 36 hpf. zfP4501A mRNA was not detected in control embryos. zfP4501A mRNA was observed in TCDD-treated embryos as early as 24 hpf and localized in the developing vasculature. zfP4501A was expressed in the vasculature of the head, trunk and tail beginning at 36 hpf in TCDD-treated embryos. Co-expression of zfAhR2, zfARNT2 and zfP4501A was most evident in the vasculature prior to the appearance of signs of TCDD toxicity. These results are consistent with previous studies suggesting that the vasculature is a primary target of TCDD toxicity during zebrafish development. (Supported by UW Sea Grant and NIEHS.)

295 ISOLATION AND EXPRESSION OF TWO ZEBRAFISH ARYL HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR (ARNT2) cDNAs WITH DISTINCT FUNCTIONS.

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In order to further establish zebrafish as a vertebrate model for studying the mechanism of TCDD toxicity it is necessary to characterize the aryl hydrocarbon receptor/aryl hydrocarbon receptor nuclear translocator (AhR/ARNT) signaling pathways in zebrafish. Previously we have cloned and characterized zebrafish AhR2 (zfAhR2). In this study two zfARNT cDNAs were isolated, expressed, and characterized. Degenerate PCR primers were used to amplify a partial zfARNT cDNA. The 974 bp amplicon was used to screen cDNA libraries derived from adult zebrafish and zebrafish liver cells. This probe failed to identify ARNT clones in either library. Rapid amplification of cDNA ends (RACE) was used to obtain the 5' and 3' ends of the cDNA. A single 0.8 kb 5' RACE product was isolated and found to be most similar to mouse ARNT2. Two 3' RACE products of 1.0 kb and 1.7 kb were amplified and found to terminate in polyA tails. Primers were designed to amplify 1.6 kb and 2.3 kb full length cDNAs named zfARNT2a and zfARNT2b, respectively. The deduced proteins zfARNT2a and zfARNT2b are identical over the first 424 amino acids but differ in their C-terminal domains as a result of alternative mRNA splicing. zfARNT2a and zfARNT2b encode proteins with apparent molecular weights of 49 kDa and 91 kDa, respectively. Both zfARNT2 splice variant mRNAs are expressed in zebrafish embryos, adult zebrafish, and liver cells. In COS-7 cells expressing zfARNT2b and zfAhR2, TCDD causes an approximately 20-fold induction of a dioxin responsive reporter gene. In cells expressing zfARNT2a, TCDD fails to induce reporter gene expression. DNA gel shift analysis suggests that the decreased function of zfARNT2a is due to inefficient binding of zfARNT2a/zfAHR2 complexes to dioxin responsive elements. The physiological significance of the two zebrafish ARNT2 proteins with different functional properties is currently under investigation. (Supported by UW Sea Grant and NIEHS.)

296 INTERSPECIES COMPARISON OF ARYL HYDROCARBON RECEPTOR (AhR) ACTIVITY IN A TRANSACTIVATION ASSAY.

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The potency of individual polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyl (PCBs) in causing toxicity correlates with their binding affinity to the AhR. While this structure-activity relationship appears to be consistent within a vertebrate class, there are differences in the relative potencies of some congeners between mammals and fish. In mammals, mono-*ortho* PCBs are the weakest group of AhR agonists among all PCDDs, PCDFs and PCBs tested and cause signs of toxicity at high levels of exposure. In fish, mono-*ortho* PCBs are nearly inactive as AhR agonists and are incapable of eliciting signs of TCDD toxicity in early life stages of rainbow trout. One possible explanation for the insensitivity to the mono-*ortho* PCBs is differences in fish and mammalian AhR structure and function. To investigate this possibility, transient transactivation assays in COS-7 cells were used to compare the potency and efficacy of PCDD, PCDF and PCB AhR agonists in inducing luciferase activity with two rainbow trout AhRs (rtAhRα and rtAhRβ), a zebrafish AhR (zfAhR2) and the human AhR with their species-specific ARNTs. For all four AhRs, differences in TCDD EC_{50} and maximal induction of luciferase activity were seen between a rainbow trout *cyp1A* DRE reporter (prt1Aluc) and a mouse *cyp1A1* DRE reporter (pGudluc1.1). Thus, responsiveness to TCDD of different AhRs is altered by changes in the context of the DREs. EC_{50s} and maximal induction levels were determined for seven PCDD, PCDF and PCB AhR agonists with each of the four AhR/ARNT pairs using prt1Aluc. The differences in potencies of the mono-*ortho* PCBs in producing toxicity in fish and mammals *in vivo*, is reflected at the molecular level. The human AhR responded to each mono-*ortho* PCBs while amongst fish AhRs, rtAhRα alone responded weakly to a single mono-*ortho* PCB. (Supported by UW Sea Grant and NIEHS.)

297 ACTIVATION OF REDOX-CONTROLLED TRANSCRIPTION FACTORS BY TCDD.

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2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the prototype agonist of the aromatic hydrocarbon (Ah) receptor, is a potent tumor promoter as well as a complete liver carcinogen. Since TCDD is not directly mutagenic, it has been proposed that it promotes neoplastic transformation through cellular signal transduction pathways resulting in the activation of proto-oncogene expression. Even though a clear oxidative stress response occurs in TCDD-treated female rats, in other animal models and in established cell lines, the oxidative stress induced by TCDD is difficult to evaluate and to quantitate by biochemical assays. To examine whether other biomarkers of oxidative stress could be more readily used to assess TCDD-mediated oxidative stress and to determine the extent of Ah receptor participation in such a response, we evaluated the effect of TCDD treatment on the activation of several transcription factors, including those in the NF-κB and AP-1 families, that are activated by changes in the redox state of cells. In an extension of prior results, we found that TCDD produced a sustained overexpression of AP-1 for at least 72 h in wild-type mouse hepatoma Hepa-1 cells, but not in Ah receptor-deficient Hepa-1-derived *c*35 cells or in CYP1A1-negative *c*37 cells. In addition, TCDD-treatment cause a significant increase in the DNA-binding activity of NF-κB, but not of the other transcription factors tested. Electrophoretic mobility supershift analyses with specific antibodies showed that increases in p50/p50 dimers accounted for most, but not all, the increases in NF-κB binding activity. TCDD-induced NF-κB increases did not take place in *c*35, *c*37, and ARNT-deficient *c*4 cells. These data suggest that TCDD causes the sustained activation of NF-κB and AP-1 possibly by an oxidative stress signal that is CYP1A1- and Ah receptor complex-dependent. (Supported by NIH Grants R01 ES06273 and P30 ES06096.)

298 CROSS-TALK BETWEEN THE ARYL HYDROCARBON RECEPTOR (AHR) AND HYPOXIA SIGNALING PATHWAYS.

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Aryl hydrocarbon receptor (AHR), aryl hydrocarbon receptor nuclear translocator (ARNT), and hypoxia inducible factor 1α (HIF-1α) are involved in two important signaling pathways in the cell. The AHR and ARNT are dimeric partners that transcriptionally up-regulate genes involved in the metabolism of xenobiotic compounds. ARNT forms a heterodimer with HIF-1α under hypoxic conditions, and together they activate a number of genes whose products regulate hematopoiesis in response to oxygen deprivation. The fact that ARNT is essential for both pathways may result in interference between these two pathways. In this report, the cross-talk between the two pathways is examined. B-1 cells, a Hep3B-derived cell line, express luciferase activity in response to hypoxia stimuli. The administration of 1.25 nM TCDD reduced the response of B-1 to hypoxia inducers by approximately 30%. H4IIE-luc cells and Hepa1 cells express luciferase and CYP1A1, respectively, in response to AHR-active compounds. The administration of a hypoxia inducer, desferrioxamine, resulted in a dose-dependent reduction of AHR-mediated luciferase activity in H4IIE-luc cells and CYP1A1 activity in Hepa1 cells. A complete inhibition of luciferase activity by 10 μ M desferrioxamine and a 67% of inhibition of CYP1A1 activity by 250 μ M desferrioxamine were observed in H4IIE-luc and Hepa1 cells, respectively. Together, these results suggest that an interference exists between the hypoxia and Ah receptor signaling pathways.

299 GENOTOXICITY STUDIES WITH BINARY, TERTIARY AND ISOLATED FRACTIONS OF COMPLEX ENVIRONMENTAL MIXTURES.

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This project addresses the development of in vitro methods for assessing the toxicity of complex environmental mixtures. Contaminated media at Superfund sites typically consist of complex mixtures of organic and inorganic chemicals. While a site may host a limited number of inorganic chemicals, literally hundreds of organic chemicals may be present at a particular site. These mixtures are difficult to characterize, both analytically and toxicologically. This project has employed a microbial genotoxicity assay to analyze binary and tertiary mixtures of polycyclic aromatic hydrocarbons (PAHs), as well as complex mixtures of PAHs and isolated PAH fractions. The model PAH carcinogen benzo(a)pyrene (BAP) induced 227 40 net revertants at a dose of 0.3 mM, while a mixture of BAP at 0.3 mM and chrysene at 1.0 mM induced 105 12 net revertants. While these responses were within the anticipated biological variability of the assay, a dose-response gradient was observed with the BAP-chrysene mixture suggesting an interaction that influenced genotoxicity. Studies with complex mixtures and isolated fractions indicate that the BAP content of the PAH mixture cannot be used to predict genotoxicity. An extract of a Manufactured Gas Plant Residue (MGPR) was separated into four fractions using Thin Layer Chromatography (TLC). The extract of Fraction 3 from site 4 contained 26.61 ppm BAP and induced 1,339 77 net revertants at a dose of 1 mg of residue per plate, whereas the extract of Fraction 4 contained only 0.88 ppm BAP and induced 1,350 86 net revertants. It is unknown whether the response observed in Fraction 4 was a result of high molecular weight PAHs or possibly alkyl substituted PAHs. (Supported by NIEHS SBRP Grant No. P42 ES004917 and ATSDR Grant No. TU68450502.)

300 NUCLEAR RECEPTOR COREPRESSOR INTERACTIONS WITH Ah RECEPTOR SIGNALING.

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Corepressors, such as silencing mediator for retinoic acid and thyroid hormone receptor (SMRT), interact with thyroid hormone receptor (TR) and retinoic acid receptor (RAR) to repress transcriptional activation. The aryl hydrocarbon receptor (AhR) mediates induction of CYP1A1 gene expression by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in human breast cancer cells. This study investigates the effects of SMRT on AhR-mediated induction of CYP1A1 in MCF-7 human breast cancer cells. TCDD induced CAT activity in MCF-7 cells transfected with Ah-responsive pRNH11c containing a human CYP1A1 gene promoter insert (-1142 to +2434) and cotransfection with pCMX (an SMRT expression plasmid) inhibited the induction response. Coincubation of AhR/Arnt with SMRT decreased formation of the AhR-Arnt-[^{32}P]-dioxin response element (DRE) retarded band in a gel mobility shift assay. A time course study showed that TCDD-induced maximal AhR-Arnt binding to [^{32}P]DRE with 2 min in the absence of *in vitro* expressed SMRT; however, after coincubation with SMRT, maximal binding was not observed until 10 min. Coimmunoprecipitation experiments showed that SMRT physically interacted with both AhR and Arnt *in vitro*. SMRT also decreased the luciferase activity of GAL4-dependent luciferase reporter with a GAL4-Arnt fusion protein in MCF-7 cells transfected with expression vectors containing GAL4DBD-Arnt, GAL4-luciferase reporter, and SMRT. These data suggest that SMRT functions as a transcriptional corepressor for AhR-mediated gene expression in MCF-7 cells.

301 PRO-OXIDANT METALS UNCOUPLE THE COORDINATE EXPRESSION OF PHASE I AND PHASE II GENES BY AH RECEPTOR LIGANDS.

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Recent reports describing the redox sensitivity of aromatic hydrocarbon receptor (AHR) activation led us to test the hypothesis that pro-oxidant metals alter AHR ligand-inducible expression of Phase I and Phase II genes responsible for xenobiotic metabolism. Our results in Hepa-1 cells demonstrate that pro-oxidant metals differentially alter 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-inducible (TCDD) *Cyp1a1* and *Nqo1* mRNA accumulation, two markers of Phase I and Phase II detoxification, respectively. $CdCl_2$ and $NaAsO_2$ did not alter *Cyp1a1* mRNA levels but enhanced TCDD-inducible levels of *Nqo1* mRNA, probably as a result of electrophile response element-dependent (EpRE) gene activation. $K_2Cr_2O_7$, on the other hand, inhibited mRNA accumulation for both *Cyp1a1* and *Nqo1*, with the greater effect on *Nqo1*. $NiSO_4$ did not alter mRNA levels for either gene. CYP1A1 and NQO1 activities were also measured to determine whether changes in mRNA accumulation resulted in altered biological activity. All enzyme activity changes mirrored the changes in mRNA levels. To test the relative contribution of AhRE-dependent and EpRE-dependent inducible gene expression, we used Hepa-1 cell lines with stably integrated AhRE-luciferase or EpRE-β -galactosidase reporters. For the AhRE-driven reporter, $CdCl_2$ increased and $K_2Cr_2O_7$ decreased the TCDD-inducible luciferase activity, while $NaAsO_2$ and $NiSo_4$ had no effect at noncytotoxic doses. For the EpRE-driven reporter, both $CdCl_2$ and $NaAsO_2$ increased β -galactosidase activity, while $K_2Cr_2O_7$ and $NiSO_4$ had no effect. These data indicate that co-exposure to pro-oxidant metals and ligands for the AHR uncouples the coordinate regulation of Phase I and Phase II genes. This imbalance between metabolic activation by Phase I genes and conjugation by Phase II genes may have important ramifications for the toxicity of complex mixtures. (Supported by NIEHS ES06273 and U.S. EPA STAR Fellowship Grant.)

302 MOLYBDATE INHIBITION OF ARYL HYDROCARBON RECEPTOR ACTIVATION.

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The aryl hydrocarbon receptor (AHR) is a cytosolic basic helix-loop-helix protein that associates with a chaperone complex including two molecules of heat shock protein 90 (HSP90). After ligand binding the AHR dissociates from its chaperone complex and translocates into the nucleus where it heterodimerizes with its DNA binding partner, the AH receptor nuclear translocator (ARNT), and activates specific genes. The gene activation of other HSP90-bound molecules such as the glucocorticoid receptor (GR) has been shown to be inhibited by molybdate, blocking the receptor's nuclear import by stabilizing the GR-chaperone complex interaction. Therefore the objective of this study was to ascertain whether molybdate inhibits AHR gene activation in a similar manner. Using HepG2 cells (human liver carcinoma) stably transfected with a luciferase reporter gene that is regulated by the cytochrome p450 1A1 promoter we demonstrated that sodium molybdate inhibits AHR gene activation in a dose-dependent manner. We confirmed this result through western blot analysis of p450 1A1 protein levels in whole cell lysates in HepG2 as well as Hepa-1 cells (murine hepatoma). Western blots of whole cell lysates also revealed that sodium molybdate could stabilize the AHR from agonist-induced down-regulation. Western blots of nuclear lysates in HepG2 cells demonstrated that sodium molybdate causes a dramatic reduction in agonist-induced nuclear AHR protein levels. Furthermore, gel mobility shift assays using a [^{32}P] dioxin responsive element and nuclear extracts from both Hepa-1 and HepG2 cells indicate that molybdate greatly diminishes the amount of active nuclear AHR/ARNT complex that can be isolated. These results suggest that dissociation of AHR from its chaperone complex is an essential aspect of AHR-mediated signaling and provide a model for further study.

303 ALTERATION OF OXYTOCIN- AND VASOPRESSIN-INDUCED CALCIUM OSCILLATIONS IN A RAT LIVER CELL LINE BY TCDD.

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A communication-competent rat liver cell line (Clone 9) was examined as a model system to investigate the effects of graded concentrations of 2,3,7,8-TCDD on the frequency of stable intracellular Ca^{2+} oscillations induced by oxytocin and vasopressin. Control Clone 9 cells treated with oxytocin (0 - 10 μM) or vasopressin (0 - 1 μM) exhibit stable, long term oscillations beginning at 10 nM or 0.1 nM for oxytocin and vasopressin, respectively. These oscillations reached maximal frequencies at 75 mHz and 100 mHz, respectively. Fast Fourier Transform analysis indicated that the inositol trisphosphate-sensitive pool contributes approximately 2/3 of the total frequency of Ca^{2+} oscillations while the contribution of a ryanodine-sensitive pool is approximately 1/3. To study the effect of TCDD on hormone-induced Ca^{2+} oscillations, cells were treated overnight with 0.1 to 10 nM TCDD and the frequency of Ca^{2+} oscillations was monitored following stimulation with dif-

ferent oxytocin or vasopressin concentrations. Results indicated that TCDD has no effect on basal intracellular Ca^{2+} levels, however, TCDD treated cells have a distinct frequency-hormone dose relationship that is significantly different from untreated Clone 9 cells. These studies indicate that, in addition to genomic effects mediated through the Ah receptor, TCDD can alter signal transduction mediated by frequency encoded Ca^{2+} signals. (Supported by NIH P42-ES04917 and P30-ES09106.)

304 ISOLATION AND CHARACTERIZATION OF NOVEL ARNT-INTERACTING PROTEINS.

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The aryl hydrocarbon receptor nuclear translocator protein (ARNT) was first identified as a heterodimerization partner of the ligand-activated aryl hydrocarbon receptor (AhR). This complex mediates the transcription of genes via the xenobiotic response element. ARNT and AhR are members of the basic helix-loop-helix Per-Arnt-Sim (bHLH-PAS) family of transcription factors. ARNT has more recently been shown to heterodimerize with the bHLH-PAS protein hypoxia inducible factor-1α, leading to activation of genes regulated by the hypoxia response element. In addition, Tango, the Drosophila ARNT homolog, forms heterodimers with the bHLH-PAS proteins Single-minded and Trachealess to regulate neurogenesis and treachea development via the central nervous system midline enhancer element. As ARNT appears to play a central role in this family, we have employed the yeast two-hybrid system to isolate novel ARNT-interacting proteins. One clone, termed ARNT interacting protein-1 (AINT-1), interacts with ARNT in vitro, as determined by GST pull-down assays and has strong transactivational ability in yeast. The full length cDNA encoding this protein reveals little homology to published sequences. Finally, Northern analyses and in situ hybridizations provide evidence that AINT-1 is important in embryonic development and tissue differentiation. These results suggest that AINT-1 may play an important role in bHLH-PAS protein function.

305 THE LACK OF ALDH3A1 INDUCTION BY TCDD IN CORNEAL EPITHELIAL CELLS MAY INVOLVE TISSUE-SPECIFIC REGULATORY PROTEINS THAT COMPETE WITH THE AHR-ARNT COMPLEX.

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The cytosolic aldehyde dehydrogenase (ALDH3A1) is inducible in liver cells by 2,3,7,8,-tetrachlorodibenzo-*p*-dioxin (TCDD), and constitutively elevated in extrahepatic tissues such as cornea, stomach, skin, urinary bladder and lungs. Induction by TCDD requires the aromatic hydrocarbon receptor (AHR) and its counterpart aromatic nuclear translocator protein (ARNT), and operates via the aromatic responsive elements (AhRE). Three corneal epithelial cell lines from human, rat and rabbit were used to study the regulation of *Aldh3a1* gene by TCDD using various deletions of the 3.2 kb 5'flanking region linked to luciferase reporter gene. Our results showed that this 5' flanking region did not respond to TCDD inducibility, although both AHR and ARNT were present and functional in all three cell lines. In addition, we found that AhRE motifs can drive high luciferase expression in corneal cells, indicating that AHR might be involved in the constitutive tissue-specific *Aldh3a1* expression. The latter can be ruled out since ALDH3A1 mRNA levels were found in high levels in the eye and stomach of AHR-knockout mice. Pax-6, a transcription factor that regulates lens determination and crystallin gene expression, is known to be present in corneal epithelium. Alignment of the Pax-6 consensus binding site with the AhRE sequences of the *Aldh3a1* gene revealed a 55-75% overlap between these two responsive elements. It is possible that Pax-6, as a corneal regulatory protein, binds to AhRE motifs and prevents the up-regulation of *Aldh3a1* via the TCDD-activated AHR-ARNT transcriptional complex. *(Supported by NIH EY11490.)*

306 THE CONTRIBUTION OF SEVERAL HYDROXY- AND DIHYDROXYBENZENES FOUND IN CIGARETTE SMOKE TO THE OVERALL CYTOTOXICITY OF THE CIGARETTE SMOKE MIXTURE.

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The complex mixture of cigarette smoke is reported to contain over 3000 chemicals representative of several chemical classes, including hydroxy- and dihydroxybenzenes. Examples of hydroxy and dihydroxybenzenes detected in cigarette smoke include phenol, catechol, resorcinol, hydroquinone, and the three isomers of cresol. Previous studies have examined the toxicity of these compounds as pure chemicals; however, there is no information on the contribution of these compounds to the overall toxicity of cigarette smoke. The specific objective of this study was to determine if the hydroxy- and dihydroxybenzenes phenol, catechol, resorcinol, hydroquinone, and cresols are major contributors to the cytotoxic potential of cigarette smoke. In a preliminary study, the cytotoxicity of each pure chemical to Chinese hamster ovary cells was assessed using the neutral red uptake assay. The order of toxicity from most to least cytotoxic was hydroquinone > catechol > cresols > phenol > resorcinol. The cytotoxicity of cigarette smoke condensate was then assessed after each single chemical was added back to the cigarette smoke condensate at concentrations up to 100 times the concentration normally reported for the chemical in cigarette smoke. The cytotoxicity of cigarette smoke was not increased when cresols, phenol, or resorcinol was added back to the condensate even at concentrations 100 times what is normally reported in cigarette smoke. The most cytotoxic dihydroxybenzenes, hydroquinone and catechol, did not increase the cytotoxicity of cigarette smoke until concentrations approximately five times normally present in cigarette smoke were added back to the condensate. These results support the hypothesis that these hydroxy- and dihydroxybenzenes are not biologically important cytotoxic chemicals in cigarette smoke.

307 SMOKELESS TOBACCO INDUCED MODULATION OF p53 GENE AND PROTECTIVE EFFECT OF ANTIOXIDANTS.

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Oral cancers account for 3% of all the cancers in the U.S.A. and is the seventh most common cancer. The p53 gene is defined as a tumor suppressor gene, and mutations in p53 occur in high frequency in most of the common types of human cancer. Previously, we have reported the oxidative stress and DNA damage in human oral cells following treatment with different concentrations of smokeless tobacco extract (STE). Apoptopic cell death of human oral keratinocytes was also increased with increasing concentration of STE. In the present study, human oral keratinocytes were treated with (0-300 μg/ml) of STE for 24 hours and changes in the expression of p53 gene was measured by reverse transcriptase polymerase chain reaction (RT-PCR). Thirty-five cycles were performed for 5 min at 94°C and β–tubulin was used as the housekeeping gene. Approximately a 2.0-fold increase in p53 gene expression was observed following incubation of the cells with 100 μg/ml of STE, beyond which the expression of p53 dramatically decreased with higher concentrations, confirming increased apoptopic cell death with a higher concentrations of STE as reported by us earlier. Furthermore, the comparative abilities of vitamin C (75 μM), vitamin E (75 μM), a combination of vitamins C plus E (75 μM each) and a novel grape seed proanthocyanidin extract (GSPE; IH636) (100 μg/ml) were determined. The results demonstrate that STE significantly modulates p53 gene expression, and approximately 22-46% decreases in STE altered parameters were observed with antioxidants. GSPE demonstrated significantly better protection as compared to vitamins C and E, singly and in combination.

308 IN VITRO RESPONSE OF MOUSE OVARIAN SMALL FOLLICLES TO EPOXIDES OF 1,3-BUTADIENE.

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1,3-Butadiene (BD), a widely used occupational chemical, is a known carcinogen in laboratory animals. The epoxide metabolites butadiene monoepoxide (BMO) and butadiene diepoxide (BDE) are thought to play a critical

role in its toxicity. Previous *in vivo* studies in our laboratory found these epoxides to be ovotoxic. Also, the rate of nicotinamide alkylation was 5 times greater for BDE than BMO. BDE also exhibited 3.8 times greater alkylating activity than that of the related ovotoxicant, 4-vinylcyclohexene diepoxide (VCD). Our laboratory has developed a method to isolate mouse small ovarian follicles for *in vitro* studies. Increased Trypan blue dye uptake ($p<0.05$) was observed in granulosa cells, but not oocytes, of small follicles incubated with VCD (76 μ M). The purpose of this study was to investigate *in vitro* effects of BMO and BDE on isolated follicles. Trypan blue dye uptake in mouse small follicles incubated with 600 μ M BMO was not significant ($p>0.05$) at any time point. Conversely, when compared with control media, follicles incubated with 76 μ M BDE exhibited increased ($p<0.05$) dye uptake into granulosa cells at 4 h (12.5± 0% follicles incorporating dye), 6 h (21± 3.5% follicles), and 24 h (45.7± 9.7% follicles). Following 24 h incubation with BDE, not all follicles were intact (oocyte surrounded by granulosa cells), however, the initial number of follicles incubated could be accounted for by counting intact follicles and isolated oocytes. There was no LDH release into the media. Thus, we propose that integrity of follicles but not cellular membranes is compromised. Our results suggest that BDE has a similar effect to VCD and is more potent than BMO. This further supports *in vivo* structure activity studies indicating that the diepoxide metabolite is the more ovotoxic form. Based on these findings, measurement of Trypan blue dye uptake in isolated small follicles incubated with a variety of environmental chemicals may provide an *in vitro* method to assist in the evaluation of ovotoxic chemicals. (Center Grant ES06694, ADCRC #9808, and CMA.)

309 THE RELATIVE TOXICITY OF PARTICLES IN RAT LUNG TISSUE SLICE CULTURES.

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The purpose of the present study was to assess the relative toxicity of instilled diesel soot (DS), residual oil fly ash (ROFA), or carbon black (CB) particles (1 mg/ml) in rat lung tissue slices. Tissue viability parameters [morphology, adenosine triphosphate (ATP), protein synthesis], and secreted cytokines [macrophage inflammatory protein-2 (MIP-2), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β)] were assessed on days 1, 4, 7, and 11 post-slicing. Transmission electron microscopy revealed particles free within alveoli, airways, and interstitium, and intracellularly within alveolar and interstitial macrophages, and alveolar type I cells. ATP content and protein synthesis in ROFA- or DS-treated slices were generally less than observed in vehicle-treated slices at early harvest time points. At harvest days 0, 1, and 4, ROFA-treated slices contained 58%, 15%, and 46% of the ATP content of control slices, respectively. DS-treated slices had a reduced amount of ATP (59% of control) only at day 4. Protein synthesis at harvest days 1 and 4 for ROFA-treated slices was 53% and 56% of controls, respectively, while that of DS-treated slices was 66% and 61%, respectively. For cytokine analysis of culture medium, MIP-2 was significantly induced at days 7 and 11 in ROFA-treated slices (213% and 218% of controls, respectively) and in DS-treated slices (157% and 202%, respectively). TNF-α did not demonstrate a significant particle effect. IL-1β was undetectable for all slice cultures throughout the culture time period. CB-treated lung slices did not produce any aberrations from values of control slices in any of these assays. Treatment of lung slices with ROFA produced greater deviations from control slices than DS, and CB-treated slices were indistinguishable from control slices, suggesting that ROFA is more toxic than DS, and toxicity of CB could not be detected in lung slice systems. (Support from DOE/OBER and NIH grant ES 07665-01.)

310 THE RELATIVE TOXICITY OF PARTICLES IN A549 CELL CULTURES.

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A549 cells were individually treated with 40 μ g/cm^2 of seven different particulate materials [nickel subsulfide (Ni_3S_2), crystalline silica (S), residual oil fly ash (ROFA), diesel soot (DS), carbon black (CB), titanium dioxide (TiO_2), and an ambient air particulate sample from Ottawa, Canada (OAA, dust EHC-93)] for 20 hr. Cultures were assessed 18 hrs post-treatment for viability [lactate dehydrogenase (LDH) concentration in the culture media, cellular adenosine triphosphate (ATP) content, and protein synthesis] and cytokines [interleukin-8 (IL-8) and growth-regulated oncogene-alpha (gro-α) proteins] in the culture media by the ELISA technique and intracellularly by immunocytochemistry and flow cytometry. Treatment with Ni_3S_2 induced the greatest decrease in viability parameters, which were significantly different from all other particle treatments and controls. Because of this high toxicity, the IL-8 and gro-α protein levels were low (similar to or lower than controls). ROFA treatment was second to Ni_3S_2 in toxicity, but induced the highest levels of IL-8 and gro-α protein. Viability parameters and cytokine analyses for treatment with DS, CB, or TiO_2, as well as vehicle-treated controls were not statistically different. Treatment with S produced LDH release similar to ROFA treatment; however, the ATP content, protein synthesis, and cytokine analyses reflected levels similar to vehicle-treated controls, DS, CB, and TiO_2. The toxicity of OAA was generally less than ROFA, but greater than vehicle-treated controls or TiO_2. In the LDH and secreted IL-8 assays, OAA particles induced a significantly higher response than DS. The toxicity of S was difficult to interpret, possibly because our bioassay system lacked macrophages, which appear to play a significant role in the pathogenesis of S-induced lung disease in vivo. This system determined the relative toxicity as follows: Ni_3S_2 > ROFA ≥ OAA ≥ DS, CB, TiO_2. (Support from DOE/OBER and NIH grant ES 07665-01.)

311 ANALYSIS OF DIFFERENCES IN THE MECHANISM OF TOXICITY OF *ORTHO-* AND *PARA*-ISOPROPYLPHENOL ISOMERS IN CULTURED RAT LIVER CELLS USING KINETIC FLUORESCENCE BIOASSAYS.

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In order to examine possible differences in the mechanism of toxicity of alkylphenol isomers, we compared the effects of *ortho* and *para* isopropylphenol (2-IP, 4-IP) on several cellular functions in cultured rat liver cells (Clone 9, ATCC) using kinetic fluorescence bioassays and a Meridian Ultima laser cytometer. Parameters monitored included intracellular glutathione (GSH), gap junction-mediated intercellular communication (GJIC), calcium levels, intracellular pH, reactive oxygen species (ROS) generation, mitochondrial and plasma membrane potentials (MMP and PMP), and cell death. Cells were exposed to concentrations of test compounds ranging from 1 to 1000 μM, and all parameters were monitored directly after addition at 15 sec intervals for at least 10 min. No differences in effect on MMP or GJIC were observed between the two isomers. Both compounds elicited a minor drop in GJIC at 1 and 10 μM, followed by almost complete inhibition of GJIC at 100 μM. Similarly, both isomers elicited a concentration-dependent decrease in MMP, with significant effects being seen at concentrations as low as 1-10 μM. However, isomer-dependent differences were seen in all other parameters monitored. 2-IP was more potent in depleting GSH, inhibiting PMP, and decreasing intracellular pH than was 4-IP. No changes in calcium levels were seen with 2-IP until the highest concentration (1000 μM), whereas 4-IP caused a significant spike in calcium at a concentration of 100 μM. In 48 h cell proliferation assays 2-IP was more cytotoxic than 4-IP (IC50s were 103 μM and 222 μM, respectively). In summary, the most significant effects observed in the present study which may contribute to differences in cytotoxic potency among alkylphenol isomers in cultured rat liver cells include the rate of depletion of intracellular glutathione and effects on PMP. (Supported in part by NIH grants ES06016 and P42-ES04917.)

312 BIDRIN-INDUCED RENAL TUBULAR CYTOTOXICITY: ROLE OF OXIDATIVE STRESS AND ANTIOXIDANT PROTECTION.

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Bidrin (BD) is an organophosphate (OP) insecticide formulation containing dicrotophos. OPs are efficient pesticides and are often misused, resulting in detrimental exposure to humans and other non-target species from environmental and occupational sources. BD's main mechanism of toxicity is inhibition of acetylcholinesterase (AChE), hindering nerve conduction. Studies have shown that OPs induce renal tubular necrosis that does not correlate with the degree of AChE suppression. This suggests additional mechanisms of toxicity. Since reactive oxygen species (ROS) are known as mediators of many toxin-induced renal injuries, we investigated whether ROS play a role in BD-induced renal tubular epithelial cell ($LLC-PK_1$) toxicity. $LLC-PK_1$ cell death, determined by lactate dehydrogenase (LDH) release, increased concentration- and time-dependently following exposure of cells to 1000-

2000ppm BD for 6-48hrs. Cell damage following incubation with 1250ppm BD for 24hrs was found to decrease in a concentration-related manner by antioxidants 2-methylaminochroman (2-MAC: 0.3-2.5μM) and desferrioxamine (DFO: 0.25-2mM). The greatest reductions in LDH (n=3, ANOVA, $p<0.05$, mean±SE) were produced by DFO 2mM and 2-MAC 2.5μM, both significantly lower than BD alone (DFO: 29.67±3.29 vs. BD: 47.67±1.46; 2-MAC: 26.67±2.61 vs. BD: 47.67±1.46, $p<0.05$). H_2O_2 levels were significantly elevated following exposure to 1250ppm BD. Significantly increased malondialdehyde (MDA) formation compared to the control was also found in BD-exposed cells indicating enhanced lipid peroxidation. MDA generation was significantly suppressed by 2-MAC and DFO. These results implicate ROS and accompanying lipid peroxidation, at least in part, in the toxic effects of BD on kidney cells and suggest that oxidative stress may play a role in the pathogenesis of BD-induced acute tubular necrosis and renal dysfunction observed in OP poisonings.

313 CADMIUM TOXICITY IN RAT HEPATIC STELLATE CELLS.

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Hepatotoxicity caused by exposure to toxic agents is often associated with the pro-oxidant activity of the agent. Cadmium (Cd) is a very toxic transition metal with pro-oxidant activity. This study examined the effects of Cd on lipid peroxidation damage, glutathione content, glutathione peroxidase and superoxide dismutase activity on rat hepatic stellate cells (CFSG-2G). Cells were exposed during 24 and 48 hs to 0.3 μ M $CdCl_2$ or 24hs to 3 μ M $CdCl_2$. Under these experimental conditions cells did not present lysosomal (neutral red probe) or mitochondrial (MTT probe) damage. Lipid peroxidation was measured as malondialdehyde production in the presence of thiobarbituric acid. Reduced glutathione was quantitated by means of the Ellman reaction, glutathione peroxidase and superoxide dismutase were determined spectrophotometrically. Intracellular Ca was determined by arsenazo III. Lipid peroxidation damage increased slightly in all experimental conditions, while GSH content diminished 22% in 0.3 μ M Cd treated cells and 47% with higher Cd concentration. Intracellular Ca increased approximately 50% in 0.3 μ M $CdCl_2$ treated cells and 135% in the 3 μ M treatment. SOD activity diminished principally in 24 hs treated cells, 51% in 0.3 and 56 % in 3 μ M $CdCl_2$ treated cells. GSH peroxidase activity decreased only in cells treated with 3 μ M $CdCl_2$ during 24 hs. No significative changes of glutathione reductase activity were found in any treatment.

Cd produced damage on rat hepatic stellate cells through an oxidative stress mechanism and altered Ca homeostasis.

314 DIFFERENTIAL EFFECTS OF SERA ON BASAL AND CHEMICAL-INDUCED CELL PROLIFERATION AND DIFFERENTIATION IN RAT EMBRYONIC MIDBRAIN AND LIMB BUD CELL MICRO-MASS CULTURE.

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Fetal bovine serum (FBS) and NuSerum have been widely used for cell culture including embryonic cell micromass culture. The type and components of sera in cell culture media have been implicated in the cell proliferation and differentiation since they contain many different growth modulating factors. FBS contains very high proteins, while NuSerum contains many hormones. In this study, we compared the effects of FBS and NuSerum on the cell proliferation and differentiation of rat embryonic midbrain (MB) and limb bud (LB) cells and their effects on chemical-induced teratogenicity. Cells derived from MB and LB of 12.5 day-old rat embryo were cultured in the medium containing 10% of FBS or NuSerum in the absence or in the presence of teratogenic compounds, ethylenethiourea (ETU) or ochratoxin A (OTA) for 4 days. Cell proliferation was assessed by neutral red staining and differentiation was determined by measuring of foci area after hematoxylin staining (MB cells) or alcian blue staining (LB cells). Cell differentiation was significantly higher in FBS medium than that in NuSerum medium while cell proliferation was not different. The concentration of ETU inhibiting 50% of cell proliferation and differentiation (IP_{50} and ID_{50}) were 11.4, 27.7 μ g/ml (NuSerum medium) and 23.0, 9.2 μ g/ml (FBS medium) in MB cells. In MB and LB cells, IP_{50} and ID_{50} of OTA were 10 fold higher in Nuserum medium than in FBS medium. IP_{50} and ID_{50} of OTA in NuSerum medium containing bovine serum albumin were corresponding with those in FBS medium. These results show that the type and components of sera in culture media may be important in the teratogenic potential of chemicals by alteration of chemical-induced cell proliferation and differentiation.

315 EXPRESSION OF HUMAN MICROSOMAL EPOXIDE HYDROLASE PROTECTS AGAINST AFLATOXIN B1-INDUCED GENOTOXICITY IN YEAST CO-EXPRESSING HUMAN CYP1A ENZYMES.

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A recent epidemiological study has suggested that a polymorphism in human microsomal epoxide hydrolase (mEH) increases susceptibility to aflatoxin B1 (AFB)-induced hepatocarcinogenesis (McGlynn et al, PNAS 92:2384-7, 1995), although studies in vitro and in laboratory animals have generally not found mEH to play an important protective role in AFB genotoxicity. However, in contrast to rodents, human liver does not express a glutathione S-transferase enzyme with high activity toward AFB-8,9-*exo*-epoxide. Thus, in the absence of significant GST activity, it is possible that human mEH could play an important protective role against AFB genotoxicity. The goal of this study was to determine if human mEH is capable of attenuating the genotoxic effects of AFB in an intact cell system. mEH was co-expressed with various human CYP 450s capable of activating AFB to the genotoxic AFB-8,9-*exo*-epoxide, in a heterologous yeast expression system. Yeast that expressed human CYP1A2, and to a lesser extent CYP1A1, exhibited a large, dose-related increase in mitotic recombination at the *trp* locus following AFB exposure, relative to non-CYP expressing yeast. Co-expression of mEH reduced the number of *trp* revertants by more than 50%. Expression of CYP1A2, but not CYP1A1, significantly increased the extent of AFB-DNA adduct formation at 0.5 and 1.25 μM AFB, and co-expression of mEH with CYP1A2 reduced AFB-DNA adduct formation by 50% or more. These results provide direct evidence that human microsomal epoxide hydrolase is capable of protecting eukaryotic DNA from human CYP mediated AFB-epoxide adduction in an intact cell system in spite of the fact that AFB-*exo*-epoxide is rapidly and spontaneously hydrolyzed in vitro. (Supported by NIH grants R01-ES05780, P30-ES07033 and T32-ES07032.)

316 SPECIES DIFFERENCES IN KUPFFER CELL-MEDIATED CYTOTOXICITY.

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Kupffer cells (KC) are the resident macrophages of the liver and represent the largest population of macrophages in the mammalian body. Recently, many reports have described KC-mediated cytotoxicity. We developed an assay to measure KC-mediated cytotoxicity and used this assay to compare the toxicity of lipopolysaccharide (LPS; *E. coli*-derived)–stimulated KC from different species. KC were isolated from rats, dogs, monkeys and human donor livers by percoll gradient centrifugation. KC and target cells (WRL68, Human Liver Embryo) previously prelabelled with BrdU were incubated with LPS in 96 well plates for 24 hours. Cell-mediated cytotoxicity was assessed by measuring BrdU-labelled DNA release to the conditioned medium using DNA fragmentation ELISA. Cytokine release (TNF-alpha, IL-1 beta and IL-6) from LPS-stimulated KC was also assessed. In another experiment, $GdCl_3$ (0, 5 and 10 mg/kg), an inhibitor of KC activity, was administrated to rats (i.v.). After 24 hours, KC were isolated for the cytotoxicity assay. The KC-mediated cytotoxicity induced by LPS was restrained by $GdCl_3$ administration in a dose-dependent manner. Target cell toxicity was greater with dog than rat, monkey or human KC. Species differences in cytokine release which correspond with toxicity were also observed. The differences between dog and rat correlated with the known difference in sensitivity of these species to LPS in vivo.

317 TOXICITY OF 1,3,5-TRINITROBENZENE (TNB) ON ASTROCYTES AND ENDOTHELIAL CELLS.

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In vitro studies with nitrobenzenes by others have focused on *m*-dinitrobenzene and its effects on cultured astrocytes and brain capillary endothelial cells. These studies have attempted to elucidate some of the mechanisms of toxicity focusing on oxidative stress and free radical production. We have used similar *in vitro* techniques with TNB to determine if cellular toxicity develops in tissue culture as observed with DNB. Primary cultures of Fischer-344 rat astrocytes were exposed to 0.0, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125 μ M TNB and 0.0, 0.5, 1, 2 mM DNB (carrier 0.5% dimethyl sulfoxide [DMSO]) for 24 hours. Commercially obtained cultures of human (umbilical vein origin) and bovine (corneal origin) endothelial cells were exposed to 0.0, 20, 30, 40, 50, 60, 70, 80, 90, 100 μ M TNB (carrier 0.5% DMSO) for 24 hours. Decreases in metabolic activity were used to assess cellular toxicity. Cellular metabolic activity was measured in astrocytes and endothelial cells via reduction of the tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; MTS] to a MTS formazan product and was recorded at 490 nm with a 96 well plate reader. Astrocytes and endothelial cells exhibited increases in metabolic activity in several of the lower dose TNB and DNB treatment groups. This stimulation with increased metabolic output occurred in response to low dose TNB (20 μ M) or DNB (1.0 mM) exposure. Astrocytes displayed a 50% decrease in metabolic activity when exposed to concentrations of TNB in the 25 to 30 μ M range. A 50% decrease in metabolic activity of endothelial cells was observed in the 50 to 55 μ M range. Cultured astrocytes displayed an increased sensitivity to the toxic effects of TNB (micromolar range) in comparison to astrocytes treated with DNB (millimolar range). Astrocytes also displayed increased sensitivity to the toxic effects of TNB when compared with endothelial cells. Abstract does not reflect U. S. Army policy.

318 EVALUATION OF TWO IN VITRO CILIATED EPITHELIAL SYSTEMS, DOG TRACHEA AND FROG PALATE, FOR POTENTIAL AS SCREENS FOR ACUTE INHALATION TOXICITY.

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Two aquatic-based *in vitro* ciliated epithelial systems, dog trachea and frog palate, have been evaluated for potential as screens for acute toxicity of inhaled chemicals. Initially, the effects of a series of nonpolar narcotic agents (ethanol, 2-butanone, 2-pentanone, 1-pentanol, 1-heptanol, 1-octanol, 2-decanone, 2-undecanone) were assessed by monitoring ciliary beat frequency under standard (static, 5 min exposure) conditions with the molar concentration eliciting a 50% reduction in ciliary beat frequency (i.e., EC_{50}) being determined. These data along with 1-octanol/water partition coefficient (log K_{ow}) were used to formulate baseline predictive models. The minimal toxicity model for the canine trachea (ct) system is log $1/ctEC_{50} = 0.79$ (log K_{ow}) – 0.002; n = 7, $r^2 = 0.987$, s = 0.15, F = 373, Pr > F = 0.0001. The frog palate (fp) baseline toxicity equation is log $1/fpEC_{50} = 0.80$ (log K_{ow}) - 0.12; n = 7, $r^2 = 0.995$, s = 0.09, F = 1112, Pr > F = 0.0001. Pre-validation of both *in vitro* systems were undertaken by regression analyses with *in vivo* mouse inhalation toxicity data. The slopes of both analyses are not statistically different than 1.0. The intercepts reveal the *in vivo* endpoint to be 10 times more sensitive. Also, a set of bioreactive chemicals (*trans*-2-octenal, heptaldehyde, benzyl chloride, 4,6-dinitro-*o*-cresol, carbaryl, malathion) was tested. Mechanisms of toxicity represented by this set of chemicals included three specific electro(nucleo)philic mechanisms, weak acid respiratory uncoupling, and reversible and irreversible acetylcholinesterase inhibition. While the relative ranking varied between the two *in vitro* systems, all bioreactive chemicals were found to have observed toxicity greater than twice that predicted by the baseline models. Based on these preliminary data, the authors feel that such a ciliary beat frequency *in vitro* system would provide valuable hazard information about potential acute effects of inhaled chemicals.

319 DETERMINATION OF IN VITRO DOSIMETRY FOR EXPOSURES OF PRIMARY RAT HEPATOCYTE CULTURES TO GLYCOL ETHER BASED COMPOUNDS.

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The US Air Force has sought the replacement of current operational chemicals with alternatives that pose less potential toxic risk. Alternatives to glycol ethers, such as diethylene glycol monomethyl ether (M-DE), a jet fuel system ice-inhibiting agent (FSII), are being investigated by our laboratory. The group of chemicals currently being evaluated include three derivatives of 1,3-dioxolane-4-methanol (M-1, M-2, and M-3) and two 1,3-dioxanes (M-26 and M-27). In addition, ethylene glycol monomethyl ether, MPDIOL glycol, and M-DE, are being evaluated as reference compounds. Our initial approach to evaluating the potential toxicities of these chemicals includes the assessment of acute toxicity in primary rat hepatocyte cultures. This set of experiments was performed to develop analyt-ical methods for the analysis of these chemicals and to assess the potential variations in aqueous concentrations of the test chemicals, due to volatilization during cell culture experiments. The vapor pressures of these compounds range over four orders of magnitude (0.001-70 mmHg at 37° C). The test chemicals were prepared at 1mM and 10mM concentrations and incubated in 6-well culture plates at 37° C for 0, 1, 2, 4, or 6 hr. One ml aliquots were taken at each time point and analyzed by gas chromatography to determine variations in test chemical concentrations over time. These data allowed us to determine the aqueous concentrations of the chem-icals, thus indicating the potential in vitro dosimetry for the primary hepatocytes in culture. The results of this study indicate that for these chemicals, for a six-hour exposure scenario, the aqueous concentrations do not vary significantly ($p>0.05$, less than 10% change) from their initial concentration. We conclude that in vitro assessment of these chemicals is reasonable, given their low potential for significant changes in aqueous concentration, due to volatilization.

320 TESTING OF GENETICALLY MODIFIED BACULO VIRUSES AND ESTABLISHED HUMAN CELL LINE.

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Baculoviruses have extensive literature documentation of their safety to mammals and have been developed and safely used as biopesticides. To enhance the insecticidal activity of the baculoviruses, *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) and *Helicoverpa zea* nuclear polyhedrosis virus (HzNPV), a gene encoding an insect selective toxin (LqhIT2) was inserted into these viruses' genome to create genetically modified organisms (GMO). These GMOs were compared to the wild-type AcMNPV and HzNPV for differences in cytotoxicity and infectivity in a human cell line. In order to test the effect on cells by a mixture of baculoviruses, cells were exposed to individual viruses as well as mixture of two viruses and compared. The test groups with a single GMO virus exposure were compared to the untreated group and the groups treated with individual parental wild type viruses. The test group that was treated with a mixture of GMO AcMNPV and GMO HzNPV was compared to the untreated group and the group treated with a mixture of wild type parental viruses. A human liver cell line was used for the infectivity evaluation. Human liver was chosen to represent systemic exposure to virus. Cells were exposed to a single dose of virus or mixture of viruses. Cells were observed for the signs of cytopathic effect (CPE) and the presence of occlusion bodies (OBs). Budded virus titers in the cell culture media at different time point after the exposure were determined by TCID50 (tissue culture infectivity dose). There was no virus associated CPE or OBs observed in any test group. Budded virus titers in media decreased with time indicating that the human cell line in this test was not able to support virus replication in any test group. The result of this study indicated that there were no deleterious effects or evidence of viral infection to human cell line from exposure to either recombinant baculoviruses or the parental wild-type viruses when treated separately or in a mixture form.

321 IN VITRO MODEL FOR INVESTIGATION OF COMPLEMENT ACTIVATION BY A PHOSPHOROTHIOATE OLIGODEOXY-NUCLEOTIDE.

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Activation of the alternative pathway of complement following rapid intravenous infusion of phosphorothioate oligodeoxynucleotides (P=S oligo) has been observed in monkeys. Investigation of the mechanism of complement activation has been hampered by the absence of activation in other laboratory animal models (rodents and dogs), and heretofore, the absence of *in vitro* models of complement activation. Monkey serum and whole blood has been used to establish a model for studying the mechanism of complement activation. P=S oligos produced concentration-dependent increase in comple-

ment split products Bb, C3a, and C5a *in vitro*, consistent with that observed in monkeys. The level of activation appeared to be greater in whole blood as compared to serum. Concentrations of oligonucleotide required to activate the alternative pathway *in vitro* were consistent with the threshold concentrations established in treated monkeys (~50 μ g/ml). Activation of complement by P=S oligo was also time-dependent, with full activation occurring within the first 30 min. Interpretation of results from these models is complicated by the inhibition of complement activation, both classical (aggregated IgG) and alternative pathways (zymosan stimulated), at higher P=S oligo concentrations (>250 μ g/ml). Slight increases in split product formation were observed at low concentrations in human serum, however, it appears to be less sensitive to complement activation than monkey serum. This model will be utilized to further study the mechanism of complement activation by P=S oligonucleotides.

322 DEVELOPMENT OF A HIGH-THROUGHPUT SCREENING ASSAY FOR HUMAN HEPATOTOXICITY.

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Besides pharmacological properties, pharmacokinetic and toxicological (ADME-Tox) properties are important determinants of a successful drug candidate. The key ADME-Tox properties include bioavailability, metabolic stability, and toxicity. Screening for desirable ADME-Tox properties, in addition to efficacy screening, should allow one to select new chemical entities with improved chances of clinical success. Research emphasis of our laboratory is the development of high-throughput screening (HTS) assays for ADME-Tox evaluation using human-based in vitro systems. Of the various in vitro experimental systems of the liver, hepatocytes represent the most relevant model for studies for metabolic stability and hepatotoxicity studies. To overcome the universal concern of limited availability of fresh human livers for experimentation, procedures were developed in our laboratory for cryopreservation of freshly isolated human hepatocytes. The cryopreserved hepatocytes had high post-thaw viability and xenobiotic metabolizing capacity (both phase I oxidation and phase II conjugation) similar to freshly isolated cells. A 96-well-based HTS assays for hepatocytotoxicity have been developed using cryopreserved human hepatocytes and using MTT as the endpoint for viability. Automated procedures were used to increase the throughput and to eliminate well-to-well differences in the 96-well plates. The assay was found to yield quantitative and highly reproducible results. Dose-dependent decreases in viability were observed for known hepatotoxic agents including tamoxifen, tranycypromine, clozapine, diclofenac and cadmium chloride. Data obtained with this human hepatocyte-based HTS for hepatotoxicity should be useful in the selection of lead drug candidates for further development.

323 NOVEL HIGH THROUGHPUT FLUORESCENT CYTOCHROME P450 ASSAYS.

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Cytochromes P450 (CYP) are the principal, hepatic xenobiotic metabolizing enzymes. We have developed microtiter plate-based, direct, fluorometric assays for the activities of the five principal human drug metabolizing enzymes, CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. For CYP1A2, CYP2C9, CYP2C19, and CYP2D6, the potency of enzyme inhibition (IC_{50}) is consistent regardless of probe substrate or assay method employed. In contrast, CYP3A4 inhibition for a specific inhibitor shows significant differences in potency (>30 fold) depending on the probe substrate being used. We have investigated these differences through the use of several structurally distinct fluorescent substrates for CYP3A4 and several classical substrate probes with a panel of known, clinically significant, CYP3A4 inhibitors. The use of multiple probe substrates appears to be needed to characterize the inhibition potential of xenobiotics for CYP3A4. This fluorometric substrate-based methodology provides a rapid means (using methodology compatible with existing high throughput screening instrumentation) to assess the potential for xenobiotics to inhibit cytochrome P450 - a mechanism for clinical drug-drug interactions and associated toxicities.

324 QUANTITATION OF HUMAN FLAVIN-CONTAINING MONOOXYGENASES ON IMMUNOBLOTS.

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The flavin-containing monooxygenases (FMOs) catalyze the NADPH-dependent oxidation of drugs, pesticides and other foreign compounds containing a soft nucleophile (*e.g.* nitrogen, phosphorous, or sulfur). The mammalian FMOs are separated into 5 families each containing about 50% sequence similarity. One or more of these enzymes are expressed in every organ examined to date. Since tissue distribution of family members can overlap, there is a need for reagents for specific quantitation of the different enzymes. We have developed antipeptide antibodies specific for human FMO1, FMO3, and FMO5. These antibodies were found to be form-specific using a panel of cDNA-expressed enzymes and western blotting. These reagents were used to quantitate levels of FMO expression in human tissue fractions. Substantial interindividual variation in expression was observed. For example, in 12 individuals, hepatic FMO3 and FMO5 levels varied 11- and 6-fold respectively. FMO1 was not detected in human liver. The ability to quantitate the levels of specific FMO enzymes should lead to a better understanding of the roles of these enzymes in human xenobiotic metabolism and toxicity.

325 DEVELOPMENT OF A RAINBOW TROUT RECOMBINANT CHIMERIC RECEPTOR REPORTER BIOASSAY TO SCREEN FOR THE POTENTIAL ESTROGENICITY OF ENVIRONMENTAL CONTAMINANTS IN FISH.

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Recently, the U.S. EPA has supported the use of estrogenic screening assays for both mammalian and wildlife species in order to elucidate the contribution of environmental contaminants in the endocrine disrupter issue. Our lab has established an in vitro rainbow trout (Oncorhynchus mykiss) bioassay to screen for compounds that act through the rainbow trout estrogen receptor. The assay utilizes a recombinant chimeric receptor consisting of the Gal4-yeast DNA-binding domain linked upstream to the ligand-binding domain (amino acids 214-576) of the rainbow trout estrogen receptor (RtGal4DEF). This cDNA is co-transfected with a Gal4-regulated luciferase reporter gene that is expressed in the presence of ligand-bound Gal4RtDEF. The transfection efficiency and performance of the Gal4RtDEF and Gal4 reporter gene was evaluated in 3 fish cell lines, PLHC-1, RTG-2 and RTH-149 and the human breast cancer cell line, MCF-7. A comparison of the ability of Gal4RtDEF and the human chimeric Gal4-estrogen receptor (Gal4HEG0) to mediate 17b-estradiol (E2)-induced reporter gene expression in the MCF-7 cells indicated that Gal4HEG0 had approximately two orders of magnitude greater sensitivity for E2 than Gal4RtDEF. This was also reflected when two different fish cell lines, PLHC-1 and RTG-2, were transfected with either construct. The PLHC-1 appeared to be the best cell line for transfection efficiency and inducibility; however, E2 was equally potent in all three cell lines. The potency of nonylphenol and benzo[a]pyrene as compared to E2 in the MCF-7 cells was similar in both the human or Gal4RtDEF; however, the overall response was consistently less with the Gal4RtDEF than seen with the Gal4HEG0. Therefore, it appears that it may be inappropriate to use a single surrogate species to monitor for estrogenicity in wildlife. In addition, this in vitro fish recombinant receptor bioassay is an excellent tool for fast, accurate, cost-effective screening of environmental contaminants or mixtures for estrogenic activities in fish.

326 HISTOLOGICAL INTEGRITY, CELL VIABILITY AND DRUG-METABOLIZING ENZYMES IN RAT LIVER SLICES AFTER CRYOPRESERVATION.

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Cryopreservation is a method for organ long-term storage used to overcome the scarcity of human tissues. We investigated the effect of cryopreservation on the morphological, biochemical and functional integrity of rat liver slices. Slices were incubated in modified University of Wisconsin solution containing 50% fetal calf serum and 10% dimethyl sulfoxide for 20 min on ice prior to a rapid freezing in liquid nitrogen. After 10-40 days, slices were thawed rapidly at 42°C. We found no changes in the lobular architecture and no microscopic findings in hepatocytes of cryopreserved liver slice sections

immediately after thawing. ATP content and MTT reduction in cryopreserved slices, measured respectively immediately and 1 h after thawing, were 40-50% that of freshly prepared slices (FPS). Total protein content decreased respectively by 40% and 60%, immediately and 24 h after thawing, relative to FPS values. To evaluate the catalytic activities of Phase I and II drug-metabolizing enzymes, antipyrine metabolism (10 mM for 24 h) was determined in cryopreserved slices. The antipyrine metabolites in FPS and cryopreserved slices were qualitatively similar but quantitatively different. The formation of Phase I and II metabolites in cryopreserved slices was 30% and 10-30% that in FPS. In conclusion, in rat liver slices cryopreserved for 10 to 40 days, i) histology was well preserved immediately after thawing, ii) viability was altered 1 h after thawing and iii) catalytic activities of drug-metabolizing enzymes were partly maintained after 24 h.

327 DETERMINING RELATIVE ESTROGENICITY BY QUANTIFYING VITELLOGENIN INDUCTION IN RAINBOW TROUT LIVER SLICES.

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Precision cut tissue slices are a valuable tool for studying protein induction or inhibition by test chemicals by modeling the entire organ. We developed parameters to quantify relative estrogenicity of chemicals by measuring vitellogenin induction, a well characterized biomarker of estrogen receptor binding, in rainbow trout liver slices. Hank's medium (phenol-red free) supplemented with HEPES, sodium bicarbonate and 1% bovine serum albumin was utilized. We optimized parameters using 1 - 1000 nM 17 -estradiol, a potent estrogen in rainbow trout that induces vitellogenin production *in vivo*. Adding trout serum and not changing the media was essential, probably to allow for the accumulation of vitellogenin in the slices and media. Histological examination and ATP analyses indicated no toxicity in control or 17 -estradiol treated liver slices after 120 hours. Induction was 4-fold greater with 25% serum containing media compared to media with 10% serum. We observed an over 100 fold induction of vitellogenin at 96 hours in liver slices and media containing 25% serum and 1000 nM 17 -estradiol. Controls without 17 -estradiol, incubated in media with 10% or 25% serum, exhibited no detectable vitellogenin production, indicating that the induction seen was not from the media or serum. We observed that 48 hours was required for significant vitellogenin induction or at least accumulation to detectable levels in the media and liver slices. Maximum induction in slices occurred at 96 hours whereas media vitellogenin levels continued to increase to 120 hours. This suggests a time delay between vitellogenin production and excretion by the liver. Our results indicate the importance of media, serum, and time selection for optimal vitellogenin induction. This study will allow for the determination of relative estrogenicity of chemicals in a controlled *in vitro* system while utilizing the advantages of precision cut slice technology. (Supported by NIH grants ES-07060, ES-03850, and ES 04766.)

328 DEVELOPMENT OF OPTIMAL CONDITIONS FOR THE CULTURE OF PRECISION-CUT RAT LIVER AND LUNG SLICES.

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The aim of this study was to compare some experimental conditions for the culture of precision-cut rat liver and lung slices. Liver and lung (prepared using an agarose instilling technique) slices were prepared from untreated male Sprague-Dawley rats using a Krumdieck tissue slicer. Tissue slices were cultured in a dynamic organ culture system for periods of 1, 24 and 48 hr in an atmosphere of either 95% O_2/5% CO_2 or 95% air/5% CO_2. Media were supplemented with 0.1 m M insulin, 0.1 m M dexamethasone, 50 m g/ml gentamicin and 2.5 m g/ml fungizone. Liver slices (diameter 10 mm, thickness around 250 m m) were cultured in Williams' medium E (WE), RPMI 1640 (RPMI), Waymouth's (WAY) and Hepatozyme (HEP) media. While levels of protein synthesis and potassium content were similar in WE, RPMI and WAY media, they were lower in HEP media. Rates of protein synthesis were similar in rat liver slices cultured in WE containing either 0.1 or 1 m M insulin, but markedly reduced in WE containing 0.01 m M insulin. Rat lung slices (diameter 8 mm, thickness around 500 m m) were cultured in WE, RPMI, CMRL 1066, Medium 199 and Minimal Essential Medium Alpha (MEMα) media containing the above additions. No marked differences were observed in potassium content and in the metabolism of 200 m M 7-ethoxycoumarin to free and conjugated 7-hydroxycoumarin. As an additional marker of functional viability, rat lung slices were cultured in MEMα with 0-20 m g/ml Aroclor 1254 (ARO) for 24 and 48 hr. ARO treatment produced a concentration dependent induction of 7-ethoxyresorufin O-deethylase activity. These studies demonstrate that it is important to use an appropriate medium and insulin concentration for the culture of rat liver slices. Like liver slices, cultured lung slices may also be employed for xenobiotic metabolism studies and to investigate the induction of xenobiotic metabolising enzymes. (Supported by EU BIOTECH Contract No. B104-CT97-2145.)

329 MAINTENANCE AND INDUCTION OF CYTOCHROME P-450 (CYP) ISOFORMS IN CULTURED PRECISION-CUT HUMAN LIVER SLICES.

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In this study we have employed a panel of monospecific antibodies to examine the stability of CYP isoforms in cultured human liver slices and the induction of CYP isoforms by xenobiotics. Liver slices were prepared from samples of human liver (surplus to transplant requirements) with a Krumdieck tissue slicer. Precision-cut liver slices were cultured in RPMI 1640 medium for 0 (i.e. a 1 hr preincubation period), 24, 48 and 72 hr in a dynamic organ culture system in an atmosphere of 95% O_2/5% CO_2. After culture, liver slice microsomal fractions were prepared for Western immunoblotting studies. Levels of CYP1A2 and CYP2C19 apoproteins declined in culture, being around 20% of 0 hr levels after 72 hr. In contrast, levels of CYP2C9, CYP2D6 and CYP3A4 apoproteins were around 50% of 0 hr levels after 72 hr culture. CYP isoform dependent enzyme activities also declined in cultured liver slices. After 72 hr, activities of 7-ethoxyresorufin 0-deethylase (CYP1A2), tolbutamide methylhydroxylase (CYP2C9) and testosterone 6β -hydroxylase (CYP3A4) were around 20, 50 and 40%, respectively, of levels in 0 hr cultured slices. Human liver slices were also cultured in control medium or medium containing 0.5 mM sodium phenobarbitone (NaPB) or 50 m M rifampicin (RIF) for 72 hr. Compared to 72 hr control liver slice microsomes, both NaPB and RIF induced levels of CYP3A4 and CYP3A5. RIF treatment also induced levels of CYP2C19, but neither NaPB nor RIF had any marked effect on CYP2D6. These results demonstrate that while levels of CYP-dependent enzyme activities fall in cultured human liver slices, there is a differential loss of specific CYP isoforms. However, this does not preclude their use for *in vitro* CYP isoform induction studies. (Supported by EU BIOMED Contract No. BMH4-CT96-0254.)

330 SODIUM ARSENITE TOXICITY IN PRECISION-CUT RAT LUNG SLICES.

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While precision-cut liver slices have been extensively used for toxicity and metabolism studies, studies using precision-cut lung slices are limited in the literature. This is probably due to the limited availability of lung tissue for such studies when using agar-instilled lung slices. Another draw back is that enzyme leakage or potassium leakage from lung tissue may not be appropriate markers of toxicity due to entrapment in agar matrix. In the present study ATP content was used to assess toxicity of sodium arsenite in agar-instilled precision-cut lung slices. Lung slices (10 mm diameter, 550-600 μ thickness) were prepared from male Sprague Dawley rats (250-300 g) and cultured for up to 24 hr in Waymouths MB752/1 medium. Lung slices were exposed to 100 nM-1 mM sodium arsenite. Control slices retained viability up to 24 hr as observed by slice ATP content and histology. Exposure to 100 nM arsenite did not result in any toxicity while 1 mM arsenite caused toxicity as early as 2 hr. Ten μ M arsenite decreased cellular ATP by 6 hr. Studies on arsenic smelter workers have shown that cigarette smoking increased the number of arsenic-induced lung cancer. Our preliminary studies show that coexposure to arsenic and cigarette smoke results in enhanced pulmonary toxicity *in vivo*. Therefore our goal is to extend these studies in the lung slice model in order to determine interactive toxicity and early sensitive molecular markers of cytotoxicity of these agents. (Supported in part by ADCRC grant 9703.)

331 LIVE TIME EVALUATION OF CELL TOXICITY IN PRECISION-CUT TISSUE SLICES USING CONFOCAL MICROSCOPY.

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Based upon the retention of organ architecture and cellular heterogeneity, precision-cut tissue slices offer a unique in vitro model to investigate site-specific effects of toxicants. The production of specific fluorescent dyes for various indices has allowed investigators to assess the live-time effect of xenobiotics using confocal microscopy in cell culture systems. However, the current studies were designed to determine if precision-cut liver slices would provide a suitable in vitro toxicity model for confocal microscopy. Male adult SD rat liver slices were exposed to 1,1-dichloroethylene (DCE). At 2, 3.5, 5 and 7 hr, DCE exposed slices were removed and stained with a combination of nucleic acid dyes. Live cells were stained with Syto-10, which is permeable to plasma membranes of living cells, while dead cells were identified with Dead Red. Dyes (1µl/500 µl medium) were made immediately prior to staining at each timepoint. Staining was for 15 min at room temperature in the dark, followed by two washes. After staining, slices were placed in a 35 mm tissue culture plate (modified to contain a glass coverslip base) and were held in place with aluminum meshing. Slices were viewed using a Leica TCS confocal microscope containing an IRBE inverted microscope and Kr/Ar laser. Simultaneous visualization of the stains was obtained by excitation with the 488/568 laser lines accompanied by FITC and TRITC emission bandpass filters. DCE lead to a selective progressive damage of the slices. Little or no dead cells were apparent at 2 hr of exposure. At 3.5 hr, the appearance of dead cells around blood vessels became apparent. The damage was progressively worse at 5 hr. However, by 7 hr, the distribution of dead cells was uniformly distributed across the surface of the slice. No damage was noted even in controls that had been incubated for up to 8 hr. These results indicate that toxicity can be observed live-time in tissue slices and offers a dynamic system for examining mechanisms of intoxication. (Supported by a Procter & Gamble Exploratory Research Grant.)

332 ESTABLISHMENT OF PRECISION-CUT HUMAN PROSTATE SLICES AS AN IN VITRO TOXICOLOGY MODEL.

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Benign prostate hyperplasia (BPH) and prostate cancer (PC) are a significant cause of morbidity and mortality. However, understanding of molecular mechanisms underlying prostate pathobiology is limited by i) the lack of a widely accepted animal model; ii) the zonal specificity of the two diseases [BPH-transitional zone; PC-peripheral zone] and iii) the complex network of stromal-epithelial interactions which limit the use of cell cultures. Recently, we have developed a novel *in vitro* approach to the prostate – the use of precision-cut human prostate slices. This system has several advantages, including the use of human tissue, maintenance of stromal-epithelial relationships, and the ability to investigate differences between the transitional and peripheral zones. Viability of stromal and epithelial (both basal and secretory) cells is maintained for up to 72 hr as assessed by histological evaluation. LDH leakage peaks at 3 hr, indicating damage due to the slicing process, however, by 48 hr media LDH activity is not detectable. Conversely, secretion of prostate specific antigen is detected throughout the 72 hr culture period. Preliminary results have shown that 1 µM cadmium, a putative prostate carcinogen, increases LDH leakage 181% in peripheral zone slices at 48 hr, but not transitional zone slices. These results suggest that human prostate slices represent a powerful tool to investigate prostate pathobiology. (Supported by Arizona Disease Control Research Commission (1-345) and an SOT Colgate-Palmolive Fellowship.)

333 IN VITRO DERMAL ABSORPTION OF JET FUEL (JP-8) AND ITS COMPONENTS.

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Military personnel are exposed to jet fuels during many activities. These kerosene-based fuels cause irritation to the skin with repeated or continuous dermal contact and high levels have been associated with renal, hepatic, neurologic, immunologic and pulmonary toxicity in animal inhalation studies. Although the local dermal toxicity of these fuels is recognized, the potential for systemic toxicity from dermal absorption due to skin exposures has not been completely assessed. The purpose of our study was to determine the absorption of JP-8 (the Air Force's primary jet fuel since 1996) and its components so that the potential for systemic toxicity can be addressed. The dermal penetration of this complex mixture was measured over a 24-hour period using static diffusion cells with Fischer-344 rat skin. Receptor solution (6% volpo/saline) was collected at two-hour intervals and assayed by gas chromatography. The sum of the peak areas at each time point was taken as the total mass of JP-8 absorbed. Flux was determined from the slope of the cumulative absorbed curve plotted over time. The steady-state flux of JP-8 was 41.4 µ g/cm^2/hr. Among the major peaks in the receptor solution, several were identified as methyl benzene, ethyl benzene, dimethyl benzene, naphthalene, methyl naphthalene, dimethyl naphthalene, tridecane and dodecane. The flux of these chemicals ranged from 0.4 to 1.4 µ g/cm^2/hr. These results show that it will be possible to estimate systemic toxicity of the individual components of this complex hydrocarbon mixture from dermal exposures. (Supported by Office of AF Surgeon General - AFMOA/SGOE.)

334 DERMAL ABSORPTION AND TOXICITY OF JET FUELS.

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Dermal exposure to jet fuels has received increased attention with the recent release of newer fuels with novel performance additives. The purpose of these studies was to assess the percutaneous absorption and cutaneous disposition of topically applied (25µ l / 5cm^2) neat JetA, JP-8, and JP-8 (100) jet fuels by monitoring the absorptive flux of the marker components ^{14}C naphthalene (N) and ^{3}H dodecane (D) simultaneously applied non-occluded to isolated perfused porcine skin flaps (IPPSF) (n=4). Absorption of these markers were also assessed using JP-8 applied to cotton fabric and from a non-volatile JP-8 fraction reflecting exposure to residual fuel that might occur 24 hrs after a jet fuel spill. Perfusate, stratum corneum and skin concentrations were assessed over 5 hrs. Cutaneous toxicity to all jet fuels was separately evaluated *in vivo* in pigs at 5 and 24 hrs. N absorption demonstrated a clear peak absorptive flux within 30 min while D had a prolonged, albeit ten fold lower, absorption flux profile. N absorption into perfusate was two-fold greater from JP-8 and JP-8 (100) compared to Jet-A, however Jet-A produced double the skin concentrations of either JP-8 fuel. D absorption was mildly suppressed in JP-8 (100) compared to Jet-A and JP-8, with Jet-A again showing the highest skin concentrations. Topical exposure to JP-8 from fabric resulted in a six-fold (N) and 20 fold (D) decrease in perfusate absorption compared to liquid JP-8 exposure, however it also produced the highest N skin levels of any exposure scenario. These studies clearly demonstrate differential jet fuel additive effects on N and D absorption as well as uncoupling of perfusate absorption from skin disposition. (Funded by the U.S. Air Force Office of Scientific Research (FQ8671-98-000462).)

335 PREDICTION OF SKIN PERMEABILITY COEFFICIENTS OF VOLATILE ORGANIC CHEMICALS FROM INFORMATION ON THEIR MOLECULAR STRUCTURE.

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Skin permeability coefficients (K_p) are required for estimating the dermal absorption of volatile organic chemicals (VOCs) using a pharmacokinetic model. At the present time, K_p of VOCs are empirically predicted by fitting molecular structure information of VOCs to experimental data on K_p. Considering the number of VOCs presents in our environment and that the resources are limited, these empirical equations have a limited use in risk assessment because *in vivo* and/or *in vitro* data on skin absorption are required to derive such empirical equations. Alternatively, mechanistic/molecular structure-based equations need to be developed for predicting K_p of VOCs. The objective of the present study was to develop a mechanistic approach for predicting the human abdominal K_p of 35 VOCs only from information on their molecular structure. The approach consisted of estimating the stratum corneum:water ($P_{s:w}$) partition coefficients (PCs), the diffusion coefficients (D), and the path length of diffusion (l) for eventually calculating K_p (= $P_{s:w}$ x D/l). $P_{s:w}$ of VOCs were predicted only from molecular structure information based on a recently validated equation used for predicting the tissue:water PCs of organic chemicals from data on oil:water PCs

and lipid and water contents in tissue. Whereas, D were predicted according to the conventional Einstein-Stoke equation using molecular structure information (i.e., molecular radius) of VOCs and data on skin viscosity as the only input parameters. Finally, l was estimated in accordance with the characteristics of the diffusion pathway in the stratum corneum (i.e., transcellular and intercellular pathways). The predicted human abdominal K_p value of several VOCs (alcohols, haloalkanes, aromatics) differed to the experimentally-determined ones with an average ratio of 1.04 (S.D. = 0.88, r = 0.85, n = 35). Incorporating the mechanistic/ structure-based equation validated in this study in a pharmacokinetic model should facilitate the risk assessment for a skin exposure to the several VOCs present in our environment.

336 PENETRATION GRADIENTS OF NICKEL SALTS ABSORBED IN HUMAN *STRATUM CORNEUM IN VIVO.*

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The standard protocol of sequential adhesive tape stripping was implemented to examine the absorption of several nickel salts through human *stratum corneum in vivo* following their application on the volar forearm and back skin of healthy adult volunteers. Exposure areas of the salts applied in low volume, open, as methanol solutions at nickel concentrations from 2 to 300 μg/cm2 were stripped at intervals from 30 min. to 24 hrs. post dosing. The strips were analyzed for metal content by inductively coupled plasma-atomic emission spectroscopy. For the nickel (II) salts chloride, sulfate, nitrate and acetate, the concentration-versus-depth profiles obtained allow the following conclusions, several in support of earlier observations: a) Accumulation of nickel in the SC was observed in function of exposure time, as the concentration gradient between the superficial and deeper layers of the SC increases commensurate with duration of exposure. b) The counter ion in nickel salts plays a major role in their passive diffusion through the SC, suggestive of ion pairing. c) While the concentration gradients of nickel retained in the stratum corneum (SC) vary with counter ion, anatomical site, dose and exposure time, for all variables tested the profiles converge towards non-detectable levels (<20 ppb) beyond the fifteenth strip, independent of these parameters. d) For most applications, mass balance based on cumulative SC analysis for nickel to the *stratum compactum* level (to include material deposited in furrows) shows a deficit. Depending on the variables of application, varying amounts of the metal applied as salt thus appear to penetrate beyond the SC, presumably via the skin shunts.

337 IN VIVO DECONTAMINATION OF METHYLENE BISPHENYL ISOCYANATE (MDI): SOAP AND WATER INEFFECTIVE COMPARED TO POLYPROPYLENE GLYCOL, POLYGLYCOL-BASED CLEANER AND CORN OIL.

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Decontamination of a chemical from skin is traditionally done with soap-and-water wash, some workplaces may have an emergency water shower. It has been assumed that these procedures are effective, yet workplace illness and even death occure from chemical contamination. This study determined whether there are more effective means of removing methylene bisphenyl isocyanate (MDI), a potent contact sensitizer, from the skin. MDI is an industrial chemical for which skin decontamination using traditional soap-and-water and nontraditional polypropylene glycol, a polyglycol-based cleaner (PG-C) and corn oil was done *in vivo* in the Rhesus monkey over 8 hours. Water and soap and water (5% and 50% soap) were partially effective in the first hour removing 51-69 percent applied dose. However, decontaminatin fell to 40-52 percent at 4 hours and 29-46 percent at 8 hours. Thus, the majority of MDI was not removed by traditional soap and water wash; skin tape stripping after wash confirmed that MDI was still on the skin. In contrast polypropylene glycol, PG-C and corn oil removed 68-86 percent MDI in the first hour, 74-79 percent at 4 hours, and 72-86 percent at 8 hours. Statistically polypropylene glycol, PG-C and corn oil were all better ($P<0.05$) than soap and water at 4 and 8 hours after dose application. These results indicate that traditional soap-and-water wash and the emergency water shower are relatively ineffective at removing MDI from the skin. More effective decontamination procudures, as shown here, are available.

338 AN INNOVATIVE METHOD TO DETERMINE DERMAL UPTAKE OF SOLVENTS FROM SOIL AND WATER IN VIVO IN HUMANS.

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Realistic estimates of dermal bioavailability following exposures to contaminated soil or water are critical to understanding human health risk. A method was developed to determine dermal uptake of volatile organics using real-time breath analysis. This data was subsequently analyzed using physiologically based pharmacokinetic (PBPK) models to estimate dermal bioavailability. For exposures lasting from 2-6 hr, soil was contained against the skin by a patch with a non-occlusive membrane, and volatilized material was trapped using activated charcoal. The water counterpart was an occlusive glass culture dish, sealed to the skin with silicone adhesive. Short-term exposures (2-4 hr) were by one hand immersion in a bucket containing circulating water or soil. In all cases, subjects were provided breathing air via a facemask to eliminate inhalation exposure. The subject's exhaled breath was analyzed using an ion trap mass spectrometer, which can quantitate individual chemicals in the breath stream in the 1-5 ppb range. This methodology was evaluated using 1,1,1-trichloroethane (methyl chloroform: MC) as a test compound. The results showed that the length of time between exposure and appearance of MC in exhaled breath varies between subject and exposure scenario, but there appeared to be a 30-60 min post application delay. The method also clearly discerned the uptake phase, peak concentrations and clearance phases. Furthermore, quantitation of MC in exhaled breath at several orders of magnitude above the detection limit of the instrument show the methodology is well suited for human dermal studies utilizing low exposures, for a short period of time, to a small percentage of the body. (Supported by a grant from US DOE, No. DE-FG07-97ER62509.)

339 ASSESSING THE DERMAL BIOAVAILABILITY OF VOLATILE ORGANICS IN RATS.

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Assessing the bioavailability of soil contaminants is critical to understanding human health risk. In this study, 1,1,1-trichloroethane (methyl chloroform: MC) was used to develop methods to assess the bioavailability of volatile organics in soil following dermal exposures in F-344 rats. Physiologically based pharmacokinetic (PBPK) modeling has been used to fully describe the dermal dosimetry. For exposures in a soil matrix, a non-occlusive patch system was developed to hold the soil against a clipper-shaved area on the flank of the rats. Volatilized MC was trapped with activated charcoal. The amount of MC in exhaled breath was determined by placing rats in sealed off-gassing chambers and sampling chamber air every 4 sec using an ion trap mass spectrometer. Regardless of dose, peak chamber MC concentrations were reached within 30 min of exposure, and concentrations dropped to <25% of peak by 2 hr of exposure. This is consistent with the volatilization of test material as determined by the analysis of MC in the activated charcoal. A chamber concentration dose-response was observed with peak MC concentrations ranging from ~800 to ~3000 ppb following application of soil containing from 0.3% to 1% MC. The permeability coefficient (k_p) for the dermal uptake of MC applied in a soil matrix, estimated using the PBPK model was between 0.34 and 0.44 cm/hr. These rat studies will be used to compare the impact of concentration, exposure duration, and soil characteristics on bioavailability of volatile organics. Human studies are being run concurrently to validate the extrapolation from rat to human using PBPK models. The end product of this research will be methodology to rapidly evaluate dermal exposures to organics in contaminated soil. (Supported by US DOE grant No. DE-FG07-97ER62509.)

340 DERMAL ABSORPTION OF N-METHYLPYRROLIDONE (NMP) IN THE RAT.

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The dermal absorption of radiolabelled material in male Sprague Dawley CD rats following a single topical application of undiluted [^{14}C]-N-

methylpyrrolidone (NMP) and also diluted at 4 concentrations in 2 dose vehicles, water and (R)-(+)-limonene, was determined. For each dose solution (constant application rate of 10 µl/cm²), 1 group of 4 male rats was sacrificed at 1, 3, 6 and 24 hours after dosing to assess the distribution of the radioactivity. The animals were individually housed in glass metabowls equipped to separately collect urine, faeces, expired air and volatile material from the dose site (carbon filters). Following a single topical application of 100% NMP, the majority of radiochemical dose (>67%) was recovered as volatile material from the dose site. Only a small proportion of dose remained in the treated skin and was washed from the skin, falling from approximately 6% to <1% of dose from 1 to 24 hours. At 24 hours after dosing, 32% of the dose had been absorbed, and largely eliminated in the urine (94%), faeces (1.6%) and expired air (0.6%). As the NMP dose solutions contained increasing proportions of water, from 35% to 97% (by vol), so the percentage of the dose absorbed decreased from 15% to <1% respectively at 24 hours after dosing. At this time point, <3% of dose remained at the dose site and <0.5% in the untreated skin and carcass for all the aqueous dose solutions, the majority of dose had evaporated from the dose site and was recovered in the carbon filters. Dilution of NMP with limonene over the range 35% to 90% limonene increased the proportion of dose absorbed compared to 100% NMP. The absolute quantity of [^{14}C]-NMP absorbed (approximately 2-3 mg/cm²) was similar for the 3 dose solutions containing 0, 35 and 70% limonene. The maximum dose absorbed (72%) was found after dosing with a mixture of 30:70, NMP:limonene. At 24 hours after dosing with this solution, >67% of the dose had been excreted in the urine with <2% remaining in the untreated skin and carcass and <0.5% at the dose site.

341 HUMAN SYSTEMIC EXPOSURE AFTER DERMAL ABSORPTION OF AHTN AND HHCB AS USED IN FRAGRANCES–EVIDENCE FOR LACK OF ABSORPTION FROM A SKIN RESERVOIR.

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7-Acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (AHTN) and 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran are the two largest volume materials of a group of substances known collectively as polycyclic musks. AHTN and HHCB are widely used in a variety of fragrances that are in consumer products. As part of the risk evaluation, the systemic exposures to these materials was determined under simulated conditions of exposure. Ring ^{14}C labeled AHTN and HHCB were each applied without occlusion to human volunteers in a hydroalcoholic solution at concentrations approximating that which might be encountered in a cologne type product. After a 6-hour period, all material was removed from the surface of the skin. Blood, feces and urine were collected over a 5-day period following application. For both materials, levels in blood and plasma were below limits of detection at all times. Based on measurements in urine and feces, the total systemic availability was 0.9% and <0.1% for AHTN and HHCB respectively although both were initially significantly absorbed into the skin. Over the period of 5 days, 14.5% of AHTN and 19.5% of HHCB was recovered from the skin in dressings over the site of application indicating that a reservoir had formed but the material in the skin was excreted either by sloughing of the skin or by reverse absorption. A mean of 24.2% (AHTN) and 21.8% (HHCB) was shown to evaporate under the conditions of exposure. Thus, while absorption into the skin occurred, most absorbed material was not available for systemic exposure.

342 EFFECTS OF AN ALCOHOL DEHYDROGENASE INHIBITOR ON CINNAMALDEHYDE ABSORPTION AND BIOTRANSFORMATION IN HUMAN SKIN.

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Cinnamaldehyde (CAld), a component of cinnamon, is an aromatic aldehyde that is commonly used as a fragrance and flavouring agent. Following skin exposure, CAld has been reported to act as a strong contact sensitizer. In this study, the biotransformation of the protein-reactive CAld to the non-protein-reactive cinnamic alcohol (CAlc) and cinnamic acid (CAcid) was investigated. A series of 24 hour percutaneous absorption and metabolism experiments were performed for CAld, in the absence and presence of the alcohol dehydrogenase (ADH) inhibitor, pyrazole. Fresh full-thickness human breast or abdomen skin (n = 3) was used in an established skin absorption model, which maintained the viability of the skin at 37°C, in a receptor fluid at pH 7.3. CAld (78 mmoles/10 µl neat) was applied to a 0.32 cm² epidermal area of skin occluded over 24h. In vehicle control and inhibition studies, 20 ml water, 80 or 320 µmoles pyrazole were applied 30min prior to CAld application. Receptor fluid samples, collected at 2h intervals, and methanol washes of occlusion cells and skin surface swabs were analyzed for metabolites by high performance liquid chromatography. In experiments with CAld, a total of ~55% of the initial dose was accounted for by a) evaporation (~0.95%), b) skin surface residue (~43.7%) and c) penetration (2.70± 0.60% CAlc, 4.66± 1.32% CAcid and 2.72± 0.68% CAld). Preincubation of an approximate 1:1 molar ratio of aqueous pyrazole resulted in a reduction in penetration of both CAlc (0.79± 0.02%) and CAcid (1.41± 0.06%) and similar penetration of CAld (3.89± 1.33%). CAld is detoxified to CAlc and CAcid in human skin, although CAld may be regenerated by ADH mediated reoxidation of CAlc. The altered profile for the penetration of CAld metabolites in the presence of pyrazole may be due to inhibition of alcohol dehydrogenase function or as a result of altered absorption kinetics for CAld in the presence of aqueous solutions.

343 PERCUTANEOUS ABSORPTION AND METABOLISM OF BUTOXYETHANOL IN RAT.

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Low molecular weight glycol ethers (ethoxylates) are used as solvents in industrial and household products. Dermal exposure and absorption of butoxyethanol (BE) can produce toxicity following metabolism to the corresponding aldehyde and acid. The aim of this study was to investigate the disposition of BE after dermal and parenteral exposures. The distribution and metabolism of BE in male, Wistar rats (n=3) was studied. BE was applied topically (88.5 mg / 9.6cm², 10µ Ci) or in saline by intraperitoneal injection (5.14 mg/g, 50µ Ci) to rats and excreta analysed for total radioactivity. At 24 hours, following topical application 28.4 % of the dose was absorbed. The major route of ^{14}C excretion was in the urine (18.6 %) with expiration in air, as $^{14}CO_2$ (5.7 %) and the faeces (0.4 %) being more minor routes. Approximately 1.34 % of the dose remained in the carcass. Overall recoveries of ^{14}C were reproducible and the total non-absorbed dose was 34.3 %. A similar profile of distribution was observed following ip administation. Whole Body Autoradiography (WBA) at 4 hours revealed widespread tissue distribution particularly in gut contents, kidney, liver, harderian gland, and intraorbital lacrimal gland following either dose route. Thin layer chromatography with phosphoimage analysis of rat urine samples indicated almost complete metabolism of BE to ethylene glycol or butoxyacetic acid, 81.7 % and 14.1 % (topical study) and 63.8 %, and 35.2 % (ip study). Several minor but distinct bands were also observed after phosphoimage analysis and could constitute a series of unidentified conjugates. By comparing profiles of BE metabolism following topical and ip administation there is little evidence of local dermal metabolism of this compound.

344 DERMAL ABSORPTION AND CUTANEOUS DISPOSITION OF 3,3',4,4'-TETRACHLOROBIPHENYL (TCB) IN A SWINE MODEL.

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The impacts of exposure variables on dermal absorption and tissue disposition of TCB were explored following topical dose of [^{14}C]TCB in pigs (40µ g/cm², n=3/dose). Application vehicles included acetone, methylene chloride (MC), water- and soil-based mixtures. An occlusion study was performed using a soil vehicle with/without antibiotics. In one group, skin P_{450} was pre-induced by benzo[a]pyrene prior to TCB exposure. Additionally, an IV study was also carried out. During the 11-day experiments, blood, urine, and feces were collected. At the experimental end, full mass balance procedures were performed. On average, 64-96% of the dose was recovered. The IV data revealed a half-life of 4-5 days in blood. Following topical exposure, the 11-day absorption (vehicle) was determined as 22% (acetone), 32% (MC), 22% (water-acetone mixture), and 33% (soil mixture) under non-occlusive exposure. The corresponding values for occluded soil were 52% (no antibiotics) and 17% (with antibiotics). A greater absorption of 29% (vs 22%) was observed if cutaneous P_{450} was pre-induced. The urine/feces

excretion (U/F) ratios ranged 0.4-1.5 and about 2/3 of the absorbed dose was found in tissues 11 days after TCB dermal exposure. Interestingly, topical doses yielded much higher ^{14}C concentrations in most tissues although the topical absorption and excretion were lower than IV as expected. TCB was absorbed much more efficiently from soil-based mixtures than from pure organic vehicles and water-organic mixture. Occlusion enhanced absorption and modified the U/F ratio (0.4 to 0.7). Skin microbial inhibition could largely offset the absorption-enhancement effect of occlusion. Addition of water to acetone changed the U/F ratio (1.3 to 0.7). In conclusion, absorption data from pure organic solvents or organic-water mixture might underestimate the risk of TCB from soil exposure. Concentration-dependent tissue uptake and/or unique skin metabolism might exist for TCB. Exposure variables may change dermal absorption via skin metabolism modification. Therefore, careful dermal exposure assessment is critical for occupational and environmental safety analysis and management. (Supported by USEPA-CR 824007.)

345 COMPARISON OF PERCUTANEOUS ABSORPTION CHARACTERISTICS FOR TWO STRUCTURALLY RELATED DIRECT HAIR DYES, HC BLUE NO. 1AND HC BLUE NO. 2, *IN VITRO* AND *IN VIVO*.

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Two structurally related direct (semi-permanent) hair dyes, HC Blue No. 1 and HC Blue No. 2, were compared for their percutaneous absorption potential using human cadaver skin mounted in Franz-type static diffusion cells in vitro. Under simulated use conditions, ^{14}C-radiolabeled dyes in hair dye formulations for which human in vivo penetration data had been obtained previously (Wolfram LJ, Maibach HI. 1985. Arch Dermatol Res 277:235-241) were applied to heat-separated epidermal membranes. The appearance of ^{14}C in 30 minute skin rinsates, in periodic receptor fluid samples, and in both a final wash and the epidermis after termination of the experiment at 48 hours was determined via liquid scintillation. For HC Blue No. 2, the absorption of dye into the receptor fluid was maximal within 4 hours after application, with a total cumulative mass absorbed of about 0.20 ug/cm^2. For HC Blue No. 1 the cumulative mass absorbed was 0.42 ug/cm^2 after 48 hours with a slope suggestive of continuing incremental absorption. The amounts remaining on or in the epidermis were similar for both dyes (approximately 1.3 ug/cm^2). Comparison of the human in vitro data with historical human in vivo data obtained under application and wear conditions indicated that for HC Blue No. 2 the in vitro receptor fluid concentration alone approximated the Day 1 in vivo value (0.23 ug/cm^2) whereas it underestimated the Day 1 in vivo value for HC Blue No. 1 (2.81 ug/cm^2). Consideration of the in vitro time course data suggested that incremental absorption of HC Blue No. 2 over time (Days 2-30) in vivo resulted primarily from re-exposure to dye leached from the hair reservoir whereas incremental absorption of HC Blue No. 1 may have resulted, at least in part, from continued mobility of dye in the epidermis.

346 IN VITRO PERCUTANEOUS ABSORPTION AND METABOLISM OF 2-NITRO-P-PHENYLENEDIAMINE IN HUMAN AND FUZZY RAT SKIN.

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2-Nitro-p-phenylenediamine (2NPPD) is a "coal-tar" dye used in semipermanent and permanent (tinting color) hair dye formulations. In previous work, we reported on the percutaneous absorption and metabolism of 2NPPD in fuzzy rat skin and intestinal tissue (Toxicologist, 36:188, 1997). These studies were expanded to include *in vitro* absorption and metabolism of 2NPPD in human skin from ethanol and from a spiked semipermanent formulation, and in fuzzy rat skin from ethanol and from a spiked permanent formulation. Dosing vehicles were applied to skin in the diffusion cells for 30 min. Absorption was measured over 24 hr by using flow-through diffusion cells (0.64 cm2) with a receptor fluid consisting of HHBSS (pH 7.4). 2NPPD metabolites were determined by HPLC. In human skin, the percentages of total applied dose absorbed (receptor fluid + skin) over 24 hr were 9.2 ± 5.7 (mean ± SD) and 9.5 ± 3.2 for the ethanol and semipermanent vehicles, respectively, with approxi-mately 3% remaining in skin. In rat skin, the percentages of total applied dose absorbed over 24 hr were 9.3 ± 1.2 (mean ± SEM) and 4.2 ± 0.1 for the ethanol and permanent form-ulation vehicles, respectively, with approximately 3% remaining in skin. 2NPPD rapidly penetrated both human and fuzzy rat skin from the product formulations with 47 and 64%, respectively, of the total absorbed dose found in the receptor fluid within 3 hr. In human and rat skin, 2NPPD was metabolized to triaminobenzene and N4-acetyl-2NPPD. 2NPPD was also metabolized to a sulfated 2NPPD metabolite in rat skin, but not in human skin. These results suggest that 2NPPD is rapidly absorbed and extensively metabolized in both human and rat skin. 2NPPD metabolism upon absorption appears to be species-dependent.

347 NATURAL RUBBER LATEX PROTEIN PENETRATION AND LOCALIZATION IN ANIMAL AND HUMAN SKIN.

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In the past few years, recognition of natural rubber latex allergies has increased dramatically throughout the world. In fact, many hospitals have taken steps to reduce exposure to natural latex products (e.g. low powder, low protein gloves) in part because of the NIOSH 1997 alert, "Preventing Allergic Reactions to Natural Rubber Latex in the Workplace." However, there remains a need to understand better the factors affecting sensitization to latex proteins. These studies begin to appraise the skin's role in latex protein allergies. Initial studies evaluated *in vitro* penetration of latex proteins through hairless guinea pig skin. Non-ammoniated latex proteins were radiolabeled with ^{125}I using Iodo-Beads™ and dialyzed against K_2PO_4 for 48 hours to remove any unincorporated label. SDS-Page and phospho-imaging were used to separate individual proteins and to identify which proteins had been radiolabeled. The protein mixture was applied to dermatomed skin sections (250 μm) that were intact or abraded via tape stripping to remove the stratum corneum. To further mimic human exposure conditions, diffusion cells were occluded for the 24-hour dosing period. The data demonstrated latex proteins penetrated significantly more through the abraded skin (3.37% ± 0.87) than the intact skin (1.36% ± 0.04). Immunohistochemistry on skin samples using a rabbit anti-latex serum localized latex proteins mainly to the stratum corneum and the viable epidermis. These results suggest that certain latex proteins can penetrate deep enough into the epidermis to sensitize and subsequently to evoke an immune response via the skin. Furthermore, the appearance of radioactivity in the receptor fluid indicates latex proteins or peptides can penetrate into the dermis, which gives rise to the possibility of systemic sensitization via the skin. Future studies will evaluate ammoniated protein mixtures and purified latex proteins in animal and human skin. (These studies were supported in part by NIOSH/NIEHS interagency agreement # Y02ES10189.)

348 IMMUNOCHEMICAL DETECTION AND LOCALIZATION OF ALDEHYDE DEHYDROGENASE ISOZYMES IN HUMAN AND RODENT SKIN.

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Human skin is exposed to potentially reactive aldehydes in the form of environmental chemicals, occupational chemicals and consumer products. These aldehydes may be absorbed across the skin and result in local and systemic toxicity. Aldehyde dehydrogenase (ALDH, E.C. 1.2.1.3.) enzymes play a toxicologically important role in the detoxification of such aldehydes. The highest activities of ALDHs are found in the liver, but these enzymes are also detected extrahepatically. In this study, the presence of ALDH isozymes in human and rodent skin has been investigated. Fresh human, mouse (BALB/c and CBA/CA strains), Fischer 344 rat and Dunkin-Hartley guinea-pig skin was homogenized in ice cold sucrose buffer, pH 6.8. Cytosol and mitochondrial fractions were prepared by differential centrifugation and subjected to SDS-PAGE and immunoblotting. For immunohistochemical analyses, 5 μm sections were cut, of formalin fixed, paraffin-wax embedded skin. Immunoblotting of human, mouse (both strains), rat and guinea-pig skin cytosol, using an ALDH class 1 antiserum, revealed a single band at ~55kDa, equivalent to the molecular weight of the ALDH protein. Immunoblotting of human skin mitochondrial fraction, with ALDH class 2 antiserum, revealed a double band at ~55kDa, while in mouse (both strains), rat and guinea-pig skin, a single ~55kDa band was observed. Immunoblotting of human, mouse (both strains), rat (but not guinea-pig), skin cytosol, using an ALDH class 3 antiserum, revealed a single ~50kDa band. Immunohistochemistry of human skin sections revealed the presence of all three classes of ALDH in the epidermis and, to a lesser degree, in the dermis. In mouse, rat and guinea-pig skin, in addition to epidermal expression, these isozymes were detected in the hair follicles/sebaceous glands,

with the exception of class 3 ALDH which was not detected in guinea-pig skin. In summary, ALDH class 1, 2 and 3 have been detected in human and rodent skin. These enzymes may serve to detoxify reactive aldehydes during percutaneous absorption.

349 FLUOROQUINOLONE ANTIBIOTIC (FQA) TOXICO-KINETICS IN MURINE SKIN AND BLOOD: ANALYTICAL METHODS, SKIN CONCENTRATION AND PHOTO-TOXICITY RELATIONSHIPS.

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Certain FQA are acutely phototoxic in experimental animals and man. The degree of phototoxicity induced by these drugs is strongly influenced by the number and location of halogen constituents. However, some variation in phototoxicity could be a function of the kinetics and deposition of these drugs in the skin. We developed successful methods to quantify FQA in murine skin and plasma. Several concentrations of ciprofloxacin, grepafloxacin, levofloxacin, lomefloxacin or ofloxacin dissolved in saline were administered to Crl:SKH1-*hr*BR hairless mice by gavage. Blood and skin samples were taken at various times after administration. Skin samples were homogenized in 0.2 M phosphate buffer, pH 6.2. Skin homogenate and plasma from anti-coagulated blood were extracted with acetonitrile and the extract analyzed by high pressure liquid chromatography using a C18 column and a water:acetonitrile:methanol:triethanolamine pH 3.0 mobile phase. All FQA were found in the plasma and skin at dose and time of dose-appropriate concentrations. The acute phototoxic potential of some of these FQA was determined by exposing mice to solar-simulated radiation, including ultraviolet radiation, 30-60 minutes after gavage dose. Skin changes (erythema, edema, flaking) were assessed for up to 3 days after exposure. "First generation" FQA such as lomefloxacin showed high skin and blood levels and elicited robust skin changes indicative of photo-toxicity. Many of the subsequently developed FQA, e.g., levofloxacin, while showing comparably high levels in the skin and blood, elicited lower or no skin changes indicative of phototoxicity. These results indicate that the phototoxic potential of these FQA appears dependent on the chemical structure of the FQA and not the deposition of the drug in the skin.

350 OCULAR AND DERMAL PHOTOTOXICITY INDUCED BY A SINGLE 8-METHOXYPSORALEN (8-MOP) AND SIMULATED SUNLIGHT EXPOSURE IN ALBINO AND PIGMENTED RATS.

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The cutaneous phototoxic response to 8-MOP and ultraviolet (UV) radiation in experimental animals is a well-described model for photo-sensitizing drugs. How well such cutaneous phototoxic responses predict adverse effects in ocular tissue has not been established. In this test system we investigated whether the threshold dose for phototoxic damage to the skin and eye was similar and whether constitutive melanin pigmentation influenced the phototoxicity response. Sprague-Dawley (Crl:CD BR VAF/Plus) and Long-Evans (Crl:(LE)BR) rats were anesthetized and the hair clipped from their backs. A 1.3cm^2 skin site was isolated with an exposure mask covering the clipped area. On the Long-Evans rats, both lightly and heavily pigmented skin sites were isolated. One hour after the rats were injected with a single intravenous 10 mg/kg dose of 8-MOP in polyethylene glycol 400, the skin and eyes were exposed to 0.5 to 2.0 MED of solar-simulated radiation (SSR), containing UV, visible, and infrared radiation. Skin responses were assessed 1, 2, and 3 days after exposure, and ocular damage assessed 3 days after exposure. Incidence and severity of skin responses (erythema, edema, flaking) were SSR dose-dependent in both rat strains, with the albino rats showing more severe responses than the pigmented rats. Skin responses were more severe in the lightly pigmented skin than the heavily pigmented skin. Incidence and severity of ocular damage (keratitis, blepharitis, iritis) were also SSR dose-dependent. As compared to the albino rats, the pigmented rats showed fewer kinds and less severe ocular damage. Retinal damage was not found in any rats. The threshold dose for cutaneous responses was lower than the dose for ocular tissue responses. The results, generated by a light source mimicking full spectrum sunlight, demonstrate the capability for generating information on eye safety related to a wide range of potentially phototoxic drugs under various light exposure conditions.

351 PHOTODYNAMIC ACTIVATION OF TOPICAL ATMPn BY UVA OR RED LIGHT IN RABBITS AND MINISWINE.

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ATMPn, a 9-ACETOXY-2,7,12,17- TETRAKIS(METHOXYETHYL) PORPHYCENE, is a photodynamic compound activated by either UVA or red light. It has been preliminarily evaluated in humans as an anti-psoriatic. The effects of a 0.1% ATMPn gel given topically for 5 days followed on day 6 by either fluorescent UVA or red light exposure has been studied for 16 days post-irradiation in the rabbit and Hanford miniswine. In the rabbit robust dermal photodynamic effects occurred with topical administration of 40 ul/cm^2/day followed by irradiation with either light sources (UVA 3-50 J/cm^2, red 6-70 J/cm^2). Post irradiation effects were erythema, edema, flaking, occasional scabbing and ulceration, acanthosis, hyperkeratosis, dermal inflammation and dermal fibrosis. Dermal responses were irradiation dependent with either source but were dose dependent only for UVA. No or minimal responses occurred at skin sites exposed to irradiation alone, ATMPn gel alone, or irradiated vehicle gel sites. Other rabbits were given topically 0.1% ATMPn (1.0 to 60 ul/cm^2 /day) followed by irradiation with 13 J/cm^2 of either UVA or red light. Dose responses to ATMPn were demonstrated. In life post-irradiation dermal responses consisted of erythema, edema, flaking and fissuring. Responses to irradiated sites alone, vehicle treated irradiated sites, or ATMPn treated sites alone were either minimal or non-detectable. In Hanford miniswine 0.1% ATMPn was topically applied for 16 weeks (60 ul/cm^2, 3 x /week, 700 cm^2) 24 hrs preceding 3 X weekly irradiation by either UVA (3, 10, and 50 J/cm^2) or red fluorescent (3, 10, and 70 J/cm^2) light. A very minimal cumulative photodynamic effect occurred consisting of slight increases in erythema and darkening in the irradiated ATMPn sites compared to vehicle treated irradiated sites, vehicle treated non-irradiated sites or ATMPn non-irradiated sites. The difference in degree of dermal photodynamic response between rabbits and miniswine can be explained by a difference in epidermal and dermal penetration of ATMPn following topical administration.

352 NINETY DAY DERMAL TOXICITY STUDY OF 1-(3-CYCLOHEXEN-1-YLCARBONYL)-2-METHYL PIPERIDINE IN RATS.

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This study was conducted to determine the dermal toxicity of the candidate insect repellent 1-(3-cyclohexen-1-ylcarbonyl)-2-methyl piperidine, designated by the United States Department of Agriculture as AI3-37220. AI3-37220 (CAS# 77251-47-9) has been proposed for use as a topical insect repellent. Entomological studies showed that AI3-37220 was as effective as N, N-diethyl-m-toluamide (DEET) against most mosquito species and better than DEET for repelling biting flies. The entomological data supports continued development of AI3-37220 as a topical insect repellent. Ninety-five male and 95 female Sprague-Dawley rats 7 to 9 weeks old were used for this study. Dosage levels were set at 0, 63, 125, 250, and 500 mg/kg/day (2 satellite recovery groups at 0 and 500 mk/kg/day were also utilized). Neat AI3-37220 was applied on a five day per week schedule (excluding weekends and holidays) for 92 calendar days and a total of 65 test compound application days. The liquid test substance was applied to the animals clipped backs covering the area with a thin, uniform film. Dermal application of neat AI3-37220 causes dose-dependent skin irritation in rats. The compound was more irritating to the skin of female rats than male rats. The effects were mild and reversible upon cessation of treatment. Subchronic dermal exposure of AI3-37220 for 90 days to male rats was associated with accumulations of hyaline (protein) droplets in the renal tubules and an increase in the regeneration of renal tubular epithelium. This effect is unique to male rats and is not seen in humans. No compound related renal effects were seen in the satellite recovery groups at 140 days indicating the reversibility of these effects. This information, along with results from other laboratory animal toxicity studies, will be utilized to predict the potential risk to human health upon exposure to this compound.

353 ADVERSE RESPONSES TO SEMISYNTHETIC METAL WORKING FLUIDS IN B6C3F1 MICE.

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About 10 million industrial workers of both sexes are exposed to Metal Working Fluids (MWFs) via inhalation or skin or both. Our preliminary results, following dermal application of 200ul of 50 % unused (neat) semi-synthetic MWF (pH 7 or pH 9.7) to the unshaved backs of 6 week old B6C3F1 mice, twice a week for 6 weeks , show significant increase in weights of the liver of both sexes. The present study is to determine if this weight change is related to oxidative stress consequent to MWF exposure. Therefore we exposed 6 month old mice of both sexes to MWFs following the above protocol except that the topical application was with 5% MWFs, 5 days a week for 13 weeks. Chemical analyses of tissues from these mice indicate significant reduction in ascorbic acid and glutathione levels in the liver of both sexes and in the gonads of the male. Malonaldehyde levels in the male liver and gonads show significant increase. The skin histamine levels are significantly increased in the female. These results suggest that MWFs are absorbed through the skin, exhibit liver toxicity, and may represent an important health risk to industrial workers exposed to these fluids.

354 RESTRAINT STRESS DIFFERENTIALLY AFFECTS CYTOKINE PRODUCTION AND EAR SWELLING IN ALLERGIC CONTACT DERMATITIS (ACD) AND IRRITANT CONTACT DERMATITIS (ICD).

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Previous studies have demonstrated that glucocorticoids suppress the immune response to antigen and that restraint stress increases serum corticosterone levels. Additional research using rats has shown that mild, acute restraint stress increases ear swelling and lymphocytic infiltration in allergic contact dermatitis (ACD), but not in irritant contact dermatitis (ICD). We hypothesized that mild restraint stress alters the murine cutaneous response to chemicals and that these changes would be reflected in the cytokine profile and be gender-specific. For ACD studies, male and female B6,129 mice were sensitized on the flank with 0.5% DNFB (Days 1 & 2) and challenged with 0.25% DNFB on the ear immediately following mild restraint stress (Day 6). For irritant studies, 5% croton oil was applied to the ear immediately after restraint. We measured ear swelling, cytokines TNF-a, IL-1b, IFN-g and IL-4 and serum corticosterone levels as an indicator of activation of the hypothalamic-pituitary axis. Restraint increased serum corticosterone levels in all mice. In the ICD studies, we measured irritant-induced increases in ear thickness and TNF-a and IL-1b production. Ear thickness and TNF-a levels were not altered by restraint stress, however, the concentration of IL-1b was suppressed at 8 and 24 hours. IFN g and IL-4 were minimally detectable in all treatment conditions. There were no gender differences in the irritant response. For ACD, in both males and females, DNFB induced ear swelling and increased TNF-a, IL-1b and IFN-g. Restraint stress coupled with DNFB treatment resulted in an additional, but transient, increase in ear thickness and, at 24 hours, increased IFN-g but decreased IL-1b. TNF-a was more elevated at 8 and 24 hours in females only, and IL-4 was not detected for any ACD treatment conditions. These data suggest a complex interaction between restraint stress-induced changes in the dermal response to chemicals that may be mediated by elevations in serum glucocorticoids in B6,129 mice.

355 USE OF GREEN FLUORESCENT PROTEIN IN A HUMAN ORGANOTYPIC STRATIFYING SQUAMOUS EPITHELIUM FOR MOLECULAR AND PHARMACOLOGICAL STUDIES OF NORMAL AND MALIGNANT EPITHELIAL GROWTH.

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We have developed a novel organotypic *in vitro* model for the study of normal and malignant human squamous epithelial cell proliferation and pharmacological response. This model utilizes expression of green fluorescent protein (GFP) in transfected epithelial cells to identify and isolate cells within an organotypic stratifying squamous epithelium. Prototypic non-malignant and malignant cell lines, BC-1-Ep/SL and SCC13y, respectively, have been developed which stably express GFP. GFP-labeled SCC13y cells (GFP13y) grown in mixed cultures with unlabeled BC-1-Ep/SL cells, do not appear to effect the normal stratification of BC-1-Ep/SL cells in organotypic cultures. Individual and groups of GFP13y cells are visualized in frozen sections using fluorescent microscopy. This report characterizes a number of standard skin differentiation and proliferation markers such as involucrin and PCNA. Data observed in studies using confocal microscopy and flow cytometry are also presented which demonstrate the growth of GFP13y cells over a period of 5-15 days in organotypic culture. This model has potential broad use with all types of stratified epithelia and associated tumors (oral cavity, cervix, trachea, cornea) and melanoma. We believe it is particularly valuable to screen for efficacy of pharmacological agents on normal and malignant epithelia cultured in a context which maintains the cell interactions present in functioning human epithelial tissue. By entirely reconstructing human epithelial cell growth within a tissue-like environment, epithelial cell growth characteristics can be monitored in a physiologically relevant context. Preliminary data characterizing the radiation response and antiproliferative response of a set of standard chemotherapeutic agents are described herein. We believe this approach may provide a more accurate assessment of epithelial cell proliferation kinetics and prove to be a valuable tool for the pharmacologic studies.

356 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCCD) CAUSES ALTERATIONS IN DIFFERENTIATION OF KERATINOCYTES IN ORGANOTYPIC CULTURE.

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Human exposure to the environmental toxin TCDD produces a severe skin pathology known as chloracne. Although several laboratories have previously reported on the aryl hydrocarbon receptor (AhR)-mediated cellular responses of normal human or rodent keratinocytes to TCDD *in vitro*, little evidence has been available to link these observations to the specific pathology observed in skin. To approximate the three dimensional microenvironment of human skin, we have established organotypic cultures utilizing BC-1-Ep/SL cells, a novel human keratinocyte cell line. The BC-1-Ep/SL cell line was isolated in our laboratory and is identical to normal keratinocytes in its growth and differentiation characteristics. These cells differentiate normally *in vitro* and are morphologically indistinguishable from normal human keratinocytes at both the light and electron microscope level. The BC-1-Ep/SL keratinocytes are immortal and have been shown to be non-tumorogenic in nu/nu mice. Upon treatment of the organotypic model system with TCDD, we have observed an altered morphology as compared to controls. In treated samples we observed a thickening of the granular and cornified superbasal layers of keratinocytes in organotypic cultures. This phenomenon was observed in organotypic culture seeded with normal human keratinocytes and subsequently with BC-1-Ep/SL keratinocytes. We present new data on the differentiation characteristics of TCDD-treated keratinocytes in organotypic culture and examine the effect of TCDD on epithelial-mesenchymal interactions *in vitro*.

357 EFFECT OF CACL2 CONCENTRATION ON THE RATE OF PROLIFERATION OF CULTURED HUMAN KERATINOCYTES.

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Topical formulations of drugs may contain $CaCl_2$ and citrate as excipients. In order to analyze the influence of calcium on the epidermal cells' behavior, the effects of $CaCl_2$ on the proliferation and cell cycle distribution of cultured normal human epidermal keratinocytes (NHEK) and HaCaT keratinocytes were investigated. The cells were continuously (24 h) or transiently (1, 2, 4 h) exposed to $CaCl_2$ (0.09, 0.2, 1, 2 and 5 mM), or to combinations of $CaCl_2$ and citrate (5 / 5 mM, and 40 / 40 mM). BrdU incorporation determined by ELISA was used as a marker for cell proliferation. Flow cytometric evaluation of the propidium iodide staining of fixed cells served for cell cycle distribution analysis. $CaCl_2$ induced a concentration-dependent decrease in keratinocyte cell growth with respect to non-treated cells. This effect was more pronounced for NHEK than for HaCaT cells. The combination of $CaCl_2$ and citrate (40 / 40 mM) strongly decreased this parameter in both cell types, and induced cytotoxicity as measured by LDH release. After 24 h treatment, NHEK and HaCaT cells incubated with 40 mM $CaCl_2$ + 40 mM citrate showed changes in the cell cycle distribution (increase in the percentage of cells in G1 phase and decrease in S phase) which were indicative

of a decrease of the proliferation rate. Although changes of the medium osmolarity played a role in these effects, their contribution was only minor. In conclusion, $CaCl_2$ modulates the proliferation rate and the cell cycle distribution of cultured human keratinocytes.

358 BIOTRANSFORMATION AND CYTOTOXICITY OF SULFAMETHOXAZOLE (SMX) AND DAPSONE (DDS) IN NORMAL HUMAN-NEONATAL EPIDERMAL KERATINOCYTES (NHEK).

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SMX and DDS are both associated with a high incidence of cutaneous drug reactions (CDR), though SMX causes a significantly greater frequency of adverse effects. Proposed mechanisms for the pathogenesis of these CDR suggest that bioactivation to a hydroxylamine metabolite is a critical initiating step. However, conventional metabolism studies demonstrating the bioactivation of SMX or DDS to systemically available hydroxylamine species have failed to adequately explain how these toxic metabolites may elicit the site-specific reactions observed clinically. Because the skin possesses substantial metabolic capacity, we hypothesized that local bioactivation of SMX and/or DDS by keratinocytes may be a contributing factor in the development of CDR. NHEK suspensions ($7x10^5$/mL) derived from cultures grown in high Ca^{+2} (2mM) media for 48 hr were able to metabolize 1mM SMX and DDS to N-acetyl and hydroxylamine derivatives, albeit at low levels. Greater amounts of N-acetyl-SMX were formed than monoacetyl-DDS (3.6 vs. 2.0 nmoles, respectively, at 12 hr), though hydroxylamine formation was more comparable (0.9 vs. 1.2 nmoles). Metabolite formation occurred in a time- dependent manner from 0.5 to 24 hr. A similar metabolic profile was also observed for SMX using NHEK cultured in low Ca^{+2} (0.15mM) media. Despite this bioactivating capability, however, evaluation of the effect of SMX and DDS (31.3μM-4mM) on NHEK viability failed to demonstrate a significant increase in cell death above controls, even after 24 hr exposure. Comparison of the cytotoxic effects of synthetic hydroxylamines toward NHEK showed that 3 hr exposure to DDS-hydroxylamine caused a concentration-dependent increase in cell death (LC_{50}=281±80μM). In contrast, NHEK demonstrated no increase in cell death when incubated with SMX-hydroxylamine (31.3μM-4mM). Considering the predominance of keratinocytes in normal human skin, local formation and release of hydroxylamine metabolites into the micro-environment of the skin may play a role in the development of CDR. (Supported by NIH Grant AI41395.)

359 COMPARISON OF DERMAL CORROSION VALUES FOR SELECTED INDUSTRIAL CHEMICALS USING CORROSITEX.

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Skin irritation is the most frequently reported occupational hazard for those employees engaged in the use and transport of industrial chemicals. The rabbit test is most common *in vivo* method used to evaluate dermal irritation and/or corrosion. Several *in vitro* test methods have been developed, with Corrositex® being the first to gain approval by a regulatory agency (DOT). Several classes of industrial chemicals, 24 formulations, were tested using the Corrositex® method and the results were compared to standard animal data for each of the formulations. Based on the results, Corrositex® accurately predicted a corrosive endpoint in 8 of the 24 formulations, when compared to the animal data, and accurately predicted a non-corrosive endpoint for 1 of the 24 formulations. The Corrositex® assay overpredicted a corrosive endpoint for 9 of the 24 formulations, and underpredicted a corrosive endpoint for 6 of the 24 formulations. In addition, corrosive classifications (DOT Packing Groups) were also evaluated. The Corrositex® assay overpredicted the Packing Group classification for 12 of the 24 formulations tested and underpredicted 7 of the 24 formulations tested.

The Corrositex® assay did not adequately predict a corrosive endpoint or Packing Group for the 24 formulations used in this study. This study was a practical application of the Corrositex® method, and the method appears to have failed for predicting the corrosive hazards of the chemical classes included in this study.

360 FURTHER EVALUATION OF THE EPIDERM™ AND EPIOCULAR™ *IN VITRO* IRRITATION MODELS.

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Both EpiDerm™ and EpiOcular™ are commercially available *in vitro* models for assessing the skin and eye irritation potential, respectively, of chemicals. The EpiDerm™ and EpiOcular™ tissue models are organotypic three-dimensional human skin and corneal analogues derived from human epidermal keratinocytes. Both are colorimetric cytotoxicity models based on the ability of chemically exposed tissues to reduce MTT. An MTT effective time-50 (ET-50; time required to decrease cell viability by 50%) is established for each chemical. The objective of this study was to evaluate both EpiDerm™ and EpiOcular™ for their ability to assess the skin and eye irritation potential, respectively, of several water soluble and insoluble compounds previously evaluated *in vivo*. All animal data was obtained from previously published animal studies. No new *in vivo* testing was performed. The following compounds were examined with both EpiDerm™ and EpiOcular™ : benzyl alcohol; dibasic ester; heptyl acetate, propylene carbonate; N-methylpyrrolidone; sodium lignosulfate; sodium alkylnaphthalene sulfonate; polyethoxylated polyarylphenol; polysorbate 85; and sodium dodecylbenzene sulfonate. The following were examined only with EpiDerm™ : 2 and 100% 1-octyl-2-pyrrolidinone and an aromatic hydrocarbon. One or 0.3% Triton X-100 were used as positive controls for EpiDerm™ and EpiOcular™ , respectively. Of the 13 compounds evaluated, EpiDerm™ correctly identified 10 (77% concordance with *in vivo* data). EpiDerm™ overpredicted the remaining 3 compounds. EpiOcular™ correctly identified 7 of the 10 examined compounds (70% concordance with *in vivo* data). The eye irritancy of 2 compounds was underpredicted, while that of the remaining compound was overpredicted. Although these systems have proved useful for evaluating the irritancy potential of water soluble compounds, further evaluation of their ability to predict the skin and eye irritation potential of various classes of water insoluble compounds needs to be conducted.

361 A STRUCTURE-ACTIVITY RELATIONSHIP (SAR) MODEL FOR ESTERS THAT CAUSE HUMAN SKIN IRRITATION.

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A structure-activity relationship (SAR) model has been developed to discriminate active from inactive esters on the basis of their ability to cause skin irritation in humans. The model is based on patch test data gathered from a critical review of the literature. Esters were selected from a broader database of human irritants and represented a large subset of homologous chemicals. Nineteen physicochemical parameters that represented transport, steric and electronic properties, were calculated for each chemical. Best subset regression analysis identified models for further analysis. Regression analyses were performed to identify all significant models ($p < 0.05$) that had variables that were also significant ($p < 0.05$). These models were evaluated using linear discriminant analysis to determine if the irritant esters could be discriminated from non-irritant esters. Cross validation was employed to estimate the sensitivity and specificity of the model. The model with the greatest discriminating ability contained the parameters density, Hansen dispersion, Hansen hydrogen bonding, and hydrogen bond donor. The overall sensitivity of the model was 0.85, while the specificity was 0.76. Active irritant esters had lower densities, lower hydrogen bond donor and lower Hansen hydrogen bonding param ters, and higher Hansen dispersion parameter. These characteristics indicate that irritant esters would tend to be less water soluble and less likely to be involved in hydrogen bonding to protein components of the skin. Thus, they are more likely to penetrate the epidermis. Absent from the model are the electronic parameters to indicate a relationship between activity and reactivity. The stability of the model was tested using random subsets of the database. The above parameters were observed with consistency over the random subsets indicating stability with regard to parameters in subsets of the data. The results imply that the model should be predictive for the irritant activity of esters toward human skin. (Supported by NIOSH-CDC #0009653058.)

362 A TWO CENTER STUDY OF THE DEVELOPMENT OF ACUTE IRRITATION RESPONSES TO FATTY ACIDS.

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A human 4 hour patch test has been developed for testing the irritation hazard potential of chemicals. This method was developed for comparative irritation assessments. Although skin irritation responses in human subjects can be quite variable, this acute irritation test method has proven robust in both intra- and inter-laboratory tests. The previous inter-laboratory studies were not optimal in that slightly differing protocols were used and the studies were not controlled for time of year or source of test chemicals. As a result, some variation in acute irritation responses were seen, that might have been reduced somewhat had these variables been controlled to a greater extent. In the present study, two laboratories conducted a direct comparison study of the acute irritation potential of 3 structurally related, concentrated fatty acids (octanoic acid, decanoic acid, and dodecanoic acid) in comparison to a benchmark positive control chemical (20% sodium dodecyl sulfate [SDS]). The studies were run within a 4 month period using the same commercial source of test chemicals. Test subjects were treated with each chemical under occluded patch conditions for gradually increasing exposure time intervals up to 4 hours. The results were then evaluated in terms of total cumulative incidence of positive responses and time response patterns. Using statistical comparisons of cumulative irritation, the rank order of irritation potential was decanoic acid ≥ octanoic acid > SDS >> dodecanoic acid. The statistical comparisons and the time response patterns for each chemical were nearly identical at the two laboratories. There were also very similar, and intriguing, variations in the inter-chemical response patterns seen in the two studies. Hence, when conducted under as controlled a set of test conditions as possible, this acute irritation protocol shows remarkably high consistency across independent test laboratories; further supporting its continued development and acceptance as an alternative skin irritation test method.

363 SKIN IRRITATION POTENTIAL OF N,N'-DI(2-HYDROXY-BENZYL)ETHYLENE-DIAMINE-N,N'-DIACETIC ACID (HBED) IN RATS.

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N,N'-di(2-hydroxybenzyl)ethylenediamine-N,N'-diacetic acid (HBED) is a promising alternative to desferrioxamine for treating Fe overload. HBED releases more Fe from Fe-loaded rats and *Cebus* monkeys at bioequivalent doses via the s.c. route. The irritation potential of the commercially available compound was assessed before proceeding with preclinical toxicity studies. Initially, young adult Sprague Dawley rats (6/sex/group) were administered HBED·HCl at 50 mg/mL in propylene glycol (PG), glycerol, or 40:60 PG:aqueous (pH 7.4) solution as a single injection to the upper back. The same volume of vehicle was applied to each rat at an adjacent site. The sites were scored on Days 3 and 8 after the injections. HBED in either glycerol or aqueous PG was slightly irritating; in pure PG, irritation by the vehicle masked any effects of the test compound. Subsequently, Na salts of HBED were prepared with (+Cl) and without (-Cl) chlorine present and tested in aqueous solution in rats using the same protocol. (+Cl) was slightly irritating whereas (-Cl) was nonirritating. The results indicate that irritation produced by commercially available HBED was due to the HCl component or to NaCl after neutralization and suggest that the chlorine-free Na salt would be better tolerated in patients receiving repeated s.c. doses. (Sponsor: NIDDK Contract No. N01-DK-3-2201.)

364 INHIBITION OF NEUROGENIC INFLAMMATION BY PEPTIDES FROM MONOSTROMA GRAVILLEI.

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Monostroma, anti-inflammatory peptides from the algae *Monostroma gravillei*, licenced by Secma Biotechnology shows strong anti-inflammatory properties. According to the characteristic structure of anti-inflammatory peptide agonists : double basic residues Arg-Lys, Lys-Arg, Arg-Arg or Lys-Lys accompanied by hydrophobic residues such as leucine, isoleucine, methionine or valine and since the peptides purified from *Monostroma gravillei* algae showed the NH2 end-sequence as given below – peptide 1 : NH_2-Glu-Ala-Ile-Leu-Asp-Pro-His-Phe-Lys-Lys-Leu-Leu-Ile-Lys- peptide 2 : NH_2-Asp-Ala-Ile-Leu-Asn-Pro-His-Phe-Lys-Lys-Leu-Leu-Ile-Lys, investigations were designed to know whether they compete with SP for binding on NK1 from human primary culture of keratinocytes and/or inhibit the leakage of SP by the dorsal root ganglion neurone (which convoy nociceptive stimuli). These peptides display a mitogenic activity as shown by the increased incorporation of tritiated thymidine in the DNA of human keratinocytes in culture by 24, 48 and 32% for respectively 2, 4 and 10μg/ml as compared to the control. The Monostroma inhibits the binding of substance P on NK1 receptors by 55, 67 and 58% for respectively 2, 4 and 10 μg/ml and reduces the substance P release by 15 and 31% for respectively 2 and 4 μg/ml as compared to the control in dorsal root ganglia.

365 LOCALIZATION OF EPIDERMAL-DERMAL JUNCTION EPITOPES IN THE MOUSE EAR VESICANT MODEL EXPOSED TO BIS (2-CHLOROETHYL) SULFIDE.

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Bis (2-chloroethyl) sulfide (sulfur mustard, HD), a potent cutaneous vesicant and bifunctional alkylating agent, produces significant time-dependent histopathologic changes in the mouse ear vesicant model (MEVM). The right ear of male CD1 mice were exposed topically to 5.0μ l of 195mM (0.16mg) HD in dichloromethane while the left ear control was dosed with 5.0μ l of dichloromethane. The ears of all mice were harvested after 6, 12, 18, and 24 hrs. Indirect immunofluorescence (IF) revealed the frequency of microvesicles in the HD-exposed ear at 6 hrs (2/8), 12 hrs (6/8), 18 hrs (7/8), and 24 hrs (6/6). In all controls and HD-treated mouse skin, IF of the epidermal-dermal junction (EDJ) stained moderately with bullous pemphigoid (BP), epidermolysis bullosa acquisita (EBA), and laminin (Lam) antibodies, and stained lightly with bullous pemphigoid 180 (BP180), fibronectin (Fn), and type IV collagen (Coll IV). Mouse anti-human monoclonal antibodies for GB3, L3d, and 19-DEJ-1 (Uncein) did not cross-react in mouse skin. Within the microvesicles, BP, BP180, and Fn exhibited focal areas of light epidermal staining and homogeneous dermal staining, while EBA, Lam, and Coll IV showed moderate dermal staining. BP and Fn staining was weaker but more inconsistent with time. Immunoelectron microscopy studies revealed similar results, with an increase in tissue damage from 6 to 24 hrs which corresponded to a decrease in antibody staining intensity. These results indicate that in the MEVM, HD causes separation in a *'zone'* between the uppermost lamina lucida and the basal cell plasma membrane/ hemidesmosome complex. While the MEVM differs significantly from other models in which the split occurs within the lamina lucida, it is still a valuable tool in the development of specific prophylactic and therapeutic strategies. (Supported by USAMRMC DAA H04-96-0086.)

366 DETECTION OF PROLIFERATING CELL NUCLEAR ANTIGEN IN THE MOUSE EAR VESICANT MODEL FOLLOWING EXPOSURE TO BIS (2-CHLOROETHYL) SULFIDE.

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Microvesicles were induced in the mouse ear vesicant model (MEVM) in response to bis (2-chloroethyl) sulfide (sulfur mustard, HD), which produces significant time-dependent histopathologic changes. The right ear of male CD1 mice were topically exposed to 5.0μ l of 195mM (0.16mg) HD in dichloromethane while the left ear was dosed with 5.0μ l of the vehicle dichloromethane (control). Tissue was harvested at 6, 12, 18, and 24 hrs post-dose. Immunohistochemical staining of the tissue fixed in 10% neutral buffered formalin and embedded in paraffin allowed quantification of the proliferating cell nuclear antigen (PCNA), a nuclear marker for cell proliferation. While the growth fraction (GF = PCNA stained cells per unit length / total number of cells per unit length) showed no significant differences ($P<0.05$) between exposure times, several interesting trends were apparent. The GF of the vehicle control ears (inner and outer surfaces, respectively) were marginally lower than the corresponding HD-treated ears (inner and outer surfaces, respectively) at 6 and 12 hrs and marginally higher at 18 and

24 hrs. Also, the inner ear (applied vehicle and HD) GF was slightly lower than the GF of the corresponding outer ear. Overall, the GF in the HD-treated ears was the greatest at 6 hrs, with a slight downward trend from 12 to 24 hrs.

367 SYSTEMIC ADMINISTRATION OF CANDIDATE ANTIVESICANTS TO PROTECT AGAINST TOPICALLY APPLIED SULFUR MUSTARD IN THE MOUSE EAR VESICANT MODEL (MEVM).

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The MEVM provides a quantitative edema response as well as histopathological and biochemical endpoints as measurements of inflammation and tissue damage following exposure to the chemical warfare agent sulfur mustard (HD). In the MEVM, several topically applied anti-inflammatory agents provided a significant degree of protection against HD-induced edema and dermal-epidermal separation. This study evaluated the protective effects of these same pharmacologic compounds when administered systemically in the MEVM. Alzet osmotic pumps were used to deliver a subcutaneous dose of the appropriate anti-inflammatory agent starting 24 h before exposure to sulfur mustard and continuing until 24 h post-exposure to HD. Twenty-four hours after pump implantation, 5 µl of a 195 mM (0.16 mg) solution of sulfur mustard (d=1.27 g/ml; MW 159; purity 97.5%) in methylene chloride was applied to the inner surface of the right ear of each mouse. HD injury in the mouse ear was measured by both edema response (fluid accumulation) and histopathological damage (necrosis, epidermal-dermal separation). The systemic administration of hydrocortisone, indomethacin and olvanil provided a significant reduction in edema (24%, 26% and 22%, respectively) from positive control. Hydrocortisone, indomethacin and olvanil also caused a reduction in subepidermal blisters compared with the HD positive control (71%, 52% and 57%, respectively). We show here that these anti-inflammatory drugs are effective when administered systemically in the MEVM.

368 COMPARISON OF SPECTROPHOTOMETRIC AND FLUOROMETRIC ASSAYS OF PROTEOLYSIS IN CULTURED HUMAN CELLS EXPOSED TO SULFUR MUSTARD.

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Histopathologic evaluation of sulfur mustard (HD) vesication suggests an involvement of proteolysis in the pathogenesis of the epidermal-dermal separation. Drug screening of candidate therapeutic compounds as medical countermeasures to vesicant agents requires the use of sensitive, reproducible assays of known biomarkers of the HD injury. Commercial protease substrates with either chromogenic or fluorogenic properties have been used to assess proteolytic activity in human PBL and HEK exposed *in vitro* to HD. In the spectrophotometric assays, PBL demonstrated significant increases in proteolysis as measured with the Chromozym TH and TRY serine protease substrates. This activity is donor specific in that 5 out of 11 donors showed marked reproducible activity. HEK presented a very different pattern of response with negligible TH or TRY activities but marked urokinase increases. Using fluorescent substrates that identify different proteases than the chromogenic substrates, PBL demonstrated a general decline in all aminopeptidase activities tested. However, in HEK, considerable variabilities were detected that might be related to culture and handling conditions. When HEK were trypsinized into cell suspensions and analyzed by flow cytometry, no changes in aminopeptidase activities were detected following HD. When the HEK were grown in 24-well plates and assayed *in situ*, certain aminopeptidase activities (K aminopeptidase B and G aminopeptidase) were increased while others (L aminopeptidase) were decreased. Assays of HD increased proteolysis in PBL with serine protease substrates are reproducible within a given donor population and can be used for screening candidate compounds. Proteolytic assessment in HEK is variable and subject to culturing and handling procedures and will require a considerable amount of research before it can be used in screening compounds.

369 PROTEIN PHOSPHATASE 2A IDENTIFIED AS THE CYTOSOLIC ENZYME INHIBITED BY THIODIGLYCOL, THE HYDROLYSIS PRODUCT OF SULFUR MUSTARD.

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Sulfur mustard (2,2'-dichlorodiethyl sulfide) is a potent vesicant used in chemical warfare. Injury to exposed epithelial tissue is delayed and ranges from erythema to massive fluid-filled blisters and necrosis in a dose dependent manner. The mechanism is unknown. However, the chemical warfare agent Lewisite and the natural vesicant cantharidin have been shown to inhibit protein (serine/threonine) phosphatase 2A (PP2A) *in vitro*. We reported several years ago that sulfur mustard inhibited enzymatic non-alkaline phosphatase related *p*-nitrophenyl phosphate hydrolysis in mouse liver cytosol, activity usually attributed to protein (serine/ threonine) phosphatases (Brimfield (1995) Toxicol. Lett. 78, 43-48). At that time we showed that the actual inhibitor was thiodiglycol (TDG), the hydrolysis product of sulfur mustard, rather than sulfur mustard itself. The IC_{50} in cytosol was 100 µM. We report here that thiodiglycol inhibits the activity of purified PP2A from human red blood cells. The methodology employed was the real time spectrophotometric measurement of *p*-nitrophenol formation by following the increase in optical density at 405 nm and 30° C. Samples contained 1.3 mg/mL *p*-nitrophenyl phosphate in 40 mM Tris buffer, pH 8.1 containing 1 mM $MgCl_2$, 1% bovine serum albumin, enzyme and various quantities of TDG. Reactions were started by the addition of PP2A in 20 mM MOPS buffer, pH 7.5 in a quantity to bring the final activity to 1.0 unit per assay. Final volume was 3.0 mL. The IC_{50}, in this initial work, was approximately 1.0 mM. This is a higher value than we observed in the cytosol and suggests that additional metabolism occurred in the cytosol to produce the ultimate inhibitor. PP2A is a target for cantharidin and Lewisite, and is the probable source for the enzymatic *p*-nitrophenyl phosphate phosphatase activity we found in mouse liver cytosol in our earlier work. PP2A inhibition by TDG provides an indication that Lewisite, cantharidin and sulfur mustard work by a common mechanism.

370 CHARACTERIZATION OF NERVE AGENT PENETRATION THROUGH PORCINE AND HUMAN SKIN AND THE EFFICACY OF A FIELDED DECONTAMINANT.

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The chemical warfare nerve agents, pinacolyl methylphosphonofluoridate (GD) and O-ethyl S-2-diisopropylaminoethylmethylphosphonothiolate (VX), are potent cholinesterase inhibitors that are rapidly absorbed through skin. Studies were performed using dermatomed skin in an *in vitro* skin penetration model, the RCR Low-Flow Cell, to determine (1) the effect of -70 C cryopreservation on the penetration rates of GD and VX through porcine skin, (2) the relative penetration rates of GD and VX through cryopreserved human skin, and (3) the decontamination efficacy of the powder fielded in the U.S. Army M291 decontamination pack against GD and VX applied onto cryopreserved human skin. The two endpoints used to assess agent skin penetration were (1) the time period between agent application and significant inhibition of acetylcholinesterase (AChE) added to the receptor fluid, and (2) gas chromatographic assay for agent extracted from the receptor fluid. Cryopreservation of porcine skin slightly but significantly slowed GD but not VX penetration. There was no significant difference between the mean times of human skin penetration, as measured by AChE inhibition, of GD (9.3 min) and VX (13.9 min). The M291 powder significantly reduced the amount of both agents that penetrated cryopreserved human skin. (Supported by USAMRDC Contract DAMD17-89-C-9050.)

371 COMPARING OCCUPATIONAL AND ENVIRONMENTAL RISK ASSESSMENT METHODOLOGIES USING PHARMACOKINETIC MODELING.

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Human health risks from occupational exposures are managed by limiting exposures to acceptable levels established by the American Conference of Governmental Industrial Hygienists or another similar body. Acceptable environmental exposures are benchmarked by values such as U.S. Environmental Protection Agency Reference Doses and Reference Concentrations. The approaches to establishing these values are different, as are the groups they are intended to protect, complicating direct comparisons.

In this evaluation, physiologically based pharmacokinetic modeling was used to predict tissue doses from acceptable/safe exposures as established by different organizations and agencies. Internal doses calculated for an agency's acceptable/safe exposures via oral and inhalation routes may differ substantially, but are sometimes in excellent agreement. Based on the 14 compounds modeled in this effort, acceptable occupational exposures almost always produce greater internal doses than acceptable environmental exposures. This finding suggests different mindsets among these groups regarding how safe is "safe." (This work was supported by Contract No. DAAA21-93-C-0046 (Task No. N.113, Subtask R1-4) to the National Defense Center for Environmental Excellence, operated by Concurrent Technologies Corporation.)

372 COMPARISON OF ACUTE INHALATION EXPOSURE LEVELS FOR CHEMICAL IRRITANTS AMONG FIVE AGENCIES IN THE UNITED STATES.

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Acute inhalation exposure limits have recently been developed by various agencies to protect the community. Many of the chemicals for which limits have been set are sensory irritants. In general, these limits have been established to give guidance regarding the need to evacuate following accidental releases. The purpose of this paper was to compare the various scientific approaches used to set these limits. Proposed one-hour exposure limits intended to prevent sensory irritation for the chemicals formaldehyde, xylene, toluene, methyl ethyl ketone and hydrogen sulfide were evaluated. Comparisons between the AIHA Emergency Response Planning Guideline (ERPG), EPA Acute Reference Exposure (ARE), Minnesota Health Risk Value (HRV), New York Air Guide-1 (AG-1), and California Acute Reference Exposure Level (REL) were made. A set of recommended criteria for calculating alternative one-hour exposure limits for these chemicals was developed. These criteria included the use of specific toxic endpoint selection, correction for short-term experimental exposure duration, and a recommendation to build a dose-response curve based on the a collection of the best published sources. Our analysis indicated that there was significant variability between short-term ambient air exposure limits developed by the five different agencies and that these were often different from our recommended values. The selection of the most sensitive adverse effect, the application of default uncertainty factors and the identification of a NOEL from a single study rather than a "blended" NOEL based on the weight of evidence accounted for the differences among the agencies. Disparities in the methodologies used to establish exposure limits will likely be resolved as more experience is accumulated.

373 VALIDATION STUDY OF AN OCCUPATIONAL EXPOSURE BAND (OEB) CLASSIFICATION SYSTEM.

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The purpose of an OEB Classification / Handling Guideline System is to provide an exposure control and containment strategy for handling substances when the available data are insufficient to establish a numerical Occupational Exposure Limit (OEL). In such a system, substances are assigned to one of several bands (OEBs) based upon the substance's presumed hazard and/or whatever limited toxicological and hazard data may be available at the time the classification is made. For each OEB there is then a general containment philosophy, a Handling Guideline (HG), specific for differing scales of operations. The HG identifies engineering controls, personal protective equipment, administrative controls, waste disposal methods, and decontamination practices generically appropriate for limiting exposure to materials with the designated level of hazard. In order to validate the OEB Classification Scheme used by Pfizer, we examined certain information contained in 29 drug substance OEL monographs approved by the corporation's Technical Review Committee (TRC) and, independent of the TRC's OEL, cast each compound into one of the 5 OEBs described by the Classification Scheme. The data considered for the banding exercise were limited to those generally available early in product development, and consisted of: clinical potency, acute toxicity, irritation / corrosion, sensitization, mutagenicity, and ≤ 30-day repeat-dose toxicity. In 27 of 29 instances, the OEB Criteria caused the compound to be classified at the same or a more conservative Band than that called for by the OEL. Indeed, in 12 of the 27 "correct" cases, the OEB and the OEL were in concurrence. In the 2 cases of non-concurrence, the cause of the failure was judged to be rounding error inherent in the OEL setting process. Based upon the high level of concordance and low failure rate in this study, the Pfizer OEB Classification Criteria have been successfully validated and adopted for corporate application worldwide.

374 DETERMINATION OF ACCEPTABLE EXPOSURE LIMITS FOR THE ANTI-VIRAL AGENT RIBAVIRIN.

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Ribavirin is a potent and broad spectrum antiviral agent commonly given in aerosol form to treat respiratory syncytial virus infections in children. Oral administration of ribavirin has been tested for teratogenicity in rats, mice, rabbits and baboons. The most susceptible test species for reproductive effects was the rabbit, with a no observable adverse effect level (NOAEL) of 1 mg/kg/day (Huntington Laboratories). Even though no adverse health effects have been reported in patients treated with ribavirin, potential fetal toxicity is a major concern among occupationally exposed health care professionals, since many of these workers are women of child-bearing age. Previous approaches to calculating a permissible exposure limit (EL) in humans were based on pharmacokinetic studies reporting an oral bioavailability of about 45%. However, a recent human study provides more relevant bioavailability data for inhalation exposures received by medical personnel. In this study, we used this recent human data that demonstrates that ribavirin bioavailability following inhalation exposure is only 0.5% of the total inhaled dose (Linn et al., 1995). We then used this human bioavailability data together with plasma and red blood cell (RBC) concentrations of ribavirin in pregnant rabbits administered a NOAEL dose of ribavirin during organogenesis. The rabbit teratology study showed that plasma and RBC ribavirin levels at the NOAEL are 2.4 and 23.6 μmol/l, respectively. An upper limit of an inhaled dose for occupational exposure was then derived using a 100-fold uncertainty factor applied to blood ribavirin levels at the rabbit NOAEL. Assuming an average female worker of 60 kg, a ventilation rate of 25 l/min, and continuous exposure during an 8-hr shift, the calculated 8-hr EL for inhalation of ribavirin was 7.4 mg/m^3.

375 THE CONCENTRATION-EXPOSURE DURATION RELATIONSHIP FOR INHALED TOXICANTS.

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When desired exposure-duration data are lacking for an assessment of acute inhalation toxicants, scaling across time may be based on the relationship between exposure concentration and exposure duration for a specific response such as lethality (LC_{50}). A log-log regression analysis similar to the probit analysis described by ten Berge et al. (1986, J. Hazard. Mat. 13:301-309) was used to extrapolate concentration-response data across different exposure times. The regression analysis by ten Berge et al. resulted in the following equation: $C^n \times t = k$, where C= concentration, t = exposure time, k = a constant, and the exponent n is = b_1/b_2 (probit regression coefficients) or the negative reciprocal of the slope of the log-log regression analysis. Data sets were evaluated for 40 different inhalation toxicants including respiratory tract irritants such as ammonia and hydrogen fluoride, systemic toxicants such as hydrogen cyanide, and combined respiratory tract irritant and systemic toxicants such as ethylene oxide. All data sets were not sufficient to conduct a regression analysis. The results showed that n fell between 0.8 and 2.5 for almost all data sets. This evaluation suggests that a default n between 1.5 and 1.7 (mid point) may be a good estimate for chemicals with insufficient data. Evaluation of these data sets also suggests that n may be based on data for a breakdown product (HCl for chloromethylsilanes) or a structurally/mechanistically similar chemical (ethylenimine for propylenimine) for chemicals with insufficient data.

376 RE-EVALUATING CANCER RISK ESTIMATES FOR SHORT-TERM EXPOSURE SCENARIOS.

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Cancer risk estimates have traditionally been calculated with the assumption that excess cancer risk is directly proportional to the cumulative carcinogen dose. For exposure to a carcinogen at a given rate, this means that the excess cancer risk is a linear function of exposure duration (e.g., exposure for 7 years is assumed to carry one-tenth the cancer risk of exposure for 70 years). The validity of this assumption was tested empirically using data from chronic lifetime and stop-exposure studies of carcinogens conducted by the National Toxicology Program. Seven compounds were identified as having data sufficient for comparison of relative cancer potencies from short-term versus lifetime exposure. In every case, the tumor incidence was higher in the stop-exposure group than the corresponding cumulatively dosed, lifetime exposure group. For both the chronic and stop-exposure studies, the data were modeled and the maximum likelihood estimates of the dose corresponding to a 10% added cancer risk (ED_{10}), as well as 95% lower confidence limits (LED_{10}), were calculated. These comparisons suggest that linear reductions in cancer risk for less-than-lifetime exposures in risk assessments may underestimate risks of short-term exposures to carcinogens.

377 USE OF LETHALITY DATA DURING CATEGORICAL REGRESSION MODELING OF ACUTE REFERENCE EXPOSURES.

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Categorical regression is being considered by the U.S. EPA as an additional tool for derivation of acute reference exposures (AREs) to be used for human health risk assessment for exposure to inhaled chemicals. Categorical regression is used to calculate probability-response functions for health effect data which has been classified into ordered severity categories such as no-observed-adverse-effect level (NOAEL), adverse effect level (AEL) and lethal effect level (FEL). The categorical regression model calculates the exposure concentrations and durations at which there is a 10% probability of adverse effects, the EC-T10, which can be used to derive AREs. Concerned that the use of lethality data would not be conservative, peer reviewers of the categorical regression approach recommended excluding lethality data from EC-T10 calculations. To determine the effect of removing lethality data, data sets for the acute toxicity of perchloroethylene, methyl isocyanate, phosgene, trichloroethylene, hydrogen sulfide and ethylene oxide were analyzed with and without lethality data. The effect of excluding lethality data seemed to be related to the amount of lethality data in the database. EC-T10s for databases with the largest amounts of lethality data were increased when lethality data was excluded, while EC-T10s for databases with the smallest amounts of lethality data were decreased. To confirm this observation, analyses were performed on hypothetical data sets with various amounts of lethality data. Calculations with hypothetical data sets did not confirm the observation on actual data sets; addition or removal of lethality data did not change the EC-T10. This study showed that the percentage of lethality data in a data set does not determine how the inclusion or exclusion of lethality data affects the ECT-10. Further investigations may examine the location of lethality points at low vs high concentrations and durations; and the intermingling of NOAEL, AEL and FEL data.

378 TOXIC EQUIVALENCY (TEQ) OF POLYCHLORINATED DIBENZO-*P*-DIOXINS (PCDDS) IN AN OVULATION MODEL: VALIDATION OF THE TEQ CONCEPT FOR ENDOCRINE DISRUPTION.

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Polychlorinated dibenzo-*p*-dioxins (PCDDs) are structural analogues, which produce a similar spectrum of biological and toxicological responses in animals, albeit with differential potencies. The gonadotropin-primed immature female rat model was used to study the effect of PCDDs on ovulation. Single doses of different PCDDs and their mixtures were given orally to 23-day-old rats. Gonadotropin from Pregnant Mare's Serum (PMSG) was injected (5 IU) 24 hr later to induce follicular maturation. Rats were decapitated at various times after PMSG, blood collected and ovarian weight measured. Serum concentrations of 17ß-estradiol (E_2), progesterone (P_4), luteinizing hormone (LH) and follicle stimulating hormone (FSH) were determined by radioimmunoassay. Ovulation was measured 72 hr after injection of PMSG by counting ova flushed from oviducts. PCDDs dose-dependently decreased the number of ova shed and reduced ovarian weight gain induced by PMSG. The slopes of the dose responses generated by individual PCDDs and/or their mixture were similar. PMSG-induced increase in serum E_2 was dose-dependently enhanced by PCDDs on the day of expected ovulation; in contrast, serum P_4 and FSH were dose-dependently decreased at that same time point. PCDDs also altered the temporal pattern of serum FSH and LH. The results indicate that all PCDDs have the same mode of action on ovulation and related reproductive hormone systems. Furthermore, parallel dose responses for the individual congeners as well as for their equipotent mixture represent a validation of the TEF concept for endocrine disruption by PCDDs.

379 ESTIMATING RISK FROM EXPOSURE-RESPONSE RELATIONSHIPS BASED ON CONTINUOUS VARIABLES.

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Health risk from a chemical hazard is typically expressed as the probability that an individual at a specified exposure level will have an adverse response. This formulation readily accommodates quantal exposure-response data (e.g., tumor incidence), where an individual is either a responder (has tumor) or not a responder (does not have tumor), and where the exposure-response relationship attained from the data provides a direct measure of risk. With continuous data (e.g., blood pressure), however, the exposure-response relationship is not directly translatable to risk. In this case, estimation of risk requires designation of what constitutes a responder by defining a response criterion. A response criterion can relate to clinically or empirically established critical levels (e.g., diastolic blood pressure greater than 90 mm Hg or a 20% decrease in nerve conduction velocity), or a statistical difference from the mean response in an unexposed population (e.g., 3 standard deviations from the control mean). Alternatively, the proportion of responders in an exposed population can be predicted without specifying a critical level (either in terms of the response variable or the proportion of controls considered to be responders) by estimating the probability density function for the control and exposed groups, ascertaining the point(s) of intersection, and taking the difference of the area under the curves. Case studies on carbon disulfide illustrate these techniques for estimating risk from continuous data and comparing the resulting risk estimates. It is concluded that each of these methods can successfully be used to estimate risk from continuous data and that characteristics of the specific data set determine which method provides the most meaningful risk estimate in a given situation.

380 ALUMINUM: AN ASSESSMENT OF ITS POSSIBLE ASSOCIATION WITH THE DEVELOPMENT OF ALZHEIMER'S DISEASE.

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Alzheimer's Disease (AD) affects the central nervous system and is the most common cause of age-related intellectual decline. The current lack of effective treatments for AD is partly due to a poor understanding of its etiology, although this appears to be multi-factorial. Endogenous factors that may be associated with the development of AD include genetic predisposition, abnormalities in neurological proteins, biochemical changes associated with oxidative stress, poor circulation, anaemia, and autoimmune disorders. External factors may also cause or promote AD, including viral infection, metal exposure or head trauma. Considerable effort has been directed towards elucidating a possible role for aluminum in AD. The totality of data do not refute a link between aluminum and AD, but it is more likely that aluminum accumulates in the brain as a result of more fundamental causes and may facilitate the neurological changes which characterize the manifestation of AD. The possible relationship between aluminum and AD has been studied using animal models and post-mortem examination of humans. Recent investigations have included the histological evaluation of aluminum-

induced neurotoxicity, mapping of aluminum accumulation in different regions of the brain and in AD-associated brain lesions, epidemiological studies of aluminum ingestion through drinking water, the effect of chelating metal ions on the progression of AD, and *in vitro* studies of lesion development. Our investigations found that, although aluminum may cause neuropathic changes, the changes were not consistent with the changes found in AD patients. Even the possibility that aluminum may have a secondary role in AD development is debatable, since aluminum is not always found in brain lesions of some AD patients. Strong reservations about inferring causality from correlative data are warranted, and other causes or mechanisms for this disease must be investigated.

381 PROPOSED NEW RISK ASSESSMENT FOR BORON.

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EPA is in the process of developing a new risk assessment for boron. Animal studies conducted using boric acid by oral exposure identified developmental toxicity as the critical effect. Studies in dogs and rodents reporting testicular effects were at higher doses or had limitations. Developmental effects occurred in rats, mice and rabbits and included high prenatal mortality (highest doses), reduced fetal body weight (lowest doses), and malformations and variations of the eyes, central nervous system, cardiovascular system and axial skeleton (intermediate doses). The RfD (Oral Reference Dose) is based on a published benchmark dose of 10.3 mg B/kg-day for reduced fetal body weight in rats. An uncertainty factor (UF) of 30 (3 for interspecies extrapolation and 10 to protect sensitive individuals) was applied to derive an RfD of 3E-1 mg B/kg-day. The UF of 3 for interspecies extrapolation was considered adequate because boric acid is not metabolized (differences in metabolism being a major contributor to interspecies differences in toxicity) and absorption, distribution and excretion are similar in rats and humans. Based on the RfD, an exposure dose of 21 mg B/day for a 70 kg person can be calculated. Most exposure to boron comes from diet and drinking water. The value of 21 mg B/day is greater than typical dietary exposure (0.5-3.1 mg B/day) and the estimated basal requirement (0.25 mg B/day) for this suspected essential element. Data were not adequate to derive an RfC (Inhalation Reference Concentration) for boron. Under EPA's 1986 guidelines for carcinogen risk assessment, boron is classified as Group D. Under the proposed 1996 guidelines, the data are considered to be inadequate. (This abstract does not necessarily reflect U.S. EPA policy.)

382 THE DEVELOPMENT OF THE CADMIUM DIETARY EXPOSURE MODEL (CDEM).

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In most human populations, diet is a major source of cadmium exposure. Since dietary intake of cadmium plays a major role in total exposure, risk associated with cadmium due to other environmental pathways can not be evaluated without quantification of the diet pathway. This presentation describes a newly developed Cadmium Dietary Exposure Model (CDEM) for estimating dietary cadmium exposure in humans. The methodology utilizes national survey data on food cadmium concentrations contained in the U.S. Food and Drug Administration Total Diet Studies (FDA TDS, 1982-1994), and food consumption patterns, derived from the Third National Health and Nutrition Examination Survey (NHANES III, 1988-1994), to estimate demographically stratified dietary intakes in the U.S. population. The CDEM predicts a central tendency estimate (CTE) of daily cadmium intake for the U.S. population of 21 μ g/day, which is consistent with estimates derived from other approaches. The CDEM has been used to construct frequency distributions for cadmium intakes, from which probability density functions can be derived for use in risk assessment. (Supported in part by U.S. EPA Cooperative Agreement CR822761-01. Methodology and conclusions do not necessarily reflect the opinions or policies of U.S. EPA.)

383 DEVELOPMENT OF COPPER TOXICITY VALUES FOR HUMAN HEALTH RISK ASSESSMENT.

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Currently USEPA does not provide reference values for copper toxicity *via* oral or inhalation routes of exposure on their Integrated Risk Information System (IRIS). While toxicity values for occupational exposure have been established, their applicability to residential populations is uncertain. USEPA does recommend an oral reference dose (RfD) of 1.3 mg/L in their Health Effects Assessment Summary Tables, based on the national drinking water standard (MCLG). Therefore, we performed a comprehensive review of the available toxicity literature and derived toxicity values (TVs) for both ingestion and inhalation exposure to copper. For copper ingestion, the most sensitive health endpoint is acute irritation of the gastrointestinal lining. Copper is unusual in this regard; for most compounds, chronic health endpoints are more sensitive than acute health endpoints. Although many of the available studies poorly quantified actual exposure levels, concentrations of 4 mg/L seem to consistently represent the lowest effect level for gastrointestinal effects. Application of default drinking water consumption rate and body weight conversion factors, as well as an uncertainty factor (UF) of 3 yields a TV of 0.04 mg/kg-day, consistent with the daily dose at the MCLG. Application of additional UFs would lower this value below the recommended "safe and adequate" nutritional level, and thus not be appropriate. For inhalation of copper, the most sensitive health endpoint is metal fume fever, which has been observed in workers exposed to 1.4 mg/m^3 copper dust. We converted this Lowest Observed Adverse Effect Level (LOAEL) to a mg/kg-day dose by applying body weight, inhalation rate, and exposure time conversion factors. We then divided this dose by appropriate uncertainty factors (UFs) to derive a TV of 0.0044 mg/kg-day for copper inhalation.

384 USE OF MERCURY VAPOR FLUX MEASUREMENTS FOR REDUCING UNCERTIANTY IN THE ASSESSMENT OF INHALATION EXPOSURES

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In this study, a risk assessment was conducted to evaluate occupational exposures to mercury vapors emitted from soil and groundwater in the mercury cell area at a large chlor-alkali plant. Evaluation of potential exposures from unregulated releases of mercury to ambient air from historical sources was complicated by permitted releases to air from facility operations. Soil and mercury vapor flux chamber samples were collected from several locations across the plant. Both long and short term, as well as day and night flux chamber samples were collected to account for anticipated diurnal variability in mercury fluxes. Mercury vapor fluxes ranged from below 1 to >894 :g/m^2/hr, and soil concentrations ranged from below 0.51 to 6,300 ppm. Mercury fluxes were 2-3 times higher in daylight than at night, but correlated well with mercury concentrations in soil. The measured emission rates were used to calculate breathing zone concentrations and potential risks for different worker groups using the point estimate approach and Monte Carlo Analysis (MCA). Mercury concentrations calculated for the breathing zone ranged between $1x10^{-4}$ and $3.3x10^{-5}$ mg/m^3, and were significantly below the OSHA PEL (0.01 mg/m^3) or ACGIH TLV (0.05 mg/m^3). Mercury concentrations at the breathing zone were associated with hazard indices ranging between 0.01 (50th percentile, MCA) to 3.8 (RME, point estimate). The results of this study suggest, that for chemicals like mercury where volatilization into ambient air is of concern and for which modeling may be unrealistic, flux chamber sampling provides more meaningful information regarding exposures by reducing the uncertainty inherent in the use of models.

385 EFFECTS OF POLYMORPHISM OF GLUTATHIONE TRANSFERASE THETA ON THE RISK ESTIMATE OF DICHLOROMETHANE IN HUMANS.

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The carcinogenic potential of dichloromethane (DCM) was linked to its metabolism by the theta (θ) family of the glutathione-S-tranferase (GSTT) system (Casanova et al., 1995). Humans, based on their ethnic background, are polymorphic with respect to this family of isoenzymes (Hallier et al., 1994). We investigated the effect of population polymorphism on the risk estimate of DCM by the application of Monte-Carlo simulation methods in

combination with physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) models. The PBPK/PD model was used to estimate the DNA-Protein cross-links (DPX) caused by metabolism of DCM (Casanova et al., 1995). Human risks were estimated based on the carcinogenic potency of DCM to mice and PBPK/PD-predicted DPX in humans. The Monte-Carlo simulations were used to provide distributions of risk estimates for a sample of 1000 PBPK/PD runs. Our results show that average and median risk estimates were about 23 to 30% significantly higher when polymorphism was not included at different inhalation doses of DCM. This increase in risk was reduced when it was based on the 95% percentile estimates for all the doses. The specific effect of polymorphism on risk estimates was further investigated by varying the probability at which a Caucasian may have a non-functional form of the enzyme at a constant dose level of 1000 ppm of DCM. As this probability increased the risk decreased correspondingly. Again, this drop in risk estimate was not as significant when the 95% percentile measure was used.

386 USING PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING TO ASSESS NON LINEARITY IN THE DOSE-RESPONSE RELATIONSHIP FOR METHYLENE CHLORIDE CARCINOGENESIS.

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Historically, chemical cancer risk assessment has been based on applying the linearized multistage (LMS) model to the results of a single, animal study at relatively high doses. In the process, a great deal of useful dose-response information generated in other studies is not used. To illustrate the utility of an alternative approach, an analysis was conducted for methylene chloride (dichloromethane, DCM). The carcinogenicity of methylene chloride has been well studied in laboratory animals. A total of twelve cancer bioassays, across three species (rats, mice, hamsters) and two routes of exposure (inhalation, oral) were used to support the analysis. The combined data set contains more than 4,800 animals and 50 treatment groups. A physiologically based pharmacokinetic (PBPK) model was used to determine several internal dose estimates for methylene chloride in the lung (peak tissue concentration, average concentration, area-under-the-curve, amount metabolized). Each dose measure was then assessed for its ability to describe a consistent dose-response relationship for DCM-induced carcinogenesis in the lung. Consistent with our current understanding of the mechanism of action for DCM, the dose-response relationship was best characterized by total amount metabolized. Several statistical models were fit to the dose-response data and evaluated with respect to goodness of fit. Graphical analysis of the results indicates that the dose-response relationship for DCM is non-linear in the low-dose region. Furthermore, several alternative dose-response models provided better fits than the LMS model to the combined dose-response data. This type of approach provides a more complete picture than do current practices of the dose-response relationship for DCM, and should be included when assessing the potential health risks associated with human exposures.

387 TRICHLOROETHYLENE HEALTH RISK ASSESSMENT: USING MECHANISTIC INFORMATION TO IMPROVE DOSE-RESPONSE ASSESSMENT.

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New approaches are being used in an updated health risk assessment for trichloroethylene (TCE), a major environmental contaminant of air, water, and waste sites. Past TCE assessments emphasized linear extrapolation from bioassays in which TCE induced liver tumors and lung tumors in mice and kidney tumors in rats. More recently, however, pharmacokinetic and mechanistic studies on TCE are able to suggest better ways to use the high-dose animal results to make inferences about human environmental exposure. In a new TCE assessment, pharmacokinetic models describe how TCE's cancer response is mediated though several metabolites. Mechanistic information may lead to identifying the key events involved in tumor formation and indicate whether linear or nonlinear extrapolation to lower doses is more appropriate. The new TCE assessment is an example of how EPA's new cancer guidelines encourage use of mechanistic information in risk assessment. These new guidelines, together with assessments like TCE, should stimulate more mechanistic research and promote better collaborations between toxicologists and risk assessors. (The information contained in this abstract does not represent EPA policy.)

388 DEVELOPMENT OF INHALATION AND ORAL BENCHMARK DOSES FOR TRICHLOROETHYLENE BASED ON CARCINOGENICITY IN MICE.

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Trichloroethylene (TCE) is an important industrial chemical and environmental contaminant commonly found at hazardous waste sites. Assessing cancer risks associated with exposure to TCE has been hampered by the absence of a cancer slope factor endorsed by the USEPA. Emerging carcinogen risk assessment methodology makes use of the benchmark dose approach. To date, the only benchmark dose reported for TCE based on carcinogenicity (Haag-Gronlund et al., Reg. Toxicol. Pharmacol., 21:261-269, 1995) was developed using the extra risk definition of increase in tumor incidence rather than the more straightforward added risk definition. In addition the benchmark dose developed by Haag-Gronlund et al. is limited to the inhalation route. In the present study, we used several data sets from chronic mouse bioassays to develop oral and inhalation benchmark doses for TCE based on liver and lung tumor incidences, respectively. Our benchmark doses were derived using the added risk definition. Our analysis resulted in oral ED_{10} values for TCE that range from 124 to 717 mg/kg/day and oral LED10 values that range from 82 to 403 mg/kg/day. The inhalation ED_{10} values for TCE ranged from 362 to 1336 mg/kg/day and the inhalation LED10 values range from 127 to 854 mg/kg/day. These benchmark doses can be used as points of departure for linear extrapolation as a cancer slope factor for TCE. Alternatively, the same benchmark doses could be used in a margin of exposure analysis to assess the risk associated with particular exposure scenarios.

389 AN AMBIENT AIR GUIDELINE FOR TETRACHLOROETHENE BASED ON NON-ONCOGENIC EFFECTS IN HUMANS.

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Air levels of tetrachloroethene in residences above dry-cleaning shops are frequently elevated compared to residences not near such shops. We reviewed the scientific literature on tetrachloroethene and derived criteria for evaluating the potential for non-oncogenic health risks from low-level chronic exposures. Animal and human data show the primary targets are the central nervous system (CNS), kidney, liver, and possibly the reproductive system. Results from cross-sectional occupational studies of central nervous system (CNS), kidney, and liver effects in dry-cleaning workers were deemed the most appropriate for use in criteria derivations. A no-observed-effect level for impaired vision and lowest-observed-effect levels for evidence of CNS dysfunction (motor and cognitive effects) and kidney and liver dysfunction were used. These workplace exposures were adjusted to continuous exposures based on inhalation rates. Uncertainty factors were applied to derive criteria for the general adult population. However, children may not be adequately protected by criteria derived for adults because they have higher inhalation rates per unit body weight than adults and their developing organs/systems may be more sensitive to the same dose than those of adults. Thus, scaling and uncertainty factors were applied to adult continuous exposure levels to derive criteria for children of 0.12 mg/m^3 (CNS effects), 0.14 mg/m^3 (kidney effects), and 0.11 mg/m^3 (liver effects). A criterion of 0.1 mg/m^3 was recommended. This criterion is estimated to provide the general population, including sensitive subpopulations, a sufficient margin-of-exposure over the air levels of tetrachloroethene associated with non-oncogenic effects in humans and animals.

390 REASSESSMENT OF THE POTENTIAL CANCER RISK OF PERCHLOROETHYLENE.

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Sponsor: B R Stern.

A physiologically based pharmacokinetics model (PBPK) was used to determine trichloroacetic acid (TCA) levels in mouse liver to generate dose-response relationships for hepatocellular carcinomas from two inhalation bioassays at low perchloroethylene inhalation exposure levels. The model was fit to existing mouse data that measured TCA and perchloroethylene blood levels. The dose-response relationships were analyzed based on the biological evidence that perchloroethylene is metabolized to TCA in the liver which in turn is responsible for stimulation of peroxisome proliferation and an increase in S-phase cells. Peroxisome proliferation leads to increases in organelle fatty acid β-oxidation resulting in the formation of hydrogen peroxide and free radicals that may reach nuclear DNA causing mutations and chromosomal aberrations. Increase cell division may enhance the number of target cells providing greater opportunity of occurrence of spontaneous and chemically induced mutations leading to cell transformations. Furthermore, the growth of transformed cells may also be stimulated by TCA, thus promoting the opportunity for further spontaneous or chemically induced transformation leading to hepatocellular carcinoma. Several biological mechanisms were examined involving interactions of TCA with a cytosolic receptor as well as the effects of TCA on enhancing levels of a natural receptor ligand. These various biological relationships were used to estimate incidences of hepatocellular carcinoma below the observable exposure range and showed different risk values when compared to estimates based on none-biological extrapolations.

391 DEVELOPMENT OF AN RFD FOR DICAPRYLPHTHALATE USING DATA FROM STRUCTURE-ACTIVITY RELATIONSHIPS AND IN VITRO STUDIES.

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Dicaprylphthalate (DCP) is a phthalate compound that does not have a dose-response value derived by the U.S. Environmental Protection Agency (U.S. EPA), and no appropriate animal studies on which to base a dose-response value. Therefore, a structurally-similar chemical was selected to represent DCP toxicity. Amongst the various phthalate compounds, dicaprylphthalate is the closest in chemical structure to di(2-ethylhexyl)phthalate (DEHP) and di(n)octylphthalate (DNOP). DEHP is considered potentially carcinogenic by U.S. EPA, whereas DNOP is evaluated as a noncarcinogen. Therefore, there are important risk implications in selecting DEHP or DNOP as the surrogate chemical. DEHP, DCP and DNOP were entered in the Quantitative Structure Activity Relationship (QSAR) program, Case Tox II, to evaluate the carcinogenicity endpoint. DEHP was found to be positive for carcinogenicity in all of the databases, whereas DCP and DNOP were negative. Studies evaluating the mechanism of phthalate ester rodent carcinogenicity indicate that DCP behaves similarly to DNOP, rather than to DEHP. DEHP is thought to cause cancer in rodents through the mechanism of peroxisome proliferation. Lake et al. (1987) showed that the 2-ethylhexyl phthalate monomer was more potent than the 1-ethylhexyl phthalate and n-octyl monomers in inducing peroxisome proliferation. This study is directly relevant to DCP, since DCP has a methyl group at the 1-carbon position, and suggests that DCP behaves similarly to DNOP. In addition, there are serious questions as to the relevance of DEHP's rodent carcinogenicity data to humans. The combined evidence indicated that the noncancer RfD for DNOP (0.02 mg/kg-day) should be used to conduct a risk assessment on DCP.

392 FORMALDEHYDE RISK ASSESSMENT BY BENCHMARK DOSE ANALYSIS USING DNA-PROTEIN CROSS-LINKS AS AN INTERNAL DOSE METRIC.

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Formaldehyde induced tumors in specific regions of the rat nose in two cancer bioassays. Analysis of inspiratory air flow and gas transport indicated that these processes play a key role in tumor site specificity. Formaldehyde also induced DNA-protein cross-links (DPX) in a dose- and a site-dependent manner in rats and monkeys. A distributed model of formaldehyde transport and formation of DPX in the rat nasal lining was developed, using computational fluid dynamics (CFD) to model airflow and regional gas uptake, and fit to DPX data. This model was used to estimate time-averaged DPX levels for each exposure concentration in the rat cancer bioassays. Benchmark dose (BMD) analysis is a statistical curve-fitting approach that can be used to determine from dose-response data the maximum likelihood estimate (MLE), or central estimate, and the lower 95% confidence limit on dose associated with a specific level of risk. This BMD is the dose or concentration predicted to result in a specified increase in risk. BMDs were calculated for the cancer response in rats using DPX values as doses. A monkey CFD model and monkey DPX data were used to estimate how DPX model metabolic parameters scale across species and this scaling was used to obtain values for a human DPX model. The human DPX model was used to estimate formaldehyde flux values that yield DPX in humans equal to the rat-based BMDs, and a human CFD model was used to estimate the corresponding exposure levels. These exposure concentrations were identified with human BMDs based on the assumption that DPX is a good metric of tissue formaldehyde levels, and that cancer risk is identical for rats and humans when tissue formaldehyde levels are the same. These results indicate that humans are somewhat less sensitive than rats to formaldehyde-induced cancer.

393 CLONAL GROWTH MODELS AND CANCER RISK ASSESSMENT: SIGNIFICANCE OF THE TUMOR GROWTH RATE FOR INTERSPECIES SCALE-UP.

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Multistage clonal growth models are being used for cancer risk assessment because they are biologically more realistic than the linearized multistage model or model-free approaches such as linear extrapolation through the dose-response origin. The time interval (or time lag) required for clonal expansion of a single malignant cell into a clinically observable tumor is one aspect of the carcinogenic process that can be described explicitly using clonal growth models. This capability allows the role of the time lag in interspecies scale-up to be evaluated. We have developed a new cancer risk assessment for inhaled formaldehyde in which a clonal growth model of nasal squamous cell carcinoma incidence data in F344 rats is scaled for the prediction of cancer risk in humans. Maximum likelihood estimation of the parameters of the rat model indicated a lag time for clinical appearance of squamous cell carcinoma of about 6 – 9 months. This represents 20 - 30% of the typical 2.5-yr lifetime of the F344 rat. A similar clonal growth model was fit to data on age-specific incidence of human respiratory tract cancer by maximizing the Poisson likelihood. A time lag of 10 - 12 yr was estimated. This lag was subsequently used in cancer risk assessment for formaldehyde-exposed people assuming that humans live for 80 yr. The human lag is 12 to 15% of human lifetime. Thus, these clonal growth models predict that, for the tumor types studied, the fraction of life span for which humans are at risk of developing clinically detectable cancer is greater than that of rats. These model predictions need to be validated by measurement of actual tumor growth rates in rodents and humans. Assuming that the predictions are found to be consistent with such data, these results indicate that rat carcinogenesis bioassays may underestimate human cancer risk to the extent that time lags in rats are significantly greater proportions of rat life span than are human lags of human life span. However, other factors, particularly the typical differences between rodent bioassay exposure levels and human environmental exposure levels, are quantitatively more important determinants of interspecies differences in risk.

394 EPOXY RESINS AND POLYAMINE DERMAL AND SYSTEMIC TOXICITY

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Dermal exposure to epoxy compounds may result in severe skin irritation, contact dermatitis and burns. This is a report of a case which also produced axillary lymph adenopathy and chronic neuralgia. An adult male was working with Metalink 5000 epoxy compound. Unfortunately the gloves he used were porous, and the effects were not immediately noticeable. Shortly thereafter his hands turned yellow. Two weeks later he developed an erythematous rash with small papules. Avoidance of the chemical and Diprolene AF cream were prescribed. The pain increased and spread to his arms. At 7 weeks he had burn-like areas over the dorsum and palms of his hands. He was treated with Medrol Dosepack and Cortisone cream and the local inflammatory reaction resolved over the next 2 weeks. Two years later he was still having pain in the forearms and proximal arm into the axilla. There was some loss of strength in his arms and grip. Prednisone temporarily

improved but did not resolve the condition. Given the history of exposure and the pain which had never existed before, it is reasonable to believe a relationship exists between the injury and his symptoms. Both direct chemical damage and immune system hypersensitivity are likely. The diagnosis of hypersensitivity reaction is supported by 1) the propensity of these chemicals to produce this effect, 2) the site of pain which corresponds with expected lymph flow, 3) delayed onset and prolonged duration of the effect, and 4) temporary response to imunosuppression with corticosteroids.

395 THE EFFECT OF AGING IN SOIL ON THE DERMAL BIOAVAILABILITY OF MERCURY.

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Soil cleanup standards based on exhaustive extraction of chemicals from contaminated soil may overestimate the risk from exposure and the extent of needed soil remediation. Instead, this study assessed the effect of chemical "aging" on dermal bioavailability from soil using mercury as an example. Experiments were performed with 203-mercury chloride on dermatomed male pig skin by flow-through diffusion cell methodology. After aging for 3 months in either a sandy or a clay soil, the bioavailability of mercury was decreased by 91-96% compared to chemical without soil. In contrast, a 40% reduction in bioavailability was observed for the compound added immediately to soil. Both soils bind mercury more strongly with time as supported by larger quantities of radioactivity in soil after aging than in soil for 16 h. Decreased mercury bioavail-ability with aging indicates lower health risk and reduced soil cleanup. (Supported in part through funding from the Hazardous Substance Management Research Center and the New Jersey Commission on Science and Technology.)

396 AN ALTERNATE VIEW OF DERMAL ABSORPTION OF HEXACHLOROBENZENE FROM SOIL.

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Estimates of the amount of contaminant absorbed systemically as the result of dermal contact with soil remain an area of significant uncertainty in risk assessment. It has been common to use default values such as 25% for volatile compounds, 10% for semi-volatile compounds, and 1% for metals to account for dermal uptake of these compounds from soil even though there is little or no experimental evidence to support these values for most compounds. Using hexachlorobenzene (HCB) as a model semi-volatile compound, a better estimate of the amount absorbed through the skin from soil was developed. Using published literature on the dermal absorption of HCB in an *in vivo* rat study (Koizumi, 1991) and information on the relative permeability of rat and human skin, an estimate of the amount of free HCB likely to be absorbed by humans was determined. In addition, extraction studies of soil contaminated with HCB were used to confirm the matrix effects as well as account for the effects of aging on the availability of this compound from soil. These results indicate that far less than 0.1% of HCB in soil is likely to be absorbed from soil under normal conditions. These findings are similar to the results of a dermal absorption fugacity model developed for soil-bound compounds by McKone and Howd (1992), and applied to HCB. Based on these results, it is likely that dermal absorption of compounds with similar physical and chemical characteristics to HCB are significantly overestimated by the default assumptions currently in use. Similar evaluation of related and unrelated compounds is needed in order to extend and generalize these findings and improve risk estimates from the dermal pathway.

397 EFFECT OF TEMPORAL ASSUMPTIONS ON RISK ASSESSMENTS FOR DERMAL TOXICITY.

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The skin can be an important route of entry for chemicals that may subsequently cause toxicity. After dermal exposures, irritation of the skin may result or if the chemical is absorbed through the skin, systemic toxicity may result. Dermal exposures can occur from contact with contaminated surfaces, spills on the skin or during showering and bathing in contaminated water. Most of these exposures are short duration and the rate of absorption does not approach steady-state. The time before chemical penetrates through the skin and becomes available for systemic absorption can be minutes to hours and is known as the "lag time". This lag time may impact duration extrapolations used for risk assessments, which assume that flux is constant over time. Using static diffusion cells and rodent skin, we measured time profiles for diffusion of dibromomethane (pure chemical and aqueous solution) into and through rat skin. Chemical concentration in the skin slowly increases and then becomes constant, without any lag time. After a fifteen-minute lag time, the concentration of chemical that comes through the skin slowly increases and then becomes constant. When he total amount of dibromomethane (in both the skin and the receptor solution) is plotted over time, the plot is linear without a lag time. These results suggest that duration extrapolations for chemical in the skin and diffusing through the skin can be inaccurate depending on the length of the lag time compared to the exposure time. (Supported by the Air Force Office of Scientific Research.)

398 POPULATION ATTRIBUTABLE RISKS FOR BLADDER CANCER ASSOCIATED WITH EXPOSURE TO CHLORINATED SURFACE WATER.

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In the US, approximately 240 million people are served by public water systems. Most of these systems rely on chlorination for disinfection. Benefits of chlorination are decreased morbidity and mortality from waterborne diseases. Some DBPs produce tumors in animals, and studies have been conducted to investigate potential carcinogenicity of exposure to disinfected water. A population attributable risk (PAR) analysis was completed to support the benefits analysis for the Stage 1 Disinfection Byproducts Rule. To date, there are fifteen bladder cancer epidemiological studies on chlorinated water. Of these, five studies were selected for analysis based on specific selection criteria. Study-specific relative risks (RR ~ 1.01-1.5) and case fractions were used to generate PARs that ranged from 2 to 17 percent. This is equivalent to 1,100 - 9,300 new bladder cancer cases per year potentially attributable to disinfection byproducts in chlorinated surface water assuming a causal relationship. This range is based on 54,500 incident bladder cancer cases expected in the US for 1998.

399 CANCER CHARACTERIZATION OF THE DISINFECTION BYPRODUCT BROMATE.

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Bromate ion may be formed in drinking water following ozonation of water containing bromide. The potential of this disinfection byproduct to cause cancer in humans was evaluated following U.S. EPA's 1996 Proposed Guidelines for Carcinogen Risk Assessment. No human studies are available which assess the potential carcinogenicity of bromate. However, three key studies demonstrate that exposure to bromate in drinking water results in kidney tumors, thyroid tumors, and mesotheliomas in rats. Evidence in rats demonstrating that bromate has the potential to be a human carcinogen includes tumors at multiple sites, a clear dose-response relationship both in tumor incidence and in severity or progression of tumors in rats, and the consistent observation of an uncommon tumor mesotheliomas. Bromate has been found to be positive in a variety of *in vitro* genotoxicity assays, although there is no evidence that bromate is directly DNA reactive. Oxidative stress may play a role in the formation of kidney tumors; treatment with bromate causes an increase of 8-hydroxydeoxyguanosine in the kidney only. Recent studies suggest that in the intact kidney, there is not a direct mechanism of DNA damage; rather, at toxic doses, bromate induces DNA damage through lipid peroxidation. However, the overall evidence is insufficient to establish lipid peroxidation and free radical production as the key events responsible for the induction of kidney tumors. In addition, no data are currently available to suggest any single mechanism, including oxidative stress, is responsible for the production of thyroid and peritoneal tumors by bromate. Therefore, bromate was evaluated as a likely human carcinogen and the default linear low-dose extrapolation was done. Using a time- to-tumor analysis and a Monte Carlo analysis to sum the cancer potency estimates for kidney renal tubule tumors, mesotheliomas, and thyroid follicular cell tumors, an upper bound cancer potency estimate for bromate ion is 0.70 per mg/kg-day. (This abstract does not necessarily reflect EPA policy).

400 CATEGORICAL REGRESSION ANALYSIS OF BROMODICHLOROMETHANE (BDCM) LIVER TOXICITY AND PATHOLOGY DATA.

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A categorical regression (CR) procedure is being investigated as an way of comparing new toxicity data with existing benchmarks, such as a Reference Dose (RfD), and as a method for predicting pathology scores from other hepatotoxic endpoints. CR was used to model data on liver toxicity and histopathology scores from female CD-1 mice exposed for 14 days by gavage to BDCM (0, 0.152, 0.305, 0.76, 1.52, 3.05 mmol/kg/day), a common by-product of drinking water disinfection processes. The toxic effects, which included both continuous endpoints (liver enzyme elevations, liver weight changes) and categorical data (centrilobular necrosis designations) were classified into ordered categories of total toxic severity (e.g., none, mild, adverse, severe) at the individual animal level. CR was then used to regress these severity categories on dose to estimate the probability of observing an adverse or severe effect (P=A,S). At the animal chronic LOAEL of 17.9 mg/kg/day based on renal cytomegaly in male mice (an effect considered minimal in the absence of renal function impairment), which was used to calculate the RfD, the (P=A,S) liver effect was 0.01 (95% CI of 6E-4, 0.14), consistent with the fact that the kidney effect should be a more sensitive endpoint. Because some additional enzyme and organ weight data were available from the same laboratory, but without corresponding pathology results, another CR model was used to predict liver necrosis designations from the enzyme and organ weight data for the additional data, which were then used to rerun the first CR model. The predictive CR model was significant, indicating that the severity category for centrilobular necrosis can be estimated from the percent liver weight and the log of ALT. (Abstract may not reflect U.S. EPA policy.)

401 DAILY WATER INTAKE IN THE U.S.

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Water intake is used in the drinking water risk assessment. In general, a default water intake for an adult has been assumed to be 2 liters. This value agrees with Ershow and Cantor's report (1989) which is based on a US Department of Agriculture survey from 1976 to 1977. To obtain current estimates of water intake, we analyzed data from USDA's 1994 - 1996 Continuing Survey of Food Intake by Individuals. This project estimates municipal tap, bottled water, and other water intake with percentile distribution by age, gender, race, socioeconomic status and geographic region. Water refers to both drinking water and water added in final preparation of beverages and foods at home or in a restaurant. Intrinsic water (water contained in foods and beverages at times of market purchase) is not included in our estimate. Preliminary results show that there has been a substantial increase in the intake of bottled water, and that the estimated 95 percentile distribution of tap water intake for people of all ages supports the assumed value of 2 liters per day. Details of our analysis will be presented. The opinions expressed are those of the authors and do not necessarily reflect U.S. EPA policy.

402 RISK ASSESSMENT FOR CHLORITE AND CHLORINE DIOXIDE BASED ON NEW NONCANCER DATA.

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Chlorine dioxide (ClO_2) and chlorite (ClO_2^-) are disinfection byproducts of chlorination treatment of drinking water. In support of Stage 1 Disinfectant/Disinfection Byproducts Rule (D/DBP), EPA has reassessed the noncancer health risk for these chemicals considering new data submitted since the 1994 proposed D/DBP rule. The 1994 proposal included a maximum residual disinfectant level goal (MRDLG) of 0.3 mg/L and a maximum residual disinfectant level (MRDL) of 0.8 mg/L for ClO_2 and a maximum contaminant level goal (MCLG) of 0.08 mg/L and a maximum contaminant level (MCL) of 1.0 mg/L for ClO_2^-. It has consistently been shown that neurotoxic/ behavioral effects are the critical effect for ClO_2 and ClO_2^-. A recent 2-generation rat study with ClO_2^- (CMA, 1997), which filled a database gap, showed alterations in auditory startle response and brain weight, supporting the studies of Orme et al. (1985) and Mobley et al. (1990) showing alterations in exploratory and locomotor activity. The weight of the evidence from these and supporting studies indicate 3 mg/kg/d ClO_2^- is the NOAEL and the LOAEL occurs at a dose of 6 mg/kg/d. The RfD of 0.03 mg/kg/d is derived from the NOAEL by application of a 100-fold uncertainty factor (UF) for interspecies extrapolation and intra-human variability. This RfD is applied to both ClO_2 and ClO_2^- because ClO_2 in drinking water rapidly degrades to ClO_2^- , and both chemicals have similar pharmacokinetics, targets of toxicity, and potency. (This abstract does not necessarily reflect EPA policy.)

403 COMPARATIVE RISKS OF DRINKING WATER TREATMENT SYSTEMS.

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Disinfection of drinking water (DW) reduces the risks associated with microbial infections, but produces several compounds whose toxicity warrants concern. To develop a method to compare these disparate risks and include costs associated with different DW intervention strategies, a cost-effectiveness analysis (CEA) approach to a public health intervention was employed. CEA is a comparative-risk approach capable of concurrently evaluating the benefits of intervention (i.e., disinfection of public drinking water), the risks of unintended side effects of the intervention [i.e., potential health risks posed by disinfectants and disinfectant byproducts (D/DBP)], and the relative costs of implementing one or more intervention strategies. CEA was applied to compare the potential health impacts from exposure to 13 D/DBP and to a representative microorganism, *Cryptosporidium*, resulting from conventional chlorination with and without a pre-ozonation step. Response-addition was assumed to estimate cancer and non-cancer human health risks from exposure to mixtures of D/DBP. Risks of cryptosporidiosis were estimated through a simple linear infectivity/sequella model. Results indicate that the implementation of a pre-ozonation step was not as cost effective as conventional chlorination; however, an uncertainty analysis showed that the results depended heavily on several key assumptions. A second iteration of the comparison will be performed to reduce uncertainties by including various source water quality indicators, a larger number of D/DBP, an assessment of D/DBP genotoxicity data, and further work on the application of various additivity assumptions as the basis for estimating mixtures risk. (Abstract may not reflect U.S. EPA policy).

404 RISK ASSESSMENT OF FURFURAL FOR THE DEVELOPMENT OF A MAXIMUM ALLOWABLE LEVEL (MAL).

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A human health risk assessment was conducted by NSF International to determine a safe concentration limit for furfural in drinking water according to ANSI/NSF Standard 60/61, Appendix A. Furfural has been detected in laboratory extractions as a leachate of coatings (Standard 61, Section 5) and adsorption process media (Standard 61, Section 7). NSF International reviewed scientific literature, including recommendations from international bodies, genetic data, subchronic toxicity testing, reproductive and developmental data, chronic and long term cancer bioassay studies, metabolism data, structure activity, and other supplemental studies. The newly proposed EPA guidelines on risk assessment methods were analyzed and incorporated as applicable. This review led to the development of a Maximum Allowable Level (MAL) in drinking water for furfural of 75 ppb. The MAL value was derived based on the LOAEL of 30 mg/kg/day from a NTP chronic rat oral gavage two-year study. A safety factor was considered to account for intraspecies extrapolation, interspecies variation, and the use of a LOAEL instead of a NOAEL. In accordance with the Standard, lack of reproductive toxicity and teratology studies were limiting factors in this analysis. Furfural is not likely to be carcinogenic to humans at or below the MAL level. MAL development was subject to peer review and discussion joint committees. Study summaries and the MAL value derivation are discussed.

405 USE OF HUMAN DATA IN THE HEALTH ASSESSMENT OF DIMETHYLAMINE (DMA).

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DMA has been identified as a contaminant in certain polymer products used

in the production of drinking water. An initial review of the existing DMA toxicology database indicated that additional toxicology studies might be required to assess the potential health effects of DMA when present as a contaminant in drinking water at part per billion (ppb) levels. However, prior to intitiating costly, toxicology studies in whole animals by the oral route, an exhaustive examination of the literature on human data was performed. It was determined that DMA is naturally present in the human diet at parts per million (ppm) levels that exceed the estimates of DMA when present as a contaminant in drinking water. DMA is endogenously produced during normal metabolic processes in the human body, and in addition, is manufactured in vivo by the gut microflora. Levels of DMA naturally present in human blood and urine exceed the estimated DMA contaminant levels in drinking water. Ingested or inhaled DMA does not undergo metabolic transformation in humans but rather is excreted unchanged in the urine. These data can be used with existing toxicology study results to complete the health assessment of DMA without the need for additional, whole animal testing. (*Now with Underwriters Laboratories Inc., Northbrook, IL, USA)

406 SPECIFIC ANTIAPOPTOTIC ACTIVITY OF THE NEURORESCUING COMPOUND TCH346.

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TCH346 (dibenzo[b,f]oxepin-10-ylmethyl-prop-2ynyl-amine) is a novel compound that is believed to possess neurorescuing properties potentially beneficial in the treatment of chronic degenerative diseases such as amyotrophic lateral sclerosis and Parkinson's disease. Although TCH346 has been proven to be non-genotoxic, its antiapoptotic activity rose the concerns that the compound might have some tumor promoting activity in various tissues outside the brain. The purpose of the present study was to evaluate TCH346 concerning its anti-apoptotic activity in different cellular model systems from different target organs/tissues. Annexin V assay, TUNEL assay, chromatin condensation and fragmentation were evaluated as parameters of apoptosis. The potential antiapoptotic activity was determined in presence of a positive reference compound. TCH346 concentrations were in the range of 10^{-11} to 10^{-6} M. The following cellular models and apoptosis inducers were used: Rat thymocyte suspensions (dexamethasone), human HaCaT keratinocytes (anti-Fas + IFNλ , or UV), rat cardiac myocytes primary cultures (atrial natriuretic peptide, ANP), rat hepatocyte primary culture (TGFβ), renal LLCPK1 cell line (rotenone), human astrocytes (rotenone) and human neuronal cell line PAJU (rotenone).TCH346 neither had an inhibitory effect on apoptosis in rapidly proliferating cells from thymus and skin, nor did it inhibit apoptosis in cells of the frequently affected cancer targets liver and kidney. In a non-neuronal astrocyte cell culture, TCH346 did not have any effect, however, in neuronal cells it effectively inhibited apoptosis. The inhibition of the ANP-stimulated apoptosis found in cardiomyocytes does not suggest a risk of heart tumor formation, since inhibition was not below the basic apoptotic rate and these cells are known not to be a target of tumorigenic responses. The results suggest that TCH346 possesses a great brain specific antiapoptotic activity and that the risk of a tumor promoting activity by inhibition of apoptosis in target organs different from the brain is very low.

407 INVOLVEMENT OF CASPASE-3-LIKE ACTIVITY IN CYANIDE-INDUCED NEURONAL APOPTOSIS.

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The caspase family has been proposed to play a role in neuronal apoptotic death. We have shown that neurons from the brain that are vulnerable to cyanide undergo apoptosis. To identify the mechanism of neuronal apoptosis after cyanide exposure, studies were conducted to determine the involvement of the caspase-3 proteases in cerebellar granule cells (CGS). Exposure to 100-300 μ M KCN, that produces apoptosis in CGS, were used to measure caspase-3-like activity by spectroflurometry. KCN-induced a concentration dependent increase of caspase-3-like activity with a maximum at 300 μ M KCN exposure. Maximum activity of the caspase-3 proteases was seen at the 24 hrs time period. The level of caspase-3 activity with various concentration of KCN treatment reciprocally paralleled neuronal apoptosis. Involvement of caspase-3 activity was confirmed by the observation that the inhibitor z-VAD abrogated KCN-induced increase of caspase-3 activity. Moreover, treatment of neurons with MK-801 (NMDA antagonist) and an inhibitor of redox transcription factor $NF\text{-}_{k}B$ (SN50 NF-kB) also blocked caspase-3 activity induced by cyanide. To determine the link between caspase-3 activity and apoptosis, cells were pretreated with agents that block the caspase-3-like proteases and MK-801 and SN50 $NF\text{-}_{k}B$. Inhibition of caspase 3-like activity (z-VAD) and blockade of NMDA receptor activation and $NF\text{-}_{k}B$ translocation all reduced apoptosis showing that caspase 3-like activity is increased in cyanide-induced apoptosis. Gel electrophoresis showed that DNA fragmentation induced by KCN was blocked by the caspase-3-like protease inhibitor, MK-801 and SN50 $NF\text{-}_{k}B$. These data suggest that cyanide induces apoptosis by NMDA receptor mediated process leading to activation of caspase cascades. (Supported by NIH grant ESO4140.)

408 PYRIDOSTIGMINE-INDUCED ACUTE AND DELAYED NEURONAL APOPTOSIS.

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Pyridostigmine Bromide (PB) is a reversible inhibitor of acetylcholinesterase (AChE) which may be a contributing factor in the Gulf War Syndrome. To determine whether PB produces neurotoxicity, TUNEL staining for apoptotic cell death was conducted in rat brain sections after treatment with PB twice daily for 4 days over the dose range of 0.25-1.85 mg/kg. PB induced a dose-related neuronal apoptosis in select brain areas. At 1.85 mg/kg, 3 of 4 rats showed marked TUNEL staining in the cortex, striatum and hippocampus. Electron microscopy was used to confirm PB-induced apoptosis. Ultrastructural changes characteristic of apoptosis were observed (chromatin margination to the nuclear membrane, chromatin clumping and cytoplasmic condensation) in the brain sections. To detect any possible delayed neurotoxicity, rats were sacrificed at 5, 10, 20 or 30 days after cessation of PB treatment (1.85 mg/kg twice daily for 4 days). At all time periods apoptotic cell death was observed in the cortex; the apoptotic process continued up to 30 days after expsoure to PB. In mechanistic studies, pretreatment with atropine (25 mg/kg, ip) blocked the apoptotic death in all brain areas. Pretreatment with PBN (antioxidant) or l-NAME (NOS inhibitor) did not alter the neurotoxic response to PB. Serum AChE was also measured as a marker of PB exposure. All doses significantly inhibited serum AchE activity, however at 5, 10, 20 or 30 days after the last PB dose, serum AChE activity was not different from control. These results suggest that apoptosis induced by PB may be mediated by excessive activity of cholinergic receptors and the apoptotic process continues up to 30 days after PB exposure. (Supported by U.S. Army grant DAM 17-97-71354.)

409 MODULATION OF NEURONAL APOPTOSIS BY AN INTERACTION BETWEEN TNFα AND NGF SIGNAL CASCADES.

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Background: High levels of inflammatory cytokines such as tumor necrosis factor-alpha (TNFα) are often associated with aging and age-related diseases such as Alzheimer's disease and Parkinson's disease. Target-derived neurotrophins (NT) such as nerve growth factor (NGF) are essential for neuronal survival. Thus, impairments in NT signal transduction may be responsible for neuronal degeneration observed during aging or chronic inflammation in the central nervous system (CNS). NGF binds to tyrosine kinase receptor A (TrkA) and to a common NT receptor $p75^{NTR}$, a member of the TNFα receptor (TNFR) superfamily. While TrkA mediates most of the NGF actions, the role of $p75^{NTR}$ is less clear; one possible role is that NGF modulates neuronal apoptosis via $p75^{NTR}$. **Objective:** Determine if TNFα-induced neurotoxicity affects NGF-mediated cell survival by interfering with the $p75^{NTR}$ signal cascade and whether high concentrations of NGF can rescue cells from TNFα-induced cell death by interfering with the TNFα signal cascade, in a Trk-independent fashion. **Experimental Design:** Rat pheochromocytoma (PC12) cells, a neuronal-like model, were treated with TNFα, NGF or both in the presence or absence of the Trk inhibitor, K252a. Cell death and apoptosis were assessed as well as levels of key signal transduction elements [nuclear factor kappa B (NFκB), protein kinase C zeta (PKCζ), caspases, n-terminal c-jun terminal kinase 1 (JNK-1) and phospholipase $A2(PLA_2)$] associated with TNFα or NGF receptors. **Results:** We found that TNFα

induces apoptosis in PC12 cells but high concentrations of NGF rescued the cells from TNFα-induced apoptosis. The Trk inhibitor K252a did not block this NGF-mediated rescue which indicates independence from TrkA signaling. TNFα-induced apoptosis was linked to inhibition of the active forms of PKCζ and NFκB, signaling elements associated with cell survival. Rescue by high concentrations of NGF abolished TNFα-induced increases of JNK-1, caspases and PLA_2, key signaling elements in the induction of neuronal cell death. **Conclusions:** We conclude that TNFα and NGF may modulate neuronal apoptosis by affecting key signal elements in each other's signal transduction pathways. These results are important for understanding the mechanisms leading to neuronal death during aging and age-related diseases. (Supported by the National Institute of Aging Grant AG13945 to GT.)

410 APOPTOSIS INDUCED BY ETHANOL AND SERUM DEPRIVATION ARE MECHANISTICALLY DISTINCT.

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Ethanol exposure results in a loss of neurons that appears to be, at least in part, due to an ethanol-mediated increase in apoptosis. PC 12 cells were used as neuronal models to compare the pro-apoptotic effects of ethanol and trophic factor deprivation. Apoptosis induced by serum deprivation occurred within 4 hr. After 24 hr the activity of Caspase-3, a principle effector of apoptosis, was increased 2.6-fold and immunoblot analysis revealed a 2.1-fold increase in the amount of the active (17 kDa) subunit of Caspase-3. In contrast, ethanol treated cells did not undergo apoptosis until 21 hr after ethanol treatment. Caspase-3 activity was increased 1.6-fold by ethanol, however, no ethanol-mediated processing of Caspase-3 to its active form was detected. The Bcl-2 family of proteins act as a rheostat for apoptosis. Immunoblot analysis of five Bcl-2 homologues revealed that serum deprivation increased the pro-apoptotic homologues Bax and Bcl-X_S and decreased the anti-apoptotic homologue Bcl-2. No ethanol-mediated change in the amount of Bcl-2, Bcl-X_L, Bcl-X_S, Bad, or Bax was detected. The second messenger ceramide can induce apoptosis that can be blocked by sphingosine-1-phosphate (SPP). SPP completely protected PC 12 cells from cell death due to trophic factor deprivation. Ethanol-induced cell death, however, was not affected by treatment with sphingosine-1- phosphate. These findings suggest that ethanol-mediated apoptosis is mechanistically distinct from apoptosis initiated by serum withdrawal. (Supported by AA06207.)

411 THE POLY (ADP-RIBOSE) POLYMERASE INHIBITOR (PARPI), BENZAMIDE IS NEURO-PRETECTIVE AGAINST SOMAN-INDUCED SEIZURE-RELATED BRAIN DAMAGE.

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Soman produces limbic seizures and seizure-related brain damage (SRBD). Soman irreversibly inhibits acetylcholinesterase, causing an elevation in acetylcholine concentration in the brain. However, there is considerable evidence that soman-induced SRBD stems from glutamate excitotoxicity. Studies have shown that metabolic impairment greatly exacerbates the excitotoxic effects of glutamate. Prevention of ATP depletion by PARPI provides considerable neuroprotection in various models. To examine the neuroprotective effects of the PARPI, benz-amide, on brain damage from soman-induced seizures, rats were challenged with 180m g/kg soman (i.e., 1.6 LD_{50}). HI-6 and atropine methylnitrate given to protect against the peripheral effects of soman. Rats received two intraperitoneal injections of 320mg/kg benzamide: 5 and 40min post-seizure onset, or 40 and 120min post-seizure onset, and euthanatized 29h after soman administration. Alternate brain hemispheres were processed for microtubule-associated protein 2 (MAP2) immunohistochemical staining or hematoxylin and eosin (H&E) histochemical staining. Macroscopic temporal lobe necrosis was assessed using morphometric image analysis of MAP2-negative immunostaining. H&E-stained sections were examined for classical histo-pathology. In all rats that received benzamide, severity of convulsions was greatly diminished within 3min following the first injection, i.e., for both the 5/40min and 40/120min groups. Despite its marked effect on convulsions, benzamide did not overtly alter ECoG evidence of status epilepticus, compared to soman-positive controls. However, a marked decrease in macroscopic temporal lobe necrosis was seen in both benzamide treatment groups, and was most pronounced in the 40/120 min treatment group. These preliminary findings suggest that inhibition of PARP by benzamide can provide neuroprotection against soman-induced SRBD.

412 CHARACTERIZATION OF DOPAMINE-INDUCED EXPRESSION OF *GADD45* AND *GADD153* IN HUMAN NEUROBLASTOMA CELLS.

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Dopamine (DA), while an essential neurotransmitter, is also a known neurotoxin and has been implicated in several neurodegenerative diseases. DA metabolism and oxidation have been demonstrated to produce reactive oxygen species (ROS). Moreover, DA can be oxidized to a reactive quinone through spontaneous and catalyzed (*via* enzymes or metals) reactions. In the present studies, human neuroblastoma (SK-N-SH) cells were killed by high concentrations of DA (100-300 mM). The sensitivity of the cells was dramatically enhanced by treatments that reduced endogenous glutathione (2 mM L-BSO). Cells undergoing DA-induced cell death exhibit molecular markers (internucleosomal DNA fragmentation, chromatin condensation, membrane blebbing) consistent with programmed cell death (apoptosis). These results indicate that apoptotic pathways are induced in response to DA toxicity. Multiplex hybridization array technology demonstrated that cells, treated with DA (300 mM for 18 hours), induced *GADD45* and *GADD153* (Growth arrest and DNA-damage inducible) gene expression by three-fold. Subsequent northern blot analysis confirmed these DA-induced changes in *GADD* gene expression. Currently, the precise function of the *GADD* gene products is unclear. It is known that these genes are upregulated in response to stress to allow the cells to repair the damage. It appears that DA, through the formation of ROS and quinones, can damage cellular macromolecules to a point of inducing *GADD* gene expression.

413 OVEREXPRESSION OF CYCLIN D1 AND METALLOTHIONEIN AND CELL KINETICS IN THE EARLY PROCESS OF URINARY BLADDER CARCINOGENESIS INDUCED BY TREATMENT WITH *N*-BUTYL-*N*-(4-HYDROXY BUTYL)NITRO-SAMINE OR SODIUM L-ASCORBATE.

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This experiment is to clarify effects of genotoxic *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) and non-genotoxic sodium L-ascorbate (Na-AsA) on significant protein expression, cell proliferation and apoptosis of the bladder epithelium during and after treatment with these chemicals. The influence of testicular castration was also investigated. A total of 320 six-week-old male F344 rats were divided into six groups. Testicular castration was conducted to groups 4-6 at 2 weeks before chemical treatment. BBN (0.05% in drinking water) was given to groups 1 and 4 for 8 weeks. Na-AsA (5% in diet) was given to groups 2 and 5 for 8 weeks. Groups 3 and 6 were used non-treatment controls. The urinary bladder was removed at the end of weeks 6, 10, 11, 12, 14 and 18, and examined by H&E, TUNEL, immunohistochemistry (incorporated BrdU, cyclin D1 and metallothionein: MT) and FCM. Overexpression of cyclin D1 and MT was induced with BBN but not with Na-AsA. Cyclin D1 positive cells were scarcely found in metallothionein-positive region. MT overexpression was seldom observed in papillomas and never found in a carcinoma, suggesting MT would protect genes from insult by genotoxic carcinogens. Cell proliferation and apoptotic parameters showed no qualitative distinctions between treatment and cessation of BBN or Na-AsA. The testicular castration only inhibited the BBN induced bladder tumor development as comparison with total number of neoplasm in non-castrated animals.

414 ROLE OF OXIDATIVE STRESS IN CHROMIUM(VI)-INDUCED APOPTOSIS IN NORMAL HUMAN LUNG CELLS.

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Particulate forms of chromium(VI) [Cr(VI)] cause lung cancer and respiratory tract toxicity in occupationally-exposed workers. Both soluble sodium chromate and particulate lead chromate induced apoptosis in cultured normal human lung fibroblasts (HLF) and small airway epithelial (HSAE) cells. To determine whether oxidative damage contributes to Cr(VI)-induced apoptosis, oxidative stress was measured in Cr(VI)-treated HLF by fluorescent imaging using dichlorodihydro fluorescein diacetate. Elevated oxida-

tive stress was observed in HLF within minutes of exposure to apoptogenic doses of sodium chromate and also at the end of a 24 hour exposure. Individual Cr(VI)-treated cells differed in the magnitude of increase in oxidative stress, suggesting heterogeneity of cellular responses. DNA from Cr(VI)-treated HLF and HSAE cells was analysed by inductively coupled plasma mass spectrometry to quantitate Cr-DNA adducts as a biomarker for exposure. A dose-dependent formation of Cr-DNA adducts was seen. Melatonin, a potent physiological free radical scavenger, abrogated Cr(VI)-induced apoptosis in both cell types. Further studies will test the following hypotheses: (i) melatonin increases clonogenic survival of Cr(VI)-treated HLF; (ii) melatonin diminishes Cr(VI)-induced oxidative stress; and (iii) melatonin modulates the formation of Cr-DNA adducts. Our results suggest that oxidative stress may play a role in Cr(VI)-induced apoptosis, and thus, may also be involved in chromium-induced toxicity and carcinogenesis. (Supported by NIEHS grant ES05304.)

415 APOPTOSIS A POTENTIAL MECHANISM OF GERM CELL DEATH IN MERCURIC CHLORIDE INDUCED REPRODUCTIVE TOXICITY.

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The potential for reproductive toxicity of mercuric chloride (HgCl2) was evaluated in Sprague Dawley rats . For twelve weeks Fo male and female rats were exposed to mercuric chloride by daily gavage. Treatment groups consisted of 0.0 :00, 0.5:0.75 and 1.0:1.50 mg/kg/day in males and females, respectively. Fo males were dosed continuously throughout mating and the females were dosed continuously throughout mating, gestation and lactation. The Fo generation showed that oral exposure to HgCl2 impairs fertility, litter size, viability and weaning indices. In the F1 generation, the absolute weights of the left and right testes in the middle dose group were significantly lower than the control. Serum testosterone levels in the 1.5 mg/kg/day for males was reduced by 60%, suggesting that steroidogenesis in these animals may have been impaired. In the present study, the role of apoptosis in the germ cell loss was evaluated. Apoptosis was assessed in control and treated animals by terminal deoxynucleotidel transferase binding to exposed 3'-OH ends of DNA Fragment End Labeling (FragELTM). Observation of oligonuclesomal DNA fragments detectable as a DNA ladder in agrose gels was barely detectable after HgCl2 exposure. However, FragEL staining of testis cross section indicated that germ cell apoptosis increased significantly after toxicant exposure, thus providing a molecular-biology based, end-labeling histochemical (or cytochemical) technique for toxicant-induced testicular injury.

416 EPOXYBUTENE AND DIEPOXYBUTANE INDUCE APOPTOSIS IN MOUSE THYMOCYTES THROUGH DIFFERENT PATHWAYS.

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1,2-epoxybutene-3 (EB) and 1,2,3,4-diepoxybutane (DB) are reactive metabolites of 1,3-butadiene, a chemical commodity and air pollutant. Chronic exposure of mice to butadiene is associated with an increased incidence of T-cell leukemia/lymphoma, bone marrow and reproductive organ toxicity, and reduced thymus cellularity. In $B6C3F_1$ mice injected ip daily with 60, 90, or 120 mg/kg of EB for up to 14 days, the number of thymocytes decreases rapidly, reaching a nadir between the 3rd and 7th doses. To investigate potential mechanisms which could lead to a loss of thymocytes, the ability of EB and DB to initiate apoptosis was determined in vitro. One million freshly isolated thymocytes from young adult C57Bl/6 mice were exposed to increasing concentrations of EB or DB and the percentage of viable, apoptotic, and necrotic cells was determined at various times using HO33342 and 7-AAD dye uptake. Dexamethasone (0.1 μM) and γ-irradiation (500 cGy) were used as positive controls. EB (1 mM) and DB (0.1 mM), both induced thymocyte apoptosis which reached a maximum level at 12 hours. Using thymocytes from homozygous p53 knockout mice (C57Bl/6 background), it was determined that apoptosis induced by EB or dexamethasone operated independently of p53, whereas apoptosis induced by DB or γ-irradiation depended on p53 function. Further, EB-induced apoptosis was blocked by actinomycin but not by cycloheximide, whereas apoptosis induced by DB was blocked by either inhibitor. It is concluded that EB and DB both induce apoptosis in thymocytes, but through different induction pathways.

417 CELL DEATH OF HUMAN HT29 COLON CARCINOMA CELLS IS ACTIVATED BY CANCER PREVENTIVE DETOXIFICATION ENZYME INDUCERS.

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Chemicals that induce the detoxification enzymes glutathione S-transferase (GST) and NAD(P)H:quinone reductase (N:QR) are under intense investigation as potential cancer chemopreventive agents with the capacity to enhance elimination of carcinogens. Representative chemicals, including allyl sulfide, benzyl isothiocyanate (BIT), butylated hydroxyanisole, dimethyl fumarate (DMF) and Sudan I, were found to activate a delayed cell death in HT29 colon carcinoma cells over the same concentration range that induces GST and N:QR enzyme activity. This cell death has some features of apoptosis, including morphological changes, an increased proportion of sub-G1 phase cells, caspase activation and a weakly expressed TUNEL-positive assay. Of the enzyme inducers tested, BIT and DMF were the most effective in promoting cell lifting by 20- and 4-fold, respectively, and a two-fold increase in caspase activation, as compared to controls. The effects of these chemicals on both enzyme activity and cell death were potentiated by pretreatment with sodium butyrate, which induces HT29 cell differentiation. Activation of cell death in cancerous or precancerous cells may provide an additional mechanism whereby these dietary constituents may protect against cancer. (Support: GM-028248, ES-09047.)

418 SIGNAL TRANSDUCTION IN PAH-INDUCED PREB CELL APOPTOSIS.

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Protein kinase C (PKC) activation has been implicated in the rescue of B cell apoptosis as well as aryl hydrocarbon receptor (AhR)-mediated signal transduction under *in vitro* conditions. We have previously reported that a prototypic polycyclic aromatic hydrocarbon, 7,12-dimethylbenz[a]anthracene (DMBA), induces apoptosis in AhR^- mouse preB (BU-11) cells grown on AhR^+ bone marrow stromal cells and that apoptosis is dependent on the AhR or AhR-regulated genes. These studies have been extended to evaluate potential signaling pathways within bone marrow stromal cells or apoptotic preB cells. Herein, it is shown that DMBA-induced BU-11 cell apoptosis was inhibited by PKC activators (PMA and mezerein) in a dose dependent manner. In contrast, the PKC inhibitors staurosporin, fisetin, quercetin and apigenin increased the percentage of apoptotic BU-11 cells when stromal/BU-11 cell cultures were co-treated with DMBA. A mitogen activated protein kinase (MAPK) inhibitor PD98059 blocked DMBA-induced apoptosis at the dose of 10 μ M. The possibility that this documented AhR antagonist inhibited apoptosis by blocking AhR activity as well as MAPK activity is under investigation. Collectively, these data implicate AhR, PKC and/or MAPK activation in either inducing a death signal from bone marrow stromal cells and/or transducing the apoptosis signal within target preB cells. (Supported by PHS grants RO1-ES06086 and 1P4-2ES07381.)

419 THE ROLE FOR APOPTOSIS IN THE CYTOTOXIC RESPONSE TO KEY MILITARY COMPOUNDS.

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We are testing the mechanisms by which two substances of military significance, sulfur mustard (SM) and jet fuel (JP-8), cause pathology. SM may cause basal cell death and detachment via induction of terminal differentiation and apoptosis. Following treatment of normal human epidermal keratinocytes with 10 μM or 100 μM SM, epidermal differentiation-specific markers are induced. Examination of biochemical markers of apoptosis following treatment of either primary or immortalized keratinocytes revealed a burst of poly(ADP-ribose) synthesis, proteolysis of poly(ADP-ribose)polymerase (PARP) *in vivo* and *in vitro* into characteristic 89 kDa and 24 kDa fragments, processing of caspase-3 into its active form, and appearance of DNA ladders. The intracellular calcium chelator BAPTA suppressed differentiation markers, while antisense oligonucleotides and chemical inhibitors

specific for calmodulin blocked both markers of differentiation and apoptosis. Modulation of p53 levels utilizing retroviral constructs expressing the E6, E7, or E6 +E7 genes of human papillomavirus (HPV) 16 revealed that SM-induced apoptosis was partially p53-dependent. Finally, immortalized fibroblasts derived from PARP -/- "knockout mice" were exquisitely sensitive to SM-induced apoptosis. These cells became SM-resistant when PARP was stably reintroduced in these cells. These results indicate that SM exerts its effects via calmodulin, p53, and PARP-sensitive pathways. We are performing similar experiments with JP-8 to determine if this compound exerts similar effects on keratinocytes and hepatocytes. (This work was supported by contract DAMD17-96-C-6065 (to DSR) and contract DAMD17-90-C-0053 (MES) from the US Army Medical Research and Development Command, and US Air Force Office of Scientific Research Grant AFOSR-89-0053.)

420 ACETAMINOPHEN (APAP) INDUCES CLEAVAGE OF POLY(AD-RIBOSE) POLYMERASE (PARP) AND DNA-DEPENDENT PROTEIN KINASE (DNA-PK) BY ACTIVATING CASPASES IN JURKAT T CELLS.

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APAP is a widely used analgesic and antipyretic drug which can be toxic at non-therapeutic doses. We have examined the effect of 10 mM APAP on cell viability of the human Jurkat T cells. Cell death was observed as early as 6 h after treatment which increased with time to reach 50% by 12 h. Hoechst staining of treated cells revealed nuclear condensation indicating that APAP was causing apoptosis in these cells. However, little DNA fragmentation was observed when analyzed by electrophoresis. APAP treatment lead to cleavage of the DNA repair enzymes PARP and DNA-PK through the activation of caspase-3. Activation of caspase-3 could be due, in part, to an activation of caspase-8 without an apparent upregulation of the Fas receptor. An early activation of PARP manifested by poly(ADP-ribosyl)ation of nuclear proteins was also observed indicating a generation of DNA breaks. Pulse field electrophoresis analysis of DNA from treated cells revealed a generation of 50 kb fragments which accumulated overtime without further processing explaining the lack of DNA ladders. This could be in part, to an inhibition of the DNA fragmentation factor (DFF-40) responsible for DNA fragmentation since its inhibitor DFF-45 was cleaved. Our results indicate that APAP activates early and intermediate events of apoptosis, however, it interferes with DNA fragmentation. (This work was supported by contracts DAMD17-90-C-0053 from the US Army Medical Research and Development Command and AFOSR-89-0053 from US Air Force office of Scientific Research.)

421 GENERATION AND DETECTION OF APOPTOSIS IN RAT LIVER SLICES.

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Apoptosis has been previously produced and detected in cell cultures. Since organ cultures reflect the whole animal more closely than cell cultures, this study was designed to determine whether apoptosis will occur in precision-cut rat liver slices exposed to specific toxic insults, and to find a compound that will induce apoptosis in liver slices in a consistent manner. Staurosporine, a protein kinase C inhibitor, is known to consistently cause apoptosis in hepatocyte cell cultures. Liver slices were prepared from 250 g male Sprague-Dawley rats and incubated with 0.5 or 10 μM staurosporine for 2-8 hr. Apoptosis was detected by DNA fragmentation, amplification of the Bax gene, and histologically. A concentration-dependent loss of LDH and K^+ was observed in liver slices. Agarose gel electrophoresis of DNA harvested from the slices contained a 'smear' with only minimal laddering. Bax gene expression was enhanced following a 2 hr exposure to 0.5 μM staurosporine, but not 10 μM. Nuclear margination of chromatin was observed in cells, however, a considerable amount of necrosis was also seen. Combined, these results indicate that although several apoptotic features were observed, staurosporine also elicited necrosis at the concentrations used in precision-cut rat liver slices. More importantly, however, the data suggests that precision-cut tissue slices present a useful in vitro system for the detection of apoptosis.

422 SPECIES-SPECIFIC INDUCTION OF APOPTOSIS IN RAT AND DOG LIVER SLICES BY SDZ 249 665.

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SDZ 249 665 is a capsaicin analogue with promising data on its pharmacological profile in the clinical indication pain. SDZ 249 665 was well tolerated in preclinical safety studies in rats but not in dogs. In dogs the compound caused severe liver toxicity in a 2 week study already at a dose levels of 2 mg/kg/day with periportal apoptosis like hepatocellular necrosis/lysis. It was the purpose of the present study to mimic the in vivo situation by an in vitro model, which can be also used for the risk assessment in man. Liver slices of rat and dog were selected in order to evaluate the SDZ 249 665 species specific effects on apoptosis. Rat and dog liver slices were incubated for 24 hours with SDZ 249 665 at concentrations of 0.1, 1, 10 and 100 μ M. TGFβ $_1$ at concentrations of 5 and 10 ng/ml was used as positive control. Apoptosis was determined by chromatin condensation (Feulgen) and DNA fragmentation (TUNEL). Lactate dehydrogenase (LDH) leakage was determined as a parameter of cytotoxicity. In both rat and dog liver slices TGFβ $_1$ caused a dose-dependent increase of apoptosis, reaching maximum values of about 2-3 fold above the basic apoptotic level of controls (1-3 %). In dog liver slices treatment at non cytotoxic concentrations of 0.1, 1 and 10 μ M SDZ 249 665 resulted in apototic rates which were about 2-3 times greater than that of controls. In rat liver slices SDZ 249 665 had no effect. Since the present results reflect the in vivo species-specific findings very accurate, risk extrapolation to man seems possible by means of human liver slices.

423 URIDINE ADMINISTRATION DOES NOT PROTECT FROM GALACTOSAMINE-INDUCED HEPATOCTYE APOPTOSIS IN RATS.

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Galactosamine is a well-known model hepatotoxin. The hepatic lesion described by Keppler *et al.* in 1968 is a scattered focal hepatic necrosis. Recent reports have demonstrated that galactosamine also induces hepatocyte apoptosis when given to rats or mice. In the present studies we wanted to examine the time-dependency for the development of apoptosis and compare it to that for development of hepatic necrosis. Further, we wished to determine whether galactosamine-induced apoptosis could be prevented by administration of a hepatoprotective dose of uridine. For the time course studies, male rats were given a hepatotoxic dose of galactosamine (800 mg/kg) by ip injection. At various times later their livers were removed and processed for microscopic examination by H&E staining (apoptosis and necrosis) and by staining for apoptosis using the ApopTag® protocol. We observed a time-dependent increase in apoptosis, with peak induction (15%) occurring at 12 hrs after galactosamine treatment, followed by a subsequent decline over the next 24 hrs. In contrast, hepatic necrosis (11%) was not evident until 36 hrs after galactosamine treatment. In additional studies, animals were treated with uridine (800 mg/kg) at the same time as galactosamine and the livers were examined for apoptosis and necrosis 12 hrs and 36 hrs later. Uridine administration effectively prevented galactosamine-induced necrosis as observed at 36 hrs, however, development of apoptosis at either 12 or 36 hrs was not prevented. These data demonstrate that development of galactosamine-induced apoptosis precedes development of hepatic necrosis. Of importance, development of apoptosis appears to be independent of uridine nucleotide depletion. (Supported by NIH DK47185.)

424 DYSREGULATION OF APOPTOSIS BY c-*myc* IN TRANS-GENIC HEPATOCYTES AND EFFECTS OF GROWTH FACTORS AND NONGENOTOXIC CARCINOGENS.

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Regulation of apoptosis is an important factor in multistage hepatocarcinogenesis. The objectives of the present study were to determine (1) whether apoptosis regulation is altered in transgenic hepatocytes that overexpress c-*myc* and (2) if growth factors or nongenotoxic carcinogens alter apoptosis regulation in c-*myc* vs. wild-type (wt) hepatocytes. Hepatocytes isolated from c-*myc* transgenic mice had 3 to 4-fold increased c-*myc* RNA and protein levels in addition to increased apoptosis compared to wt hepatocytes. The increased apoptosis in c-*myc* hepatocytes was accompanied by

increased p53, bax, and bak and decreased bcl-2 protein levels. Hepatocytes overexpressing c-*myc* were more sensitive to bleomycin-induced apoptosis but less sensitive to TGF-b-induced apoptosis. TGF-a inhibited apoptosis, increased bcl-xL, and decreased bak protein levels in c-*myc* hepatocytes but not wt hepatocytes. IGF-II did not affect apoptosis in c-*myc* or wt hepatocytes. Phenobarbital inhibited apoptosis in c-*myc* hepatocytes but not wt hepatocytes and decreased p53 and bax and increased bcl-2 protein levels. Nafenopin inhibited apoptosis in both c-*myc* and wt hepatocytes, while TCDD did not inhibit apoptosis in either wt or c-*myc* hepatocytes. This study demonstrated that overexpression of c-*myc* altered the response to apoptotic stimuli in transgenic hepatocytes. Furthermore, phenobarbital and TGF-a inhibited c-*myc*-induced apoptosis, potentially resulting in a selective growth advantage for an initiated cell population and representing a mechanism for tumor promotion.

425 POLY (ADP-RIBOSE) POLYMERASE MODULATORS 4-AMINOBENZAMIDE (AB) AND NOCOTINAMIDE (NICO) PROTECT AGAINST ACETAMINOPHEN (AAP)-INDUCED HEPATOTOXICITY IN MICE BY INFLUENCING EXPRESSION OF BCL-XL AND p53.

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It is well known that the oncogene bci-X_L and anti-oncogene p_{53} play a role in apoptotic death *in vitro* and *in vivo*. This study was designed to investigate whether agents that modulate DNA repair through poly (ADP-ribose) polymerase (PARP) activity (AB & NICO) can protect against AAP-induced DNA fragmentation and hepatotoxicity, and do so by altering expression of the apoptosis-regulating genes, bci-X_L and p_{53}. Male ICR mice (3 months old, 30-40 gms) were administered AAP (500mg/kg, ip) or AAP plus one of the protective agents one hr later (AB, 400mg/kg, ip; NICO, 250mg/kg, ip). Chlorpromazine (CPZ, 25mg/kg, ip), a Ca^{2+}-antagonist acting through another mechanism was also used. Animals were sacrificed 24 hrs following the drug administration. Serum chemistry (ALT) and hepatic histopathology, DNA and western blot analysis for bci-X_L was downregulated and p_{53} was upregulated by AAP. The effect of AAP on bci-X_L was antagonized and that on p_{53} was synergized by both PARP-modulating agents, whereas CPZ was without effect on both bci-X_L and P_{53}. These results suggest that antagonism of AAP-induced hepatocellular apoptosis and/or necrosis by modulators of PARP may be mediated by their influence on bci-X_L and/or p_{53}.

426 ARYL HYDROCARBON RECEPTOR REGULATION OF CERAMIDE-INDUCED APOPTOSIS IN MURINE HEPATOMA CELLS.

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The relationship between aryl hydrocarbon receptor (AHR) content and susceptibility to apoptosis was examined in the murine hepatoma 1c1c7 cell line and a series of variant cell lines having different levels of AHR expression. Exposure of 1c1c7 cultures to N-acetyl-sphingosine (C2-ceramide), a cell permeable analog of ceramide, caused a concentration-dependent inhibition of cell proliferation and induction of apoptosis, as monitored by analyses of caspase activation and DNA fragmentation. A variant line (Tao) having ~10% of the AHR content of 1c1c7 cells also arrested following exposure to C2-ceramide. However, Tao cells were resistant to C2-ceramide-induced apoptosis. Modulation of Tao and 1c1c7 AHR contents by transfection of *Ahr* sense and antisense constructs, respectively, confirmed the relationship between AHR content and susceptibility to C2-ceramide-induced apoptosis. The AHR ligands 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and alpha-naphthoflavone neither induced apoptosis nor modulated the development of apoptosis in C2-ceramide treated 1c1c7 cultures. Ceramide also induced the apoptosis of BP^{r}c1 cells, a variant line having normal AHR contents, but lacking a functional aryl hydrocarbon receptor nuclear translocator (ARNT) protein. These results suggest that the AHR modulates aspects of ceramide signaling associated with the induction of apoptosis but not cell cycle arrest, and does so by a mechanism that is independent of its interaction with ARNT. (This work was supported in part by NIH grant CA34469.)

427 PARTICIPATION OF SERTOLI CELL-EXPRESSED FASL IN THE INITIATION OF GERM CELL APOPTOSIS IN YOUNG RAT TESTIS AFTER EXPOSURE TO MONO-(2-ETHYLHEXYL) PHTHALATE (MEHP).

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The Fas/Apo-1/CD95 signaling system is an important regulator of germ cell apoptosis in mammalian testis. Germ cell apoptosis occurs to limit their numbers in order to match the Sertoli cell's supportive capacity. Sertoli cells, via expression of FasL, initiate apoptosis of Fas-bearing germ cells (*Endocrinol.*, 1997, 138:2081). In this study, alterations in the expression of FasL were measured in 28-day-old Fisher rats after exposure to the widely studied Sertoli cell toxicant, mono-(2-ethylhexyl) phthalate (MEHP, 2 g/kg, p.o). After 3 h of MEHP exposure, immunohistochemical staining of FasL was changed from a Sertoli cell basal membrane to an adluminal membrane localization. The intensity of theFasL staining increased over the exposure time course (3, 6 & 12 h). It is along the adluminal membrane of the Sertoli cell where apoptotic germ cells are observed after MEHP treatment. Western blot analysis demonstrated a time-dependent increase in the production of the soluble form of FasL after MEHP exposure (129, 144 & 161% at 3, 6 &12h, respectively). The ability of FasL to bind to Fas is abolished in *gld* mice which have a point mutation near the C-terminus of the coding region for FasL. Exposure of *gld* mice to MEHP did not result in a significant increase in germ cell apoptosis up to 24 h after exposure. However, in control mice there was a 2.5 fold increase in apoptosis. These data support the direct involvement of Sertoli cell-expressed FasL in the initiation of MEHP-induced germ cell apoptosis. (Supported by NIH/NIEHS ES09145 & ES07784.)

428 ALTERATIONS IN TESTICULAR CYTOSKELETAL AND SIGNALING PROTEIN DISTRIBUTION DURING GERM CELL DEATH.

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Previous work on the testis lesion induced by ethylene glycol monomethyl ether (EGME) has found that several stages of spermatocytes are vulnerable, although pachytene spermatocytes are the most sensitive . Trans-cellular calcium movement has been implicated as an early event. Still unknown are other early responses to toxicity, and changes associated with cell death. In these studies, we used adult F344 rats killed 4, 8, 16 and 24 hrs after a single dose of 200 mg/kg EGME. Apoptotic death was histologically detectable 16 hrs after administration of EGME, so the 16 and 24 hr timepoints were used for subsequent studies. Reasoning that cytoskeleton and cell-cell signalling proteins would likely be involved in this cell death, we used immunohistochemistry to determine changes in the distribution of cytoskeletal (actin, tubulin) and signaling proteins (β -catenin, pp120, src, paxillin, E-cadherin, talin, α -catenin, vinculin, tensin). In addition, since one blocker of PKC had been previously shown to inhibit this lesion, we explored the distribution of a panel of antibodies to various PKC isoforms (μ , ζ , γ , α , β , ε , θ , λ , δ) in both control and EGME-treated rats. These studies found an increase in the staining intensity of PKC-μ , ζ and γ , pp120, actin, src, and paxillin around and/or in the dying germ cells at 16 and 24 hrs after treatment. With the exception of reduced src staining in Sertoli cells seen at 4 hrs after treatment, none of the changes preceeded visible apoptosis. These results identify at least some of the proteins involved in the development of this lesion.

429 BREATH VOLATILE ORGANIC CHEMICALS (VOCs) AS BIOMARKERS FOR HEPATIC CYTOCHROME P450 (CYP) ISOZYME ACTIVITIES.

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These studies were designed to determine if certain breath components are reflective of the activity of hepatic CYP enzymes. We have reported methodology for collection of VOCs in rat breath and analysis for over 100 consitiuent components (Raymer et al., Toxicol Methods 4:243-258, 1994). Changes in the profiles of VOCs present were determined after treatment with a general inhibitor of hepatic CYP isozymes (1-aminobenzotriazole, ABT), as well as isozyme-selective inhibitors. The major rat hepatic CYP isozymes monitored included CYP1A2 (<5% of P450), 2B1 (1%), 2C6

(20%), 2C11 (40%), 2E1 (10%), and 3A1 (20%). ABT is an inhibitor of CYP 2C6, 2C11, 2B1, 3A, 1A2 and 2E1 in rat. ABT treatment led to marked increases in a broad spectrum of VOCs (Mathews et al., Biomarkers 1:196-201, 1996). Isozyme-selective inhibitors were next used to link individual isozymes with specific components in breath. Inhibition of CYP1A2 with furafylline gave rise to only minor changes in the VOC profile and similarly, only minimal changes were linked to inhibition of CYP2C6 and 2C11. There was no effect on breath volatiles following inhibition of CYP3A. Inhibition of CYP2E1 with trans-1,2-dichloroethylene led to several-fold increases in n-hexane (26-fold) and methylketones (Mathews et al., Toxicol Appl Pharmacol 146: 255-260, 1997). Percent increases of methylketones in response to inhibition were inversely related to chain length, with acetone and 2-butanone > 2-pentanone >>2-hexanone > 2-heptanone. The remainder of the VOCs associated with general P450 inhibition with ABT, but not CYP2E1, were associated with a group of gas chromatography peaks eluting with eight-carbon volatile chemicals, including 1-octene, n-octane, 3-octene, 2-octene, 1,3-octadiene, and 3-methyl-1-heptene. These breath VOCs also increased following inhibition of CYP 2B1, but these inhibitors were not completely selective for 2B1. It is likely that these C8 breath components reflect the hepatic activity of this phenobarbital-inducible CYP. Thus, selected VOCs in breath can serve as biomarkers for the activity of CYP2E1 and possibly CYP2B1, but no breath markers for isozymes of the 1A, 2C, or 3A families were found.

430 EXPRESSION, CHARACTERIZATION, AND INHIBITION BY TELLURITE (TeO_3) OF RECOMBINANT HUMAN SQUALENE MONOOXYGENASE (SQUALENE EPOXIDASE).

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Tellurium is an element with a growing number of industrial applications, including the manufacture of semiconductors, doping of metal alloys, and vulcanization of rubber; however, little is known about its toxicology. In weanling rats tellurium produces a transient peripheral demyelination due to the inhibition of squalene monooxygenase, an enzyme in the pathway for cholesterol biosynthesis. Squalene monooxygenase is a membrane-bound flavoprotein that uses NADPH-cytochrome P450 reductase as a source of reducing equivalents. To study the potential mechanisms of tellurium toxicity in humans, the cDNA for human squalene monooxygenase was cloned and expressed in *Escherichia coli*. Expression was enhanced by deleting the first 110 amino acids from the N-terminus, based on studies by others with the rat enzyme. The enzyme was expressed as a chitin binding domain-intein fusion protein and purified by affinity chromatography. Squalene monooxygenase activity was determined by measuring the formation of 2,3-oxidosqualene from ^{14}C-radiolabeled squalene; the products were separated by thin layer chromatography and visualized by electronic autoradiography. Preliminary results indicate a Km of 10 μ M for squalene, similar to published values for the rat and pig enzymes. An estimated IC_{50} of 17 μ M was obtained with sodium tellurite (TeO_3); tellurate (TeO_4) was not inhibitory. Thus, squalene monooxygenase is a potential target of tellurium toxicity in man. (Supported by the American Heart Association, Kentucky Affiliate; KY EPSCoR NSF Cooperative Agreement; and NIEHS Training Grant ES-07266.)

431 EFFECTS OF XENOBIOTICS ON STEROIDOGENIC CYTOCHROME P-450 (CYP450) ENZYME EXPRESSION IN HUMAN H295R ADRENOCORTICAL CARCINOMA CELLS.

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The H295R cell line was deployed to investigate potential effects of xenobiotics on the expression and catalytic activity of several steroidogenic CYP450s. H295R cells, cultured in medium containing steroid-free serum, produce many steroid hormones and express numerous steroidogenic CYP450s, including CYP11A, CYP11B1, CYP11B2, CYP17, CYP19 and CYP21. We examined the effects of several environmental contaminants on the levels of messenger RNA and the catalytic activity of CYP11A (cholesterol side-chain cleavage) and CYP19 (aromatase) in untreated or 8-bromocyclic AMP-(8BrcAMP)-treated H295R cells. mRNA levels were determined semi-quantitatively using RT-PCR and catalytic activities were determined using specific substrates and inhibitors. 8BrcAMP induced CYP11A and CYP19 in H295R cells (mRNA levels 3- and 10-fold relative to β-actin; catalytic activities 2- and 10-fold, respectively). Established pharmacological inhibitors were able to inhibit the activity of both enzymes by >90%. Exposure of H295R cells to 100 μM tetrachlorodibenzo-*p*-dioxin (TCDD) 24 h prior to treatment with 8BrcAMP led to a significantly reduced ability of 8BrcAMP to induce CYP11A mRNA (about 40% reduction; $p<0.05$). The same was true for benzo[a]pyrene. TCDD and benzo[a]pyrene exposure resulted in a moderate reduction in the ability of 8BrcAMP to induce the catalytic activity of CYP11A (about 30% reduction). No effects of exposure to the two compounds were observed on basal activities of CYP11A. Significant effects on CYP19 mRNA levels or activities (with or without induction) were not observed. At present, potential effects of a number of environmentally important pesticides including atrazine and several of its metabolites are being investigated in the H295R cell line. In summary, our results indicate that the H295R cell line is suitable for the detection of potential effects of xenobiotics on the expression and activity of steroidogenic CYP450 enzymes.

432 INFLUENCE OF TAMOXIFEN ON HORMONALLY REGULATED CYTOCHROME P450 ENZYME EXPRESSION IN RATS.

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Tamoxifen, a partial estrogen antagonist in humans used widely in the treatment of breast cancer, has been reported to suppress growth hormone (GH) secretion in both rats and humans and therefore may alter expression of hormonally regulated cytochrome P450 (CYP) enzymes. The purpose of this study is to investigate the long-term hormone-mediated effects of tamoxifen on CYP enzymes in rat liver. Adult female rats were injected s.c. with tamoxifen at 0.5, 5, 20, 100, or 200 mg/kg b.w. for two consecutive days and hepatic microsomes were prepared 35 days later. Administration of tamoxifen at all doses tested decreased somatic growth in male and female rats. The testosterone 7α-hydroxylase activity and CYP2A1 protein content in hepatic microsomes from female rats that received doses greater than 5 mg/kg was decreased by approximately 40% and 35%, respectively, as compared to the control group. The same treatment had no effect on the testosterone 6α-, 6β-, 16α-, 16β-, 2α-, and 2β-hydroxylase activities and on the hepatic microsomal content of CYP 2B1, CYP2B2, and CYP3A. In adult male rats, the testosterone 7α-hydroxylase activity was increased by approximately 40%, but the hepatic microsomal CYP2A1 protein level was not affected significantly in the treatment group receiving doses of 200 mg/kg. The testosterone 16α- and 2α- hydroxylase activities were decreased by approximately 44% and the hepatic microsomal CYP2C11 content was decreased by approximately 30%. The same treatment had no effect on the testosterone 6β-, 16β-, and 2β-hydroxylase activities and on the hepatic microsomal content of CYP 2B1, CYP2B2, and CYP3A. Thus far, analysis of plasma GH concentrations suggests that the effect of tamoxifen on specific hepatic CYP enzymes was not mediated by changes in plasma GH levels. In conclusion, tamoxifen treatment resulted in a long-term alteration of specific hepatic CYP enzymes in male and female rats, which has not been reported previously and is different from the direct, inductive effects of tamoxifen on hepatic CYP enzymes.

433 DETECTION OF CATECHOL ESTROGENS BY GAS CHROMATOGRAPHY / ELECTRON CAPTURE (GC-ECD).

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Catechol estrogens, the oxidative metabolites of estrogen, have been shown to play important roles in both physiologic and pathologic processes. Catechol estrogens may be further metabolized via cytochrome P450 (P450)-catalyzed reactions to quinones that can undergo redox cycling reactions, producing oxygen radical species that can cause oxidative DNA damage. Thus, P450-catalyzed estrogen metabolism may play an important role in carcinogenesis, and environmental chemicals that alter P450 activities may contribute to oncogenesis through this mechanism. We have modified a GC-ECD method to detect 2-hydroxyestradiol (2-OHE2) and 4-hydroxyestradiol (4-OHE2) metabolites in rat liver microsomes. The modified method employs a simple, multiphase extraction procedure which first extracts the analytes from microsomal preparations (quenched with 2% sodium sulfate solution (2% Na_2SO_4)) into dichloromethane (DCM), followed by solvent exchange into acetonitrile-saturated with hexane (ACN/HEX) . Metabolites are then derivatized by acylation to heptafluorobutyrl esters in the ACN/HEX extract, the derivatization reaction quenched by addition of 2% Na_2SO_4, and the analytes extracted into n-hexane. Derivatized catechol estrogens are then separated by capillary column gas chromatography and quantified by electron capture detection. 4-bromoestradiol (4-BrE2) was chosen as a surrogate. This protocol optimizes sample clean up, improves

chromatography, and increases column life while maintaining extraction efficiency. A method quantitation limit normalized for microsomal protein content was 1.2 ng/μ g. All compounds achieved an acceptable level of reproducibility, with an average coefficient of variation of 8.6%. Ongoing studies are measuring 2- and 4-OHE2 in liver microsomes from animals exposed to chlorinated hydrocarbon inducers of P450 enzymes. (Support from NIEHS ES 09455-01.)

434 RAPID PURIFICATION OF BACULOVIRUS EXPRESSED HUMAN CYTOCHROME P-450 AROMATASE BY PERFUSION CHROMATOGRAPHY.

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Cytochrome P-450 aromatase catalyzes the conversion of androgens to estrogens. Because of the importance of estrogens in hormone dependent diseases such as breast cancer, and the enigmatic mechanism involved in estrogen formation, aromatase has been the subject of much clinical and academic interest in the recent past. We have developed a method to purify human aromatase using classical chemistries of adsorptive chromatography, but applied via high flow perfusion chromatography. *Tricoplusia ni* TN5 cells expressing aromatase were solubilized in buffer containing 1.1% sodium cholate and centrifuged. After removal of detergent by dialysis, lipids and suspended fines could be rapidly removed from the supernatant with complete recovery of the target protein by passage of the solution through a pad of Cell Debris Remover. At least 13 nmol P-450 per run could then be applied to a high-density phenyl perfusion hydrophobic interaction chromatography column at 6 mls per minute, washed, and eluted in under 15 minutes with 80-85% yield. The column eluate could then be directly applied to a high-density sulfopropyl perfusion cation exchange column at 7 mls per minute, washed, and eluted in about 5 minutes with 80-85% recovery. This preparation was applied to a hydroxyapatite column at high flow rates, washed, and eluted in about 20 minutes. All chromatographic purification steps, including equilibrations, could be performed in approximately two hours, with total yields of 50% being typical. This method yielded preparations which were homogenous when deglycosylated with peptide-N-glycosidase F and were suitable for kinetic analysis. (This project was supported in part by NIH Grant No. GM32165 (SDN), and NIH Training Grant No. GM07750 (CAG).)

435 FINGERPRINTING CYTOCHROME P450 AND mEH GENE EXPRESSION IN HUMAN LEUKEMIA CELL LINES.

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The present study was conducted to validate human peripheral blood cells as a model to assess chemical induction of cytochrome P450 gene expression and to investigate the association between cell lineage and inducibility. We compared three different human blood cell lines, the erythroleukemia cell line HEL 92.1.7., the promyelocytic leukemia cell line HL-60 and the monocytic leukemia cell line THP, each representing a different lineage of human hemopoiesis. Using a highly sensitive quantitative-competitive RT-PCR assay, we established gene expression patterns for seven different CYP enzymes and microsomal EH. All cell lines exhibited relatively high constitutive mRNA levels of CYP2D6 and CYP2E1 (1.6-5.8x104 molecules/μg total RNA) and mEH (0.9-4.7x105 molecules/μg total RNA). CYP1A1 and CYP3A4/5 were expressed at very low levels, 2x102 and 103 molecules per μg total RNA respectively. CYP1A2, CYP2A6/7 and CYP2F1 were only marginally detectable. Overall, we observed low variation (1.5-4.4 fold) in basal expression profiles between the three cell lines. Treatment (24 and 48 hours) with different concentrations of β-naphthoflavone (βNF) and AROCLOR 1254 (ARO) revealed no significant increase of CYP1A1 or 1A2 message or detectable response in CYP3A mRNA expression. In contrast, corresponding treatment of the human hepatoma cell line, HepG2, induced CYP1A1 mRNA 200 fold (107 molecules/μg total RNA). These results indicate that human leukemia cell lines are not substantially responsive to the prototypic inducers, βNF and ARO, and that CYP expression patterns essentially are conserved across cell types. (Supported by NIH grant ES04696.)

436 OXIDATIVE INJURY IN HUMAN BREAST TUMOR CELLS VIA CATECHOL ESTROGEN METABOLISM.

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Recent literature has suggested a role for catechol estrogens in oxidative cellular injury, and their possible role in carcinogenesis. We have investigated the ability of catechol estrogens to produce oxidative DNA damage, via cytochrome P450 (CYP) mediated metabolism, in an estrogen receptor positive human breast tumor cell line, MCF-7. We measured the following endpoints after exposure to a CYP1 inducer, dioxin (TCDD), with and without 2-hydroxy (2OHE2) and 4-hydroxyestradiol (4OHE2) treatments: 1) CYP1 enzyme activity; 2) DNA fragmentation. TCDD induced a 25-fold increase in CYP 1 enzymatic activity in MCF-7 cells, as measured by the aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin -o-dealkylase (EROD) assays. Co-treatment with alpha-napthoflavone (A-NF), an aryl hydrocarbon receptor (AhR) antagonist, abolished induction of CYP1 by TCDD. While 4OHE2 did not alter CYP1 induction by TCDD, 10μM 2-OHE2 decreased CYP1 activity approximately 2-fold. Inhibition of CYP1 activity by 2-OHE2 does not appear to be related to feedback inhibition by 2-OHE2 methylation, as the catechol-o-methyl transferase (COMT) inhibitor, RO 41-0960, failed to reverse this inhibitory effect. 10μM 4-OHE2, but not 10μM 2-OHE2, resulted in a 3-fold increase in DNA strand breaks in MCF-7 cells after 12 hours treatment, as measured by immunoassay/ELISA. Surprisingly, 10nM TCDD produced a 2-3 fold increase in DNA strand breaks, even in the presence of A-NF. Following CYP1 induction by TCDD, treatment with 10μM 4OHE2 alone, or 10μM 4-OHE2 and the peroxide cofactors necessary for quinone cycling, did not increase DNA fragmentation over TCDD alone. To conclude, dioxin appears to produce DNA fragmentation in MCF-7 cells through a non-AhR pathway, while quinone cycling does not appear to play a significant role in the production of oxidative DNA damage by catechol estrogens. Ongoing studies include measuring the production of oxidized DNA base adducts following these cell treatments. (Support from College of Vet Med/CSU and USACEHR.)

437 EFFECTS OF DDT ON THE HEPATIC METABOLISM OF TESTOSTERONE.

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DDT is considered to be an endocrine disruptor, therefore, we studied the effect of single doses of technical DDT (0, 0.1, 1, 5, 10 and 100 mg/kg body weight) on the hepatic microsomal metabolism of testosterone *in vitro*. DDT was administered by gavage to male or female adult Wistar rats. Animals were sacrificed 24 h later. Microsomal hydroxylation of [4-^{14}C]-testosterone was assayed *in vitro*. Testosterone and its metabolites were extracted with ethyl acetate, analyzed by TLC and quantitated by radioscanning. DDT increased the rate of testosterone metabolism without following a clear dose-related response. The maximum effect (2.2-fold) was obtained at 1 mg/kgbw. Female rats metabolized [4-^{14}C]-testosterone faster (20%) than males at doses of 0.1 and 1 mg/kgbw. DDT also altered the hydroxylation pattern of testosterone in a sex-related manner. In males, the synthesis of 6β - and 6α - hydroxylated metabolites (CYP3A2- and 2A1-mediated, respectively) was increased ~4-fold over control values and 2-fold in 16α - (CYP2C11- and 2B- mediated). In females, the synthesis of 16α -, 16β - (CYP2B1-mediated) and 6β - was increased ~4-fold over control values. 15α -OHT (CYP2A2-mediated) synthesis increased 2-fold and the oxidation of testosterone to 6-dehydrotestosterone increased ~4-fold in both sexes. No significant effects were observed on 7α -OHT and androstenedione formation. Western blot densitometric analysis showed a preferential induction of CYP2B1 (17- fold in males and 42-fold in females). CYP3A1 was induced 32-fold and CYP3A2 18-fold in females. CYP2C11 increased 2.2-fold in males. Testosterone hydroxylation in positions 6α - and 15α - suggest an induction of CYP2A1 and 2A2, respectively. These results suggest that one of the mechanisms by which DDT may disrupt endocrine functions is its ability to modulate the expression of CYPs involved in the metabolism of sexual hormones and the induction of sex-specific CYPs normally non-expressed in a particular sex. Supported by the John D. and Catherine MacArthur Foundation.

438 THE EFFECTS OF DIET RESTRICTION ON EXPRESSION OF HEPATIC CYTOCHROME P450 ENZYME mRNA LEVELS IN THE RAT.

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Utilization of moderate diet restriction (DR) in life time rodent cancer bioassays results in a more appropriate model to assess the human safety of xenobiotics. Moderate diet restriction improves survival and reduces the variability of spontaneous tumor incidences thereby potentially increasing the sensitivity of the assay to detect weaker carcinogens. However, DR has been reported to affect the maximum tolerated dose (MTD) for carcinogenicity testing. This suggests a possible influence of DR on hepatic biotransformation enzyme activity. The purpose of the present study was to define the influence of moderate diet restriction, specifically 75% of ad libitum control, on basal and induced levels of mRNA for the major liver cytochrome P450 (CYP450) isoforms and UDPGT in 5-7 week old male and female rats. After four weeks, mean body weights with DR were 19% (male) or 15% (female) less than respective ad libitum controls, whereas mean relative liver weights between the two groups were similar. The mean basal relative mRNA expression levels of the CYP1A1 and UDPGT enzymes were elevated in the DR group as compared to controls. In male, but not in female, DR rats, 3MC was found to induce a lower mean level of CYP1A1 mRNA expression as compared to controls. Differences in induced mRNA expression levels of the CYP3A1 isoform were also observed between DR and control rats of both sexes. Evaluations of other liver CYP450 isoforms did not reveal meaningful differences between ad libitum and DR rats. This data suggests that differences may exist in the basal and xenobiotic-induced levels of hepatic biotransformation enzymes between ad libitum and DR rats.

439 CYTOTOXICITY OF HCFC-123 IN ISOLATED RAT HEPATOCYTES.

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The hydrochlorofluorocarbon 1,1-dichloro-2,2,2-trifluoroethane (HCFC-123), a close structural analogue of the hepatotoxic anaesthetic halothane, is bioactivated by microsomal cytochrome P450 (P450) to reactive metabolites both oxidatively1 and reductively2. Since more recent work in our laboratory has shown that HCFC-123 is also cytotoxic to isolated rat hepatocytes, we investigated the mechanism of HCFC-123 cytotoxicity and the role played in it by P450. Hepatocytes from Wistar male rats were incubated, both under aerobic and hypoxic (4% O2) conditions, with HCFC-123 (1-10 mM). A time- and dose-dependent increase in LDH leakage was observed, which reached its maximal level after 60 min with 10 mM HCFC-123. At the same time, a dose-dependent depletion of total cellular glutathione was observed. The intensity of these effects is apparently not related to O2 levels. LDH leakage was prevented by the free radical scavenger PBN (30 mM) under both aerobic and hypoxic conditions, whereas glutathione depletion was partially reduced by PBN under hypoxic but not aerobic conditions. A complete protection against cytotoxicity was provided also by the aspecific P450 inhibitor metyrapone (100 M), and a partial protection was observed in presence of the P450 2E1 inhibitor 4-metylpyrazole (500 M) or the P450 3A2 inhibitor troleandomycin (100 M), under aerobic condititons. No effect of these inhibitors was observed on glutathione depletion. These results indicate that HCFC-123 hepatocytotoxicity is dependent on its activation to reactive metabolites by P450 2E1 and 3A2, and that, as observed for the microsomal system, free radical intermediates may be also involved in cell damage.

1. Harris et al (1991) *Proc Natl Acad Sci USA*, 88: 1407-1410.
2. Ferrara et al (1997) *Toxicol Appl Pharmacol*, 143: 420-428.

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440 USE OF RAT ANTI-CYTOCHROME P450 ANTIBODIES TO DETECT THE PRESENCE OF DOG LIVER CYTOCHROME P450 ISOZYMES.

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Cytochromes P450 are important enzyme systems for Phase I metabolism of xenobiotics and the biotransformation of endogenous substrates. Many forms of cytochrome P450 have been isolated from various species. The most well-studied species include rats and rabbits. However, much less is known about the cytochromes P450 of the dog despite its use in preclinical toxicology studies. Recently in the literature the isolation and purification of various dog cytochrome isoforms have been described. In addition, the production of polyclonal antisera against dog cytochrome P450 isozymes has been described but as yet these antibodies are not commercially available. In our laboratories, we use a Western blot assay to quantitate the levels of various cytochrome P450 proteins in microsomal preparations after treatment of an animal with a given drug. This paper describes the use of commercially available rat cytochrome P450 antibodies to detect the presence of various cytochrome P450 isoforms in dog liver microsomal preparations. The dog microsomal proteins were first separated using SDS-PAGE, followed by blot transfer and subsequent immunoblotting. Antibodies against rat cytochromes 1A1, 2B1, 2C6, 2E1, 3A2 and 4A1 were used to assess the ability of rat antibodies to recognize the corresponding dog cytochrome P450 isozymes. Rat microsomes with induced levels of the target cytochrome P450 isozymes were used as positive controls. All of the rat antibodies tested recognized dog proteins of the same molecular weight as the rat positive controls. We conclude that commercially available rat antibodies can be used to detect the presence of a particular cytochrome P450 isozyme in dog liver microsomes.

441 CYTOCHROME P-450 INDUCTION IN PRECISION-CUT RAT LIVER SLICES EXPOSED TO 5-LIPOXYGENASE (5-LO) INHIBITORS IN DYNAMIC ORGAN CUTURE.

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Hepatomegaly and induction of cytochrome P-450 (CYP) enzymes have been reported in rats exposed to the N-hydroxyurea-containing 5-LO inhibitors CJ-11,802 and zileuton (Beierschmitt *et al.*, 1998). The present study investigated the potential of these and tetrahydropyran-containing 5-LO inhibitors have to modulate CYP activities in precision-cut rat liver slices maintained for 96 hr in dynamic organ culture. Liver slices were exposed to medium containing zileuton (0, 5, 50, 100, or 300 μM), CJ-11,802 (0, 5, 50, or 100 μM), CJ-12,918 (0, 5, 20, 50, or 100 μM), or CJ-13,454 (0, 5, 20, 50, or 100 μM). Liver slice viability, as assessed by maintenance of intracellular K^+, was maintained under these conditions except for 50 and 100 μM exposures to CJ-12,918 and CJ-13,454. The O-deethylation of ethoxyresorufin (EROD), methoxyresorufin (MROD), and pentoxyresorufin (PROD) were assessed at 96 hr in liver slice microsomes as markers of CYP1A1, CYP1A2, and CYP2B activities, respectively. CJ-11,802 (50 μM) was the most potent inducer *in vitro* producing a significant ($p<0.05$) concentration-related increase in EROD (80-fold), MROD (12-fold), and PROD (5-fold). Zileuton (300 μM) increased EROD (10-fold), MROD (5-fold), and PROD (1-fold), while CJ-13,454 (20 μM) and CJ-12,918 (20 μM) produced little or no change in CYP. The modulation of CYP1A1 *in vitro* by these 5-LO inhibitors followed their rank order of potency *in vivo*. CJ-11,802 (200 mg/kg/d) and zileuton (300 mg/kg/d) induced EROD 26- and 4-fold, respectively while CJ-12,918 and CJ-13,454 (250 mg/kg/d) had little activity (<2-fold). Although CJ-11,802 and zileuton both induced PROD *in vivo* they did not show the same correlation *in vitro*. These studies show that the rank order potency of 5-LO inhibitors to induce CYP1A1 *in vivo* is preserved *in vitro* and further illustrate the use of precision-cut liver slices to assess the potential of pharmaceutical drug candidates to modulate liver CYP expression.

442 EFFECT OF ORGANIC SOLVENTS ON *IN VITRO* cDNA-EXPRESSED HUMAN CYTOCHROME P450 ACTIVITIES.

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Organic solvents are typically utilized to add lipophillic chemicals to *in vitro* incubations. The effects of methanol, ethanol, DMSO and acetonitrile were studied *in vitro* on the catalytic activities of nine individual, cDNA-expressed cytochromes P450: CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4, in commercially available microsomes. These data show that specific solvents have enzyme-selective effects on P450 activities. Methanol did not substantially inhibit (≤10%) any of the activities at 0.3%, but did inhibit CYP1A1, CYP2B6, CYP2C9, and CYP2D6 by 12-26% at 1%. In contrast, 0.1% ethanol inhibited CYP1A1, CYP2B6, and CYP2C19 by 20-30%. Ethanol at 1% did not inhibit CYP1A2, CYP3A4, CYP2C8, and CYP2C9. DMSO inhibited CYP3A4, CYP2C19, and CYP2D6 by 15-25% at 0.1%. However, DMSO

had little effect on CYP1A2, CYP2A6, and CYP2C8. Acetonitrile, like methanol, did not inhibit any P450 activity at 0.3% solvent except for CYP1A1 (26%) and CYP2B6 (13%). At 1%, acetonitrile decreased activities of these enzymes by 40-60%, and inhibited CYP2A6, CYP3A4, CYP2C19, and CYP2D6 activity by 10-20%. Acetonitrile also increased CYP2C9 activity by 10-15% above control values at 1-3% solvent. Excluding solubility considerations, methanol and acetonitrile appear to be the most suitable solvents for the introduction of substances to cytochrome P450 incubations for *in vitro* toxicology or metabolism studies.

443 THE EFFECT OF LEPTIN ON CYTOCHROME P450, CONJUGATION, AND ANTIOXIDANT ENZYMES IN THE OB/OB MOUSE.

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Leptin is a hormone that is secreted by adipocytes and regulates body weight through it's effect on satiety and energy metabolism. The ob/ob mouse is deficient in this protein and is characterized by obesity and other metabolic disorders. This study investigated the alterations of several hepatic cytochrome P450-related, conjugation and antioxidant enzymes in lean and ob/ob mice and the role leptin may play in the modulation of these enzymes. Lean and ob/ob male mice were injected with leptin (100μg) or PBS (100μl) for 15 days. Mice were euthanized and livers removed and processed to assess the various enzyme activities. Liver microsomes from ob/ob mice, when compared to lean controls, displayed significantly lower total hepatic cytochrome P450 levels (22%) and chlorzoxazone 6-hydroxylation activity (27%); however, 2β- 7α- and 16α- testosterone hydroxylation and pentoxyresorufin *O*-dealkylation activities were significantly higher (34%, 47%, 22% and 39%, respectively). Leptin administration corrected alterations seen with all activities except 2β-testosterone hydroxylation and total hepatic P450 levels. Dealkylation of ethoxyresorufin (EROD) and ω-hydroxylation of lauric acid activities from ob/ob and lean mice were not statistically different; however, leptin exposure significantly increased EROD activity in lean mice (14%) and decreased the activity in ob/ob mice (36%). UDP-glucuronosyltransferase and glutathione S-transferase activities were not altered with obesity or leptin exposure. The antioxidant enzymes, catalase (11%) and glutathione peroxidase (26%), as well as glutathione reductase (17%) were lower in the ob/ob mice and leptin treatment corrected these alterations. Total glutathione levels were not affected by obesity or leptin treatment. The results of this study demonstrate alterations in constitutive expression of numerous xenobiotic metabolizing enzymes in ob/ob mice which were restored to lean control values following leptin treatment.

444 MOLECULAR CLONING, HETEROLOGOUS EXPRESSION, ENZYMATIC CHARACTERIZATION AND TISSUE DISTRIBUTION OF A NOVEL MOUSE CYTOCHROME P450.

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A cDNA encoding a new cytochrome P450 designated CYP2J9 was isolated from a mouse brain library. Sequence analysis reveals that this 1,958-base pair cDNA has an open reading frame and encodes a 502-amino acid protein that contains the highly conserved heme binding peptide. Based on the deduced amino acid sequence, this protein shares less than 31% identity with members of the CYP1, CYP3 and CYP4 families, 40-49% identity with other members of the CYP2 family, and 70-91% identity with other CYP2J subfamily P450s. Reverse-transcriptase polymerase chain reaction analysis of total RNA prepared from various mouse tissues using CYP2J9 sequence-specific primer pairs shows that CYP2J9 transcripts are present in brain, kidney, liver, intestine, heart, lung, and ovary. Northern analysis using the full length CYP2J9 cDNA probe shows that CYP2J9 mRNAs are most abundant in brain and kidney. Western blots using a polyclonal antibody prepared against a CYP2J9-specific peptide demonstrates abundant CYP2J9 protein in microsomal fractions prepared from brain and kidney. Recombinant CYP2J9 protein was co-expressed with NADPH-cytochrome P450 oxidoreductase (CYPOR) in *Sf9* insect cells using a baculovirus expression system. Microsomal fractions prepared from CYP2J9-CYPOR transfected insect cells metabolize arachidonic acid primarily to 19-HETE as the principal reaction product. We conclude, based on this data, that CYP2J9 is an enzymologically distinct mouse P450 that is expressed primarily in extrahepatic tissues. We speculate that CYP2J9 products play important functional roles in the brain and kidney. (Supported by the Division of Intramural Research, NIH/NIEHS.)

445 INDUCTION OF CYP2K1 EXPRESSION IN STARVED MUMMICHOG: A PROBLEM FOR USE AS A BIOMARKER OF PEROXISOME PROLIFERATION?

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In addition to peroxisome proliferation, peroxisome proliferating agents (PPAs) are known to induce the expression of lauric acid hydroxylase (LA-OHase) cytochromes P450. In mammals, LA-OHase expression can also be regulated by endogenous fatty acids and ketotic states such as starvation and diabetes. In fish, induction of LA-OHase by starvation could be problematic for using induction as a biomarker of PPA exposure and effect. The purpose of this study was to assess the effect of starvation on fish LA-OHase (CYP2K1) expression. The effect of dimethylsulfoxide (DMSO), a vehicle for waterborne PPA exposure, was also investigated. Wild-caught, female mummichog (*Fundulus heteroclitus*) were acclimated in the laboratory for at least two weeks prior to the start of the experiment. Treatments (9 fish each; n=3) consisted of non-treated/fed, non-treated/not fed, DMSO/fed and DMSO/not fed. After 4 days, microsomes were prepared and frozen at –80°C until used for SDS-PAGE and immunodetection using trout anti-LMC2 (CYP2K1; gift of D. R. Buhler, Oregon State University). Starvation for 4 days resulted in significant ($P<0.001$) induction of CYP2K1 that was approximately 4-fold higher than induction ($P<0.01$) in the presence of DMSO. There was no difference between the non-treated/fed and DMSO/fed groups suggesting that DMSO has no effect on basal CYP2K1 expression. The results indicate that DMSO may decrease induction and that CYP2K1 is induced by starvation. Caution is suggested when using CYP2K1 expression as a biomarker of PPA exposure and effect.

446 METABOLISM OF PHENANTHRENE BY HOUSE FLY CYP6D1 AND DOG MICROSOMES.

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Polycyclic aromatic hydrocarbons (PAHs) consist of a ubiquitous class of environmental contaminants and phenanthrene is a model PAH. A novel fluorometric method for measuring phenanthrene metabolism *in vitro* was developed and verified with direct measurement of [^{14}C]phenanthrene using mammalian microsomes. The fluorometric assay and direct measurement of [^{14}C]phenanthrene metabolism were used to show that CYP6D1, a house fly cytochrome P450, is the major house fly P450 involved in phenanthrene metabolism. Phenanthrene was metabolized by microsomes from the LPR house fly strain that over-expresses CYP6D1, but metabolism was not observed in the CS strain that has a lower level of CYP6D1. Furthermore, phenanthrene metabolism was inhibited completely by a CYP6D1 specific antibody. This study increases the number of known substrates of CYP6D1, identifies PAHs as potential substrates of CYP6D1, and enhances the utility of CYP6D1 as an agent in bioremediation.

447 ACETAMINOPHEN HEPATOTOXICITY CAUSED BY SHORT-TERM TREATMENT WITH ETHANOL PLUS ISOPENTANOL: PROTECTION BY TRIACETYL-OLEANDOMYCIN.

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We have previously reported that pretreatment of rats with ethanol plus isopentanol for 7 days resulted in a synergistic increase in acetaminophen (APAP) hepatotoxicity compared to pretreatment with ethanol alone. We have now found that combined treatment with ethanol and isopentanol triggered APAP hepatotoxicity at a very low dose (0.25 g APAP/kg) even when rats were pretreated for only 3 days with this alcohol combination. When rats were pre-exposed to a mixture of isopentanol with 6.3 % ethanol for 3 days, a dose of isopentanol as low as 0.1 % was sufficient to cause an increase in APAP hepatotoxicity. Treatment of rats with triacetyloleandomycin (TAO, 500 mg/kg), which forms a metabolic intermediate complex with CYP3A, 2 h before administration of APAP protected against the development of liver

damage, as seen histologically and by decreases in serum levels of ALT. CYP3A, measured immunohistochemically, appeared to be destroyed by APAP and, as expected, protected by TAO. Our findings suggest that CYP3A has a major role in alcohol-mediated increases in APAP hepatotoxicity and that alcoholic beverages rich in isopentanol may be a risk for developing liver damage from APAP. (Supported by the Dept. of Veterans Affairs.)

448 MULTIPLE FACTORS REGULATE PROMOTER ACTIVITY OF THE NADPH CYTOCHROME P-450 OXIDOREDUCTASE GENE.

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The mixed function oxidase system is composed of NADPH-cytochrome P-450 oxidoreductase (P450R) and the cytochromes P-450 (CYPs). Unlike the CYP gene superfamily, P450R is encoded by a single gene with a TATA-less, GC-rich promoter containing multiple Sp1 consensus sites, similar to many housekeeping genes. Transient transfection analyses demonstrated that basal expression of the P450R gene was dependent on multiple cis-acting elements. Transcription factor binding sites located within the minimal promoter include two upstream Sp1 motifs, a CACCC element containing overlapping Sp1/Egr-1/CACCC box motifs, and a site designated the Oxidoreductase Upstream element (ORU). More than one Sp1 site was necessary for maximal promoter function, although in longer constructs containing upstream motifs, distal sites were able to compensate for loss of proximal sites. Analysis of the CACCC region revealed that mutation of the Sp1 motif produced little change in promoter function, whereas mutation of the Egr-1 site resulted in ~90% drop in activity, indicating a factor other than Sp1 stimulated transcription under basal conditions. Finally, mutation of the bases within ORU demonstrated that this element played an essential role in promoter function. The P450R promoter thus differs from a number of TATA-less promoters; Sp1 was not the primary regulator, rather, the CACCC and ORU elements were critical for basal activity. Furthermore, although multiple sites were necessary, Sp1 enhancer activity was independent of position. (Supported by Grants CA22484 and CA0920.)

449 INFLAMMATORY CYTOKINE-MEDIATED DOWN REGULATION OF CYTOCHROME P450 BY POKEWEED MITOGEN IN MICE.

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Inhibition of cytochrome P450 (P450)-mediated metabolism by immunostimulation may involve one or more mediators. Inflammatory cytokines and nitric oxide were suggested as mediators of down-regulation of P450 during immune stimuli. These cytokines are synthesized and released by activated mononuclear phagocytes from various sources. Therefore liver macrophages (Kupffer cells), via their cytokines, may affect P450. We demonstrated previously that suppressive effects of B and T lymphocyte mitogens on the activities of several isozymes of mouse hepatic P450s might be different and B cell mitogens such as lipopolysaccharides and pokeweed mitogen (PWM) selectively depress the expression of P450. In the present study, we investigate the role of Kupffer cells and inflammatory cytokines in PWM-induced down-regulation of the microsomal activities of P450 in mice. Treatment of PWM significantly reduced hepatic microsomal total P450 contents and activities of dealkylation of ethoxyresorufin, methoxyresorufin, benzyloxyresorufin, and aniline hydroxylation, a representative activity of P4501A1, P4501A2, P4502B1/2, and P4502E1, respectively. However, when the mice treated with gadolineum chloride, the inhibitor of Kupffer cells function, P450 activities were less suppressed by PWM alone. In this investigation, we also examine the expression profiles of inflammatory cytokines in the liver, after following the treatment of PWM. The reduced these microsomal monooxygenase activities in PWM-treated mice were accompanied by increase of transcripts for IL-1α , IL-1β , IL-6 and TNF-α in RT-PCR analysis. These results show that the inflammatory cytokines that are induced by PWM-activated Kupffer cells do play an important role in the PWM-induced down-regulation of P450. (Supported by KOSEF Grant 961-0505-044-2.)

450 EXPRESSION OF DRUG METABOLISM ENZYMES IN HUMAN RENAL PROXIMAL TUBULAR CELLS.

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Expression and activity of several GSH-dependent enzymes and cytochrome P450 (CYP) monooxygenases in freshly isolated and primary cultures of human proximal tubular (hPT) cells were determined to assess the drug metabolism capabilities of these cells and to validate their use as *in vitro* models. hPT cells were isolated from human renal cortical slices by enzymatic dissociation from minced cortical tissue, filtration to remove tissue fragments, and then low-speed centrifugation to pellet hPT cells. In freshly isolated hPT cells, γ-glutamyltransferase activity, a marker enzyme for PT cells, was 18.8 – 3.3 mU/mg. Enzymatic activities were also assessed for γ-glutamylcysteine synthetase, glutathione reductase, glutathione peroxidase, and glutathione S-transferase (GST). Previous studies showed that CYP4A11, CYP4F2, and CYP3A5, but not CYP2E1, CYP2A6, CYP2C8, CYP2C9, CYP2C19, or CYP3A4, are expressed in human kidney microsomes. Western blot analysis of hPT cell lysates demonstrated the presence of CYP4A11, GSTA, and GSTP, but not CYP2E1 or GSTM. CYP4A11 activity in freshly isolated hPT cells, as determined by rates of lauric acid hydroxylation, was 0.474 – 0.053 nmol/min per mg protein. Primary cultures of hPT cells maintained expression of GSH-dependent enzyme activities and cytokeratin for 7 days. After several passages, however, some GSH-dependent enzyme activities were lost. Primary cultures of hPT cells maintained expression of CYP4A11 for up to 4 days in primary culture and preliminary studies suggested that exposure of cultures to ethanol (50 mM) for 48 hr induced CYP4A11 expression. These results provide the first accounting of drug-metabolizing capability of both freshly isolated and cultured hPT cells. Furthermore, this is the first demonstration of CYP4A11 expression in freshly isolated or cultured hPT cells.

451 CYTOCHROME P450 INDUCTION BY p,p'-DDE IN THE DEER MOUSE, VOLE AND LABORATORY RAT.

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It has been previously reported that 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (*p,p'*-DDE) induces cyp2B activity but not cyp1A1 activity in the laboratory rat (*Rattus rattus*) (Nims et al, 1998). We have observed significant increases in benzyl-resorufin-O-dealkylase (BROD), ethoxyresorufin-O-dealkylase (EROD), and methoxyresorufin-O-dealkylase (MROD) but not pentoxyresorufin-O-dealkylase (PROD) activity in deer mouse dosed *per os* with p,p'-DDE. In this study, we dosed both prairies voles (Microtus) and deer mice (*Peromyscus maniculatus*) with five environmentally relevant doses of p,p'-DDE. Hepatic microsomes were prepared from each animal, solubilized, and run on SDS-PAGE. These gels were transferred to nitrocellulose, blocked and sequentially probed with goat anti-rat P450 1A1 and goat anti-rat 2B. Anti-goat antibodies labeled with alkaline phosphatase were used as secondary antibodies in conjunction with a photoluminescence substrate to visualize the blots. These blots showed that p,p'-DDE induced only cyp1A1 protein in the deer mouse but both cyp1A1 and cyp2B (predominantly) in the vole. Immuno-inhibition studies showed that EROD, MROD and BROD activities were inhibited by anti-cyp1A1in the deer mouse but anti-cyp2B was ineffective at neutralizing these activities. In the vole, anti-cyp1A1 inhibited EROD activity but not PROD or BROD activities. Anti-cyp2B inhibited PROD and BROD but not EROD activity in the vole. These studies indicate that conclusions about isozyme induction cannot be based solely upon substrate activity.

452 DIFFERENTIAL EXPRESSION OF CYTOCHROMES P450 1A1 AND 1B1 IN HUMAN LUNG FROM SMOKERS, NON-SMOKERS AND EX-SMOKERS.

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Cytochromes P450 1A1 and 1B1 are known to bioactivate procarcinogens such as polycyclic aromatic hydrocarbons (PAH) found in cigarette smoke, and are known to be inducible via an Ah-receptor mediated mechanism. The

aim of this study was to examine levels of P4501A1 and P4501B1 expression in human lung samples from smokers, non-smokers and ex-smokers. Using highly specific polyclonal IgG and immunoblot analysis of microsomes from lung tissues, we determined the specific content for P4501A1 and 1B1. P4501A1 was determined to have mean levels of 53.3 pmols/mg microsomal protein (n=12) in smokers, 7.5 pmols/mg microsomal protein (n=4) in non-smokers, and 32.9 pmols/mg microsomal protein (n=6) in ex-smokers. P4501B1 was determined to have mean levels of 4.2 pmols/mg microsomal protein (n=12) in smokers, 1.4 pmols/mg microsomal protein (n=4) in non-smokers, and 4.3 pmols/mg microsomal protein (n=6) in ex-smokers. These results indicate that both P4501A1 and P4501B1 are at higher levels of expression in lung tissues from smokers and ex-smokers when compared with non-smokers. In conclusion, induced expression levels for P4501A1 and P4501B1 may be due to smoking and exposure to PAHs present in cigarette smoke, such that smokers may be more susceptible to chemical carcinogenesis. Additional studies are underway to determine the variance, activity and cellular localization of these proteins.

453 CYTOCHROME P450 1A1 AND 2B1 IN THE RAT LUNG: EXPOSURE TO SILICA AND INDUCERS OF XENOBIOTIC METABOLISM.

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Pulmonary exposure to respirable silica causes alveolar type 2 cell proliferation. Cytochrome P450 (CYP) is present in type 2 cells and increased levels of CYP1A1 are reported in silicotic rats. Because carcinogen activation by CYP1A1 may contribute to human lung cancer and because silica is often present in occupational mixed dust exposures, we hypothesized that new type 2 cells in silicosis are potential sites of CYP1A1 induction and carcinogen activation. Male, Sprague-Dawley rats received intratracheal silica (20mg) or vehicle (saline). The silicotic and control rats were then exposed to inducers of xenobiotic metabolism by the intraperitoneal injection of phenobarbital (PB), β-naphthoflavone (NF), NF and PB, or vehicle (corn oil). 7-ethoxyresorufin (ER)-O-deethylase (EROD) and 7-ethoxycoumarin (EC)-O-deethylase (ECOD) activities were increased by NF but unaffected by PB. Morphometrically, the number of cells expressing immunoreactive CYP2B1 was not significantly affected by silica, NF, and/or PB. CYP 1A1 positive airway and alveolar epithelial cells were absent in controls, present after NF exposure but not significantly increased by PB or silica. Although we had hypothesized that induction of type 2 cells would lead to more xenobiotically active cells in silicotic rats, NF-exposed silicotic rats had significantly less EROD activity than NF-exposed control rats. We conclude that the new type 2 cells of silicosis were not a major site of 1A1 induction in combined exposures in this rat model.

454 REGULATION OF CYP1A EXPRESSION BY NICOTINE IN THE RAT: DOSE- AND ROUTE OF EXPOSURE-DEPENDENCE.

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The induction of pulmonary, renal and hepatic CYP1A expression by the major tobacco constituent nicotine was examined in rats administered either a single subcutaneous (SC, 2.5 mg/kg) dose, a vapor (VP, 200 μ g/m^3) dose or a 30-day, low (20 mg/kg), medium (60 mg/kg) or high (200 mg/kg) dietary dose of the alkaloid. SC nicotine caused a lung-specific but transient upregulation of CYP1A1 mRNA, protein and catalytic activity, as did VP nicotine, with the induction peaking (15-fold) 12 h post exposure. Dietary nicotine caused a dose-dependent induction of pulmonary, renal and hepatic CYP1A1 and of hepatic and renal CYP1A2. The hepatic CYP1A1 induction was not accompanied by elevated mRNA abundance and occurred only at the high nicotine dose, at which the pulmonary and renal induction was 21-fold and 55-fold, respectively. Nicotine bound weakly to the Ah receptor in a gel shift assay, suggesting some involvement of transcriptional mechanisms in the induction. Nicotine-fed rats had plasma levels of nicotine and cotinine comparable to those reported in heavy smokers. However, administered cotinine did not induce CYP1A1. The findings suggest that CYP1A induction by cigarette smoke may be contributed by nicotine. (Supported by ES06414 and DK43135.)

455 COMPARISON OF LOW-OXYGEN MEDIATED CYP1A1 INDUCTION IN 3 HEPATOMA CELL LINES.

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Hepatoma cell lines are useful *in vitro* models for understanding liver metabolism of xenobiotics. However, when standard cell-culture incubation conditions (5%CO2/95% air) are used, oxygen concentrations are generally higher than the physio-logical range. This may have an impact on the responsiveness of cells and general applicability for toxicological testing. When oxygen was lowered to 10%, we found that 3-methyl-cholanthrene (3-MC)-mediated induction of ethoxyresorufin O-dealkylase (EROD) activity in human HepG2 cells was significantly enhanced (14-fold). Immunoprecipitation studies using specific antibodies showed that the low-oxygen effect was mediated primarily by the CYP1A1 isoform. Interestingly, the specific CYP1A1-specific inducer, 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) did not show a similar enhancement in EROD activity, but may have been more acutely toxic to the cells. The low-oxygen effect was also tested in hepatoma cell lines derived from mouse (1c1c7) and rat (H4IIE). The mouse cell line showed a similar response but it was much less pronounced than in the human HepG2 cell line. The rat-derived cell line was totally unresponsive. These results indicate that physiological oxygen tension may be an important regulator of inducer-mediated drug metabolism in cultured hepatoma cell lines; however, the response is inducer- and species-specific.

456 INDUCTION OF CYP1A1, CYP1A2, AND CYP1B1 IN PRECISION-CUT HUMAN LIVER SLICES BY 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN AND OMEPRAZOLE.

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Precision-cut human liver slices incubated in dynamic organ culture were utilized as an *in vitro* model to investigate the ability of the environmental pollutant TCDD and the clinically relevant proton pump inhibitor omeprazole to modulate the cytochrome P450s 1A1 (CYP1A1, 1A2 (CYP1A2), and 1B1 (CYP1B1). These cytochrome P450s have been specifically implicated in the metabolic activation of compounds, such as the polycyclic aromatic hydrocarbons and heterocyclic aromatic amimes, into carcinogenic species. Liver slices were incubated in dynamic organ culture for up to 96 hours in Waymouth's medium (supplemented with 25 mM HEPES, 25 mM glucose, 5% horse serum, 5% fetal calf serum, and penicillin and streptomycin) containing either 10 nM TCDD (initial 24 hr), 50 μM omeprazole, or vehicle alone (0.1% DMSO). The slices remained viable throughout the incubation period maintaining an intracellular potassium content of 40 μmoles/g liver or greater. The levels of CYP1A1, CYP1A2, and CYP1B1 proteins and corresponding activities were measured in microsomal preparations from the human liver slices and the levels of CYP1A1 and CYP1B1 mRNA were quantitated by reverse transcriptase-polymerase chain reaction. Induction of CYP1A1, CYP1A2, and CYP1B1 was observed following exposure to either TCDD or omeprazole. Interestingly, variability in responsiveness was observed between different individual liver specimens. For example, the fold induction of CYP1B1 mRNA following exposure to 50 μM omeprazole for 48 hours ranged from ≈5 to 18, compared to control. Likewise, the same liver specimens displayed a ≈7 to 41 fold induction of CYP1A1 mRNA following omeprazole (50 μM) treatment for 48 hours, compared to control. The present study demonstrates the utility of precision-cut human liver slices as *in vitro* model for studying the modulation of cytochrome P450s by xenobiotics. Furthermore, the ability of omeprazole to induce the recently identified CYP1B1 is a novel observation which warrants further investigation.

457 SEA OTTER (*ENHYDRA LUTRIS*) CYTOCHROME P450 1A GENE EXPRESSION IN PERIPHERAL BLOOD MONO-NUCLEAR CELLS AS A BIOMARKER OF EXPOSURE TO CRUDE OIL.

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The nearshore ecosystem of the Prince William Sound (PWS) serves as a

repository for the spilled oil from the *Exxon Valdez* tanker. Responses associated with exposure to residual oil may include transcriptional activation of mRNA for cytochrome P450 1A. Here, we report on the isolation, cloning, and sequencing of a PCR product for sea otter (*Enhydra lutris*) cytochrome P450 1A. Additionally, we developed a quantitative RT-PCR technique to quantify the level of P450 1A expression in sea otter peripheral blood mononuclear cells (PBMC). Using this quantitaive RT-PCR method with PBMC isolated from sea otters captured in oiled (n=20) and non-oiled (n=21) areas of the PWS, we detected a significant difference ($p < .001$) in the level of P450 1A expression between the two study areas. The mean level of expression in PBMC from sea otters (molecules/100 ng total RNA) was 1.96×10^6 for the oiled area and 0.12×10^6 for the non-oiled area. Analysis of the expression of P450s in circulating mononuclear cells by RT-PCR represents a sensitive and non-lethal method for evaluating potential exposure to environmental contaminants.

458 INHIBITION OF CYP1A1 ENZYME ACTIVITY IN MOUSE HEPATOMA CELL CULTURE BY SOYBEAN ISOFLAVONES.

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These studies investigated the mechanisms by which soybean- and soybean isoflavone-enriched diets inhibit carcinogenesis. We found that genistin and daidzin, and their respective aglucone forms daidzein and genistein, block the TCDD-inducible activity of CYP1A1 and prevent CYP1A1-mediated covalent binding of benzo[*a*]pyrene (BaP) metabolites to DNA. Although daidzein and genistein appear structurally similar to known aromatic hydrocarbon receptor (AHR) agonists and antagonists, gel mobility shift assays indicated that the isoflavones do not inhibit dioxin-induced activation of the AHR or the accumulation of CYP1A1 mRNA, suggesting that the isoflavones do not act at the transcriptional level. We therefore evaluated the isoflavones for direct effects on the CYP1A1 enzyme. Daidzein and genistein are noncompetitive with the CYP1A1 substrate BaP for microsomal BaP hydroxylation, with apparent K_i values of 325 μM and 140 μM, respectively. The extent of CYP1A1 inhibition increases with time of preincubation at 37°C, but not at 4°C, in the presence of isoflavone plus NADPH; after 60 min preincubation the inhibition remains noncompetitive, with apparent K_i values of 55 μM and 50 μM, respectively. Inhibition is neither prevented nor reversed by the thiol antioxidant dithiothreitol, nor by the iron chelator deferoxamine. Repeated washing of the microsomes does not reverse the inhibition. These results suggest that the isoflavones undergo NADPH-dependent formation of metabolites that inhibit CYP1A1 activity, contributing to the mechanism by which these soybean isoflavones protect against carcinogenesis. (Supported by NIH Grants P30-ES06096, RO1-AG09235, RO1-ES06811 and RO1-ES06273.)

459 ETHANOL-MEDIATED INDUCTION OF CYP1A1/2 IN RAT SKELETAL MUSCLE.

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This study tested the hypothesis that feeding rats an ethanol-containing diet would induce CYP2E1 and/or CYP1A1/2 in skeletal muscle tissue; these cytochrome P450 enzymes were also analyzed in liver, as a positive control tissue. Enzymatic assays, *p*-nitrophenol (PNP, for CYP2E1) and ethoxyresorufin O-deethylase (EROD, for CYP1A1/2), as well as Western blot analysis, demonstrated that a liquid diet containing 6% ethanol and delivered via a feeding system using glass bottles and rubber stoppers with a stainless steel tube (Feeding System 1) induced CYP2E1 and CYP1A1/2 in liver tissue. Immunocytochemistry studies confirmed induction of these liver enzymes by the ethanol-containing diet. Immunohistochemical analysis of skeletal muscle (soleus, plantaris and diaphragm) indicated ethanol-containing diets administered by Feeding System 1 induced CYP1A1/2, and immunohistochemical staining was predominantly localized to capillaries surrounding myofibers. However, antibodies to CYP2E1 did not detectably react with skeletal muscle tissue from animals receiving a control or ethanol-containing diet. Identical results were obtained when highly purified ethanol was prepared and administered in the diets. However, when liquid diets were administered via Feeding System 2 (all-glass feeding system), CYP1A1/2 was not induced by ethanol-containing diets in liver or in skeletal muscle tissue; CYP2E1 was induced in liver by Feeding System 2. The results indicate that feeding ethanol can mediate induction of CYP1A1/2 in liver and in capillaries of skeletal muscle; however, this induction was not attributed to ethanol *per se*, but rather to an unidentified compound(s) apparently solubilized by 6% ethanol from rubber stoppers or stainless steel tubing.

460 IN VITRO INHIBITION BY PRENYLATED FLAVONOIDS OF HUMAN CYP1A2-MEDIATED METABOLISM OF ACETANILIDE AND AFLATOXIN B_1.

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Three prenylated flavonoids, xanthohumol (XN), isoxanthohumol (IX), and 8-prenylnaringenin (8PN), have recently been isolated from hops (*Humulus lupus*) and their presence detected in beer. Studies with human breast cancer cell lines indicate possible cancer chemopreventive activity of these compounds. We examined these flavonoids for their ability to inhibit cDNA-expressed human CYP1A2 activity toward the diagnostic substrate acetanilide and the carcinogen, aflatoxin B_1 (AFB_1). At 10 μM concentration, IX and 8PN eliminated more than 90% of the acetanilide 4-hydroxylase activity of CYP1A2 compared to ETOH-treated controls. XN inhibited this P450 activity by 68%. When the inhibitory activity of the flavanone, IX, and the chalcone, XN, were compared in a dose-response study, they gave IC50 values of 4.5 and 31.4, respectively. Since the metabolism of AFB_1 is also catalyzed, at least partially, by CYP1A2, IX and 8PN were further examined for their effect on this activity. When added to incubation mixtures at 25 μM for 8PN and 100 μM for IX, both flavonoids were potent inhibitors of the conversion of AFB_1 to tris dihydrodiols and AFM_1, with virtually none of the diols and only 4-5% of the AFM_1 remaining as compared to ETOH-treated controls. Even at 5 μM, 8PN and IX inhibited production of AFM_1 by 65.3 and 38.3%. These results suggest possible use of these flavonoids as chemopreventive agents and additionally, as diagnostic inhibitors of CYP1A2. (Supported by the Hop Research Council and NIEHS grant ES00210.)

461 CYP1A1 AND CYP1B1 IN BREAST CELL LINES.

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Eight human breast tumor cell lines differing with respect to estrogen receptor (ER) status and indicators of progression (invasiveness, chemotaxis), were assessed for expression levels of CYP1A1 and CYP1B1 and for AhR, AhR nuclear translocator protein (ARNT),. The response to TCDD was measured with respect to each of these genes. In selected lines, we measured the response to TCDD with respect to AhR nuclear translocation and activation from AhR/ARNT complexes with the transcription response element (gel mobility shift assay). Four of the lines utilized were characterized as more progressive, and differed collectively from three lines typically categorized as in the earlier stages of tumorigenesis, with respect to AhR mRNA levels and maximal induction of CYP1A1. AhR mRNA was low in early, ER-positive lines, and basally undetectable CYP1A1 induced to very high levels. In the later, ER-negative lines, AhR mRNA was at modest to high levels, while CYP1A1 was poorly induced. Basal and induced levels of CYP1B1 were also lower in the late lines. The MCF 10A line, like primary human breast epithelial cells, expressed high AhR, but CYP1A1 and CYP1B1 levels that were typical in the early lines. Expression of ARNT mRNA was inversely related with AhR mRNA. TCDD effected downregulation of AhR protein in all cells to about the same extent but did not affect AhR or ARNT mRNA levels. Although early T47D and later Hs 578T cells differ extensively in AhR activity and expression, amounts of TCDD-induced AhR/ARNT/XRE complex formed from nuclear extracts was similar. This study indicated that expression levels of these four genes provides a marker of breast tumor progression. The correlations between their expression levels suggests a coordination of AhR/ARNT expression and activity that changes from early to late stage tumor cells. (Supported by grants DOD DAMD17-94-J-4054 (CRJ), NIH 144-FR43 (CRJ), NIEHS 144EN46 (CRJ), and NRSA 1-F32-ES05733-03 (WA).)

461A MOLECULAR INDENTIFICATION OF CYTOCHROME P450 1B IN LIVER OF SCUP (*Stenotomus chrysops*) AND STRIPED DOLPHIN (*Stenella coeruleoalba*) AND CYTOCHROME P450 1A1 IN STRIPED DOLPHIN TESTIS.

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Cytochrome P450 (CYP) enzymes transform a variety of endogenous substrates and environmental contaminants. The CYP1 family (CYP1A1, CYP1A2 and CYP1B1) is the most active in oxidation of polychlorinated biphenyls and polycyclic aromatic hydrocarbons. CYP1As have been cloned from numerous species (fish, birds and mammals) while CYP1B1, prominent also in estradiol hydroxylation, has been identified only in human, rat, and mouse. We investigated the presence of CYP1B-like sequences in fish and cetaceans. RT-PCR using degenerate primers amplified a 461 base pair (bp) fragment from scup liver and a 388 bp fragment from dolphin liver. The scup sequence shares 62% amino acid (aa) identity and 65% nucleotide (nt) identity with human CYP1B1 and 58% aa and 63% nt identities with both rodent CYP1B1s. The dolphin fragment has 89% aa and 85% nt sequence identities with human CYP1B1 and 86% aa and 82% nt sequence identities with rodent CYP1B1s. Scup and dolphin CYP1Bs share 49% aa and 64% nt sequence identities. A 392 bp fragment also was amplified from dolphin testis and has 77% aa and 83% nt sequence identities with human CYP1A1. The significance of CYP1A1 expression in dolphin testis is unknown. Analysis of function and regulation of fish and cetacean CYP1Bs may indicate further the endogenous and toxicological significance of the CYP1B subfamily. (Supported by Sea Grant NA46RG0470-R/P61,EPA R829890-01, and NIH ES04696.)

462 COMBINATION ANTICONVULSANT TREATMENT OF SOMAN-INDUCED SEIZURES.

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Diazepam is the currently fielded anticonvulsant drug for treatment of nerve agent seizures in the U.S. military, but several drawbacks limit its usefulness. This has fueled the search for alternative therapeutic approaches. One approach being considered is to supplement diazepam with a second anticonvulsant drug. These studies investigated the effectiveness of combination treatment of soman-induced seizures with a benzodiazepine and an anticholinergic drug. The anticholinergic drugs considered were biperiden, scopolamine, trihexyphenidyl, and procyclidine; the benzodiazepines were diazepam and midazolam. Male guinea pigs were surgically implanted with cortical screw electrodes to monitor and record electrocorticograms. Pyridostigmine (PB; 0.026 mg/kg, i.m.) was injected as a pretreatment to inhibit erythrocyte AChE by 30 - 40%. Thirty min after PB, 2LD50s (56 μg/kg) of soman was injected s.c. followed 1 min later by i.m. treatment with atropine (2 mg/kg) + 2PAM (25 mg/kg). Electrographic seizures occurred in all animals. Anticonvulsant treatment combinations were administered i.m. at 5 or 40 min after seizure onset. Combination treatment consisted of diazepam or midazolam plus one of the above mentioned anticholinergic drugs. Doses of the treatment com- pounds were utilized that exhibited little or no antiseizure efficacy when given individually. The combination of a benzodiazepine and any of the anticholinergic drugs was effective in terminating soman-induced seizure, whether given 5 or 40 min after seizure onset. The results suggest a strong synergistic antiseizure effect of combining benzodiazepines with centrally active anticholinergic drugs and support the concept of using this approach for treatment of nerve agent-induced seizures.

463 CHOLINESTERASE ACTIVITY: MUSCARINIC RECEPTOR RATIOS IN CANINE AND FELINE BRAINS.

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In response to the findings that brain cholinesterase (ChE): muscarinic receptor density (mChR) ratio is highly conserved between bird species and is useful to diagnose acute organophosphate (OP) toxicosis in birds for which normal brain ChE is unknown (J.Wildlife Dis. 32(2),1996,216-224), our current study investigated the conservation of brain ChE:mChR between mammalian species and assessed the ratio's usefulness for diagnosis of OP toxicosis in dogs. Whole brains of 7 normal greyhounds, 13 normal non-greyhound dogs, 9 normal cats, 2 beagles with subacute (~72 h) OP toxicosis, and 1 greyhound with acute (<4 h) OP toxicosis, were assayed for ChE, mChR and protein. The same assays were made on cerebrum, cerebellum and brainstem from the greyhounds and 6 of the non-greyhound dogs. The Ellman assay was used to measure ChE, mChR were labelled with tritiated quinuclidinyl benzilate, and protein was measured by Lowry assay. The mean brain ChE :mChR (umol/min/mg protein:nmol/mg protein) for non-greyhounds was 1:16.8 (sd 5.5, n=14, range 1:9.9-1:24.8), for greyhounds 1:21.9 (sd 6.4, n=7, range 1:11.9-1:32.5), for cats 1:11.6 (sd 5.36, n=8, 1:6.2-1:20.5), for the greyhound with acute OP toxicosis 1:30.31, and for the 2 beagles with subacute toxicosis 1:68.8 and 1:76.8. Trends for cerebrum, cerebellum and brainstem were similar. Only in cerebrum was ChE:mChR in acute OP toxicosis distinctly different to that of normal greyhounds. Brain ChE:mChR was not highly conserved within or between the species. There was as great a difference in mean ChE:mChR between non-greyhounds and greyhounds as between non-greyhounds and cats. The ChE:mChR was profoundly altered in 2 cases of subacute OP toxicosis, but only slight to moderate change occurred in the acute OP toxicosis. Measurement of ChE:mChR is not a significant improvement to ChE assay alone and is unlikely to be useful in endangered felids or canids for which normal ChE levels are unknown, because it cannot be assumed that endangered species would have the same ChE:mChR as their domestic relatives.

464 EVALUATION OF THE NEUROTOXICITY OF LINDANE USING A FUNCTIONAL OBSERVATIONAL BATTERY.

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Lindane (γ -hexachlorocyclohexane) is a chlorinated hydrocarbon pesticide that produces an inhibition of the GABA-ergic neurotransmitter system. The neurotoxicity of Lindane in the rat was assessed using a Functional Observational Battery (FOB). In a 2-week preliminary study, mortality and convulsions were noted in females at the beginning of the study, whereas at the same dose-levels, no signs of neurotoxicity were observed in males. In the neurotoxicity study, male animals only were studied. Male Sprague-Dawley rats were given Lindane daily by the oral route for four weeks at 0, 30, 40 and 55 mg/kg/day in corn oil. The FOB was performed in the pre-treatment period, and in weeks 1, 2, 3 and 4 of the treatment period. During weeks 1 and 2, there were marked, dose-related signs of neurotoxicity. After this, the animals appeared to become habituated to Lindane treatment: in weeks 3 and 4 the dose-relationship was less clear and fewer animals showed signs of neurotoxicity. The principal functions altered by Lindane were autonomic functions (soiled fur, ptyalism), muscle tone and equilibrium (gait on tiptoe, round back), central nervous system activity and excitability (hyperactivity, hyperreactivity to the touch escape and to handling, convulsions), sensorimotor functions (hyperreactivity to the touch and tail pinch), and physiologic functions (lower body weight gain in treated groups compared to control group). No variation of spontaneous motor activity was recorded in treated groups using the open field apparatus. The majority of these neurotoxic effects (excepting convulsions and ptyalism, both of which were observed just after administration) could be detected and quantified using the FOB approach.

465 ASSESSMENT OF NEUROTOXICITY IN PESTICIDE-EXPOSED WORKERS USING AUTOANTIBODY (AAb) PROFILES.

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Previously, in human and animal studies, serum AAb to nervous system (NS) proteins were shown to be indicative of metal neurotoxicity. In the present study agricultural workers exposed to pesticides (n=100; 17±6 y exposure) and a reference population from a food processing plant (n=50, no direct exposure) were assessed for possible neurotoxicity using serum AAb detection against neurofilaments (NF), myelin basic protein (MBP), desmin (D), and acetylcholinesterase (AChE). Pesticide exposures included organophosphates (OPs; e.g., metamidophos), carbamates (e.g., ethiophencarb) and pyrethroids (deltamethrin). Blood AChE activity and blood lead levels (PbB) levels were also determined. AAb IgG to NF, neuronal intermediate filaments, and MBP predominated in pesticide workers. IgG to D, a muscle intermediate filament, and to AChE were detected in 70% of the pesticide-exposed group. AChE activity was 40-60% of normal (26 IU/g Hb).

PbB, determined to rule out other neurotoxicants, was 12 ± 5 μg/dl. In contrast, few of the reference population had detectable IgG levels, 40% had IgM to NF. None had either IgG or IgM to MBP, D, or AChE. IgG levels to NF correlated with exposure duration. In addition, IgG levels to NF and D correlated with peripheral sensorimotor deficits (muscle tremor and reflexes). There was some correlation with PbB in a subset of the population. The detection of AAb to NF and AChE suggests neuronal involvement, while detection of AAb to D suggests some myopathy. This is consistent with the sensorimotor deficits detected. AChE inhibition, particularly by OPs, has been shown to induce muscle damage. Some of these OPs are also known to produce a delayed neuropathy which results in AAb. This study suggests that AAb detection may be used in monitoring pesticide neurotoxicity.

466 INFLUENCE OF DITHIOCARBAMATES ON THE DEVELOPMENT OF ORGANOPHOSPHATE INDUCED DELAYED POLYNEUROPATHY (OPIDP).

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Some esterase inhibitors interfere with OPIDP development. By inhibiting neuropathy target esterase (NTE), the target of OPIDP, they protect from OPIDP when given before the neuropathic organophosphate; they promote OPIDP when given afterwards. If they do not inhibit NTE they may promote OPIDP regardless of the dosing sequence. Protection and promotion are associated with inhibition of NTE and M200 (the target of promotion), respectively. Hens were treated with dithiocarbamate herbicides molinate, thiobencarb, thiocarbazyl, or cycloate because they are metabolized to sulfones and sulfoxides. Inhibitions (mean%±SD, n=3) of NTE and M200 in the nervous system measured 24 h after dosing were: molinate (180 mg/kg sc) 84±11 and 99±1, thiobencarb (1350 mg/kg sc) 29±13 and 50±13, thiocarbazyl (1150 mg/kg sc) 17±15 and 3±7, and cycloate (1090 mg/kg sc) 42±1 and 25±10, respectively. No neuropathy developed in paired treated hens. Hens (n=5-9 per group) were treated with either diisopropyl fluorophosphate (DFP, 0.5 mg/kg sc) or dibutyldichlorovinylphosphate (DBDCVP, 0.4 mg/kg sc) followed by the above doses of a dithiocarbamate 24 h later. Molinate or thiobencarb promoted neuropathies by DFP (clinical score from 1 to 3, 0-8 point scale) and DBDCVP (clinical score from 1 to 3, molinate only tested). When given before a neuropathic dose (1 mg/kg sc) of either DFP or DBDCVP, molinate protected from OPIDP (clinical score from 4 and 2, respectively, to 0) whereas thiobencarb promoted both DFP and DBDCVP neuropathies (both scores 8). A new class of compounds has been found to interfere with OPIDP. Data are consistent with the concept that NTE is the target for initiation and protection whereas M200 is the target of promotion.

467 PROTECTION OF CEREBELLAR PURKINJE CELLS IN ORGANOPHOSPHORUS COMPOUND-INDUCED DELAYED NEUROTOXICITY (OPIDN) BY PHENYLMETHANESUL-FONYLFLUORIDE (PMSF).

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Previous work has shown that the effects of OPIDN on the adult rat cerebellum can be potentiated by posttreatment with PMSF. We sought to determine if pretreatment with a sub-potentiating dose of PMSF would protect against these effects. Groups of male Wistar rats (8-10 mo old) were pretreated with PMSF (90 mg/kg sc) followed by di-*n*-butyl-2,2-dichlorovinyl phosphate (DBCVP; 6 mg/kg sc) and 2 doses of PMSF (125 mg/kg/day sc). All treatments were separated by 24 hr intervals. Additional groups were treated with PMSF or vehicle (glycerol formal) alone in the indicated doses and intervals. 48 days following the first doses of PMSF an additional group of rats received harmaline (40 mg/kg/day ip in saline for 2 days) to serve as positive controls for vermal Purkinje cell degeneration. On day 50, all rats were perfused with 10% neutral buffered formalin and brains were processed for light microscopy. Sagittal sections of cerebellum were immunostained with a monoclonal antibody directed against calbindin to visualize Purkinje cells. Cerebellar Purkinje cell degeneration was seen only in animals treated with harmaline and was characterized by extensive loss of vermal Purkinje cell bodies and their dendritic processes. These results confirm and extend earlier work showing that pretreatment with OPIDN potentiators such as PMSF protects against the effects of this disease. Future work will include the use of more potent potentiators than PMSF to examine the mechanism of neuroprotection. (Supported by Dow AgroSciences, PHS-NIH grants ES07062, and ES60103.)

468 ACETYLCHOLINESTERASE ACTIVITY IN SPECIFIC REGIONS OF THE CANINE BRAIN.

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Suggested revisions to the federal guidelines on the evaluation of cholinesterase inhibitors indicate the need to use a more accurate enzyme assay and to determine better biological indicators of human risk. The principal biological target of cholinesterase inhibiting pesticides, of concern in risk assessment, is the enzyme present in the brain, which is primarily acetylcholinesterase (AChE). Several AChE isozymes exist in the brain, but little is known about the specificity of action of pesticides on brain regions. Enzymatic activity in brain tissue was assessed utilizing a modified Ellman procedure [US EPA, Federal Register April 26, 1996; Vol. 61:(82), 18593], which is devoid of the inaccuracies of traditional assays. To investigate the regional activity of AChE, male and female canine brains were dissected using recognized visual landmarks to obtain the cerebellum, cortex, hippocampus and caudate-putamen. AChE activity was similar between males and females, though whole brain activity was not established. AChE activity [mean (± S.E.)] in the canine cerebellum was 17.5 (± 0.9) IU/mL of homogenate, which is approximately equal to that of the whole brain of the rat. Enzymatic activity in the canine cortex and hippocampus was approximately 15% and 40%, respectively, of the cerebellum value. AChE activity in the canine caudate-putamen was approximately 480% of the cerebellum value. This work provides data characterizing regional AChE activity in canine brain, and is a necessary precursor to assessing the regional specificity of action of pesticides, which could further lead to the identification of more appropriate indicators of the potential risk of pesticides to humans.

469 ACETYLCHOLINESTERASE INHIBITION AND [^{3}H]OXO-TREMORINE BINDING IN NEONATAL AND ADULT RAT HEART FOLLOWING REPEATED ORAL CHLORPYRIFOS EXPOSURES.

M Stiles and C Pope. *Div. Toxicol., Northeast LA Univ., Monroe, LA.*

Chlorpyrifos (CPF) is a commonly-used organophosphorus pesticide (OP) whose primary mode of action is inhibition of the enzyme acetylcholinesterase (AChE, EC 3.1.1.7). It has also been reported that some muscarinic receptor subtypes (e.g., m2) may be additional direct sites of action for OPs. Several laboratories have reported marked age-related differences in sensitivity to OPs including CPF. As the heart contains primarily m2 muscarinic receptors, these putative direct interactions may be important in the age-related toxicity of OPs. We studied the comparative AChE inhibition and downregulation of muscarinic m2 receptor ([^{3}H]oxotremorine) binding in neonatal (7 days of age) and adult (90 days of age) rat heart after repeated oral CPF exposures. Neonatal (n=3-6/group) and adult (n=3/group) rats were given CPF (0, 0.15, 0.45, 0.75, 1.5, 4.5 or 7.5 mg/kg/d for 14 d) by gavage in peanut oil and sacrificed 4 hrs after the last exposure for biochemical assays. Relatively similar levels of AChE inhibition were noted in both age groups after repeated CPF exposures ($ED_{50\%}$ 1 mg/kg/d). In contrast, [^{3}H]oxotremorine binding was only reduced in adults (22-35% reduction at 1.5-7.5 mg/kg/d). While the muscarinic agonist carbachol was a potent blocker of specific [^{3}H]oxotremorine binding sites in adult cardiac membranes *in vitro*, the active anticholinesterase metabolite of CPF (CPF-oxon) only marginally affected [^{3}H]oxotremorine binding (≈ 10% reduction at 5 uM). These results suggest that cardiac muscarinic receptors in neonatal and adult rats are differentially modulated by AChE inhibition and that direct binding by the oxon to cardiac receptors is of lesser importance. Differential modulation of cardiac muscarinic receptors in neonates and adults could contribute to age-related differences in response to OPs. (Supported by U.S. EPA R 825811-01-0.)

470 COMPARATIVE EFFECTS OF PARAOXON, CHLORPYRIFOS OXON AND MUSCARINIC AGONISTS ON HIGH AFFINITY CHOLINE UPTAKE IN RAT CORTICAL AND STRIATAL SYNAPTOSOMES.

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High affinity choline uptake (HACU) is the rate limiting step in acetylcholine synthesis and has been reported to be regulated by cyclic adenosine monophosphate (cAMP) levels in the cholinergic presynaptic terminal. The muscarinic m2 and/or m4 receptor subtype is coupled to inhibition of adenylyl cyclase and some organophosphorus (OP) anticholinesterases appear capable of direct activation of these receptors in vitro. We studied the possi-

ble direct activation of synaptosomal muscarinic receptors and consequent modulation of HACU in cortex and striatum by the active anticholinesterase metabolites of the common OP pesticides parathion and chlorpyrifos (paraoxon and chlorpyrifos oxon) in comparison to the well-known muscarinic agonists carbachol, oxotremorine and *cis*-dioxolane. Neither paraoxon nor chlorpyrifos oxon (1-1,000 uM) had any effect on choline uptake in this system. In contrast, all three agonists inhibited HACU in a concentration-dependent manner, with potencies cis-dioxolane ($IC_{50\infty}$ 100 uM) > oxotremorine ($IC_{50\infty}$ 400 uM) > carbachol ($IC_{50\infty}$ 1 mM). While muscarinic agonists all inhibited HACU (presumably through inhibition of cAMP formation), addition of exogenous cAMP (8-bromo-cAMP, up to 3 mM) had no effect on choline uptake. We conclude that muscarinic receptor activation can modulate HACU in cortical and striatal synaptosomes but neither paraoxon nor chlorpyrifos oxon appear capable of directly activating this muscarinic receptor subtype. (Supported by U.S. EPA R 825811-01-0.)

471 EFFECTS OF ORAL CHLORPYRIFOS ON CORTICAL AND STRIATAL MUSCARINIC AUTORECEPTOR FUNCTION IN NEONATAL, JUVENILE AND ADULT RATS.

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Several laboratories have reported marked age-related differences in sensitivity to organophophorus pesticides including chlorpyrifos (CPF). We previously reported that muscarinic receptor-mediated inhibition of acetylcholine release (i.e., muscarinic autoreceptor function, MAF) is disrupted in a time- and OP-selective manner in adult rat striatal slices. The modulation of MAF was studied in cortical and striatal slices of neonatal (7 days of age, n=3-4), juvenile (21 days of age, n=3) and adult (90 days of age, n=3-5) rats at 1 and 4 days following oral CPF exposure. Preliminary studies determined LD_{10} values for each age group (neonate=15 mg/kg; juvenile=47 mg/kg; adult=136 mg/kg) and these respective dosages were used throughout. MAF was studied in brain slices prelabeled with [^{3}H]choline, superfused in the presence of physostigmine (10 μ M), and stimulated twice (S1 and S2) with buffer containing high potassium (15 mM), with atropine (1 μ M) added 10 min prior to the second stimulation (S2). Either an increase in S1 or a decrease in S2/S1 is an indicator of impaired MAF. There were no significant differences in release (either S1 or S2/S1 ratio) in neonates at either timepoint in either brain region. In juveniles, CPF exposure increased (185-224% of control) striatal acetylcholine release (S1) at both timepoints indicating possible impairment of MAF, but no change in the S2/S1 ratio was noted. Cortical release (S1) was only increased (172% of control) at 1d after exposure. Again, no change in S2/S1 was noted in cortex. Adults treated with CPF exhibited no change in striatal or cortical release (S1) at 1 d after exposure but release in both regions was increased (127-149% of control) at 4 d. Reductions in the S2/S1 ratio were also noted in both regions at 4 d after treatment. The results suggest that MAF may be affected in an age- and time-dependent manner by CPF and that alterations in acetylcholine release could contribute to age-related differences in response to CPF. (Supported by U.S. EPA R 825811-01-0.)

472 DIFFERENTIAL ALTERATIONS IN CORTICAL NICOTINIC RECEPTOR-MEDIATED ACETYLCHOLINE (ACh) RELEASE FOLLOWING *IN VIVO* AND *IN VITRO* ORGANOPHOSPHATE (OP) EXPOSURE.

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Parathion (PS) and chlorpyrifos (CPF), two structurally-similar OP insecticides, have been previously reported to produce differing degrees of toxicity in the presence of similar degrees of acetylcholinesterase (AChE) inhibition, suggesting one or both OPs has an additional biochemical target with which interaction can influence anticholinesterase toxicity. The ability of nicotinic autoreceptors, a potential alternative OP target, to positively modulate acetylcholine (ACh) release after OP exposure was evaluated in cortical synaptosomes preloaded with [^{3}H]choline. Nicotinic autoreceptor function (NAF) was measured 4, 24 and 48 hrs after *in vivo* PS (18 mg/kg, sc) or CPF (280 mg/kg, sc) exposure. Synaptosomes were superfused with physiological buffer (in the presence of atropine, 1μM) to determine basal (6mM KCl) and stimulated (KCl + 10μM ACh) [^{3}H]ACh release. Under these conditions, ACh caused 100-125% increase in [^{3}H]ACh release which was completely blocked by mecamylamine (100μM). Neither OP altered [^{3}H]ACh release 4 hrs after OP exposure. CPF, however, reduced (~20%) ACh-stimulated [^{3}H]ACh release 24 hrs after exposure, compared to either control or PS, and both OPs reduced (~25%) ACh-stimulated transmitter release 48 hrs after exposure. *In vitro*, PO (100nM) enhanced ACh-stimulated release but inhibited release at higher concentrations (10-100μM) while CPFO had no effect at any concentration (10nM-100μM). The results suggest both OPs may alter NAF, depending on the specific conditions of exposure. Further, the earlier reduction (24 hrs vs. 48 hrs) of stimulated neurotransmitter release by CPF *in vivo* and the increase in transmitter release by PO *in vitro* (100nM) are both consistent with the higher incidence of toxicity noted following PS exposure. The differential effects of PS and CPF, or their oxon metabolites, on NAF could contribute to the differential toxicity of these agents with similar levels of AChE inhibition. (Supported by U.S. EPA R 825811-01-0.)

473 COMPARISON OF NEUROCHEMICAL EFFECTS OF CHLORPYRIFOS AND METHYL PARATHION ON THE CHOLINERGIC SYSTEM IN EARLY POSTNATAL RATS.

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The effect of repeated chlorpyrifos (CP) and methyl parathion (MPS) exposures on brain acetylcholinesterase (AChE) activity and muscarinic acetylcholine receptor (mAChR) density were studied and compared in early postnatal rats gavaged with CP or MPS in corn oil from postnatal day (PND) 1 through PND 21. In CP treatment groups, young rats were divided into 3 groups and received CP every other day starting at 3 mg/kg and increasing gradually to 6 mg/kg (for medium dosage group) to 12 mg/kg (for high dosage group). In MPS treatment groups, young rats were divided into 3 groups and received MPS every day starting at 0.3 mg/kg and increasing gradually to 0.6 mg/kg (for medium dosage group) to 0.9 mg/kg (for high dosage group). Brain AChE activity was assayed in brain homogenates. Brain mAChR density was assayed in brain synaptosomes with ^{3}H-*N*-methylscopolamine (NMS) and ^{3}H-quinuclidinyl benzilate (QNB) as ligands. Control groups for both treatments showed almost the same brain AChE activities and mAChR densities, indicating that the vehicle or dosing regime did not affect these parameters. In CP treatment, about 29%, 57%, and 63% of AChE activities were inhibited in the low, medium, and high dosage groups, respectively, 1 day after the last dose; reductions of ^{3}H-NMS were observed on PND 6, PND 14, PND 22, and PND 25. In MPS treatment, about 25, 42 and 55% of AChE activities were inhibited in the low, medium and high dosage groups, respectively, 1 day after the last dose; reductions of ^{3}H-NMS were only observed on PND 22. The results indicate that repeated exposures to CP or MPS will result in prolonged inhibition of brain AChE activities and reductions in mAChR densities. The reduction of mAChR density may be differentially regulated by CP and MPS exposures. (Supported by NIH RO1 ES04394.)

474 EFFECTIVENESS OF DETERGENT SOLUBILIZATION OF CHOLINESTERASE FROM PERIPHERAL TISSUES IN THE RAT.

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The assay of cholinesterase (ChE) activity in peripheral target tissues following exposures to anticholinesterase insecticides may yield more useful information regarding toxic impacts than the traditional analyses of ChE in plasma or red blood cells. However, because of the low ChE activity and the presence of higher levels of connective tissues, a colorimetric assay is problematical. Colorimetric assays were conducted by two independent laboratories using acetylthiocholine as a substrate in homogenates of brain, skeletal muscle, lung, heart, diaphragm and salivary glands prepared with or without detergent (1% TritonX-100) and which had been centrifuged to yield a membrane pellet and supernate. The following percentages of activity of the whole homogenate were present in the supernate for each tissue in the non-detergent-containing and detergent-containing preparations, respectively: brain, 13, 88; skeletal muscle, 30, 66; lung, 37, 65; heart, 40, 79; diaphragm, 26, 49; and salivary gland, 36, 77. Therefore, in contrast to brain, appreciable activity is retained in the membrane fraction of these peripheral target tissues even in the presence of detergent. Homogenization at higher motor speeds or for longer periods of time did not solubilize appreciably greater amounts of enzyme activity. Since these insoluble fractions will not be sampled by the small bore pipet tip of clinical autosamplers, a significant amount of ChE activity in some peripheral tissues will not be analyzed using these machines. It is not known whether these solubilized and insoluble preparations of ChE would contain the same percentage of inhibited ChE from anticholinesterase-treated animals.

475 EFFECTS OF DPX-MP062 and DCJW ON THE NEURONAL NICOTINIC ACETYLCHOLINE AND GABA RECEPTORS IN MAMMALIAN NEURONS.

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Little is known about the mechanism of action of a novel insecticide DPX-MP062, indeno(1,2-*e*)(1,3,4)oxadiazine-4α (3*H*)-carboxylic acid, 7-chloro-2,5-dihydro-2-(((methoxycarbonyl)-((4-trifluoromethoxy)phenyl) amino)carbonyl)-methyl ester, except for the blocking action on insect neuronal sodium channels. We studied the effects of DPX-MP062 and its decarbomethoxyllated metabolite, DCJW, on the mammalian neuronal nicotinic acetylcholine receptors (nAChRs) and $GABA_A$ receptors which are known to be the targets of some other insecticides. Currents induced by ACh and GABA were recorded using the whole-cell patch clamp technique from rat cortical and dorsal root ganglion (DRG) neurons in primary culture, respectively. DPX-MP062 applied in bath at 1 or 10 μ M caused irreversible reductions of ACh-induced, rapidly desensitizing, α -bungarotoxin(α -BuTX)-sensitive currents in a dose-dependent manner. By contrast, ACh-induced, slowly desensitizing α -BuTX-insensitive currents were augmented by 1 or 10 μ M DPX-MP062 in dose-dependent, use-independent, and reversible manners. In addition, current decay was accelerated. DCJW at 1 or 10 μ M had similar but less potent actions than DPX-MP062 on both α -BuTX-sensitive and α -BuTX-insensitive currents. In contrast, the peak amplitude of GABA-activated currents was not affected by either 1 or 10 μ M DPX-MP062. However, the decay phase of GABA current was accelerated in a dose-dependent and reversible manner. DCJW at 1 and 10 μ M increased the peak current only slightly without any effect on current decay. These observations suggested that neuronal nAChRs are one of the target sites of DPX-MP062. This insecticide appears to be different from any other insecticides in terms of its mechanism of action.

476 EFFECTS OF THE TRIAZINE HERBICIDE CYANAZINE ON $GABA_A$ RECEPTOR LIGAND BINDING TO RAT BRAIN MEMBRANES AND GABA-STIMULATED $^{36}Cl^-$ FLUX IN SYNAPTONEUROSOMES.

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Triazine herbicides disrupt luteinizing hormone (LH) release in female rats following *in vivo* exposure. Although the mechanism of action is unknown, inhibition of LH release by triazines may be mediated by effects in the central nervous system. $GABA_A$ receptors are important for neuronal regulation of gonadotropin releasing hormone and LH release. The ability of the triazine herbicide cyanazine to interact with $GABA_A$ receptors was examined by measuring effects of cyanazine on [^{3}H]muscimol, [^{3}H]Ro15-4513 and [^{35}S]*tert*-butylbicyclophosphorothionate (TBPS) binding to rat cortical membranes and on GABA-stimulated $^{36}Cl^-$ flux into rat cortical synaptoneurosomes. Cyanazine (1-200 μM) was without effect on [^{3}H]muscimol or [^{35}S]TBPS binding. However, cyanazine inhibited [^{3}H]Ro15-4513 binding with an IC_{50} of approximately 50 μM (n = 4). At concentrations of 1 and 5 μM, cyanazine shifted the EC_{50} for GABA-stimulated $^{36}Cl^-$ flux into synaptoneurosomes from 28.8 μM to 22.9 and 19.4 μM, respectively (n = 5). Diazepam (10 μM) and phenobarbital (100 μM) potentiated GABA-stimulated $^{36}Cl^-$ flux to 138% and 184 % of control, respectively. At concentrations of 50 and 100 μM, cyanazine decreased potentiation by diazepam to 119 and 107% of control, respectively, and decreased potentiation by phenobarbital to 167% of control (n = 5). These results suggest that cyanazine interacts with the $GABA_A$ receptor at the benzodiazepine, but not the muscimol (GABA receptor site) or TBPS (Cl^- channel) binding sites. Furthermore, at low concentrations, cyanazine may be a weak agonist at the benzodiazepine site, but at higher concentrations, cyanazine antagonizes the effects of diazepam and phenobarbital.

477 LESSONS LEARNED FROM STUDYING TOXICANT-INDUCED IRREVERSIBLE TESTICULAR INJURY.

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In the last quarter century, our understanding of male reproductive biology and toxicology has advanced to the point that potential mechanisms of toxicant action can be proposed and tested (without too much laughter in the back of the room). This talk will highlight lessons learned from the mechanistic examination of 2,5-hexanedione-induced testicular injury, an intensely studied model system. LESSON #1: Hypotheses are both gross oversimplifications and useful tools. Since 2,5-hexanedione exposure results in relatively specific injury of the nervous system and testis while reacting globally with primary amines throughout the body, this requires an hypothesis by which a global modification can produce a specific injury. LESSON #2: The most exciting observations are those at odds with basic assumptions. The assumption that 2,5-hexanedione-induced irreversible testicular injury is a consequence of killing all the germ cells is just plain wrong. Primitive germ cells are present and happily proliferating after toxicant exposure—they just die instead of mature. Furthermore, "irreversible" injury is reversible with appropriate hormonal manipulation, opening up new avenues for exploring the basic biology of spermatogenesis, and for treating idiopathic azoospermia. LESSON #3: Life is a balance. Sertoli cells not only make growth factors (i.e., stem cell factor) that support germ cells, these "nurse" cells also make death factors (i.e., Fas ligand) to kill germ cells. The goal of the presentation will be to make these specific realizations broadly generalizable.

478 MECHANISM OF ACTION OF NICOTINE ON NEURONAL ACETYLCHOLINE RECEPTORS: FROM MOLECULE TO BEHAVIOR.

T Narahashi. *Department of Molecular Pharmacology and Biological Chemistry, Northwestern University Medical School, Chicago, IL, USA.*

Nicotine has long been known to interact with nicotine acetylcholine (ACh) receptors since Langley used it extensively to chart sympathetic ganglia a century ago. It has been used as an effective insecticide. However, it was not until 1990's that the significance of nicotine was increasingly recognized from the toxicological, pharmacological and environmental points of view. This is partly because studies of neuronal nicotinic ACh receptors are rapidly coming out of orphanage as fueled by several lines of developments. Since Alzheimer's disease is known to be associated with down-regulation of the cholinergic activity in the brain, a variety of nicotine derivatives are being tested and developed for the treatment of the disease. Public awareness of the adverse effects of nicotine has reached the highest level recently. Since incest resistance to insecticides is one of the most serious issues in the pest control arena, it is urgently needed to develop newer insecticides that act on target sites not shared by the existing insecticides. The neuronal nicotinic ACh receptor is one of them, and new nicotinoids are being developed. Thus time is ripe to discuss the mechanism of action of nicotine from a variety of angles including the molecular, physiological, and behavioral points of view. This is the major focus of the present Symposium.

479 REGULATION OF ACETYLCHOLINE RECEPTOR DESENSITIZATION AT LOW CONCENTRATIONS OF NICOTINE.

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Tobacco-related concentrations of nicotine produce two major effects through neuronal nicotinic receptors (nAChRs): augmentation of neurotransmitter release and receptor desensitization. Following chronic exposure to low doses of nicotine, the number of CNS (4(2 nAChR binding sites increases while their functional responsiveness decreases. It has been argued that desensitization underlies these changes. We show here that desensitization of (4(2 nAChRs in Xenopus oocytes is strongly regulated by the cellular level of phosphorylation. For example, activation of PKC by PMA markedly enhances the rate of recovery from desensitization, following a 30 min exposure to 300 nM nicotine. Moreover, a mutant receptor, with a PKC consensus sequence on the (4 subunit removed exhibited little recovery from desensitization. These data are consistent with the idea that dephosphorylation may lead to a "permanently" inactive form of the receptor. In addition we have found that the relative increase in [3H]-nicotine binding to (4(2 receptors (and other subtypes) during a 24-48 hr exposure to nicotine is strongly correlated with the level of functional desensitization. Because, in addition, the mutant nAChR is more readily upregulated than the wild-type receptor, we suggest that a desensitized and dephosphorylated receptor conformation may act as a trigger for upregulation and explain the long-lasting decrease in receptor function after chronic treatment with low concentrations of nicotine. (Supported by PHS Grant NS31669.)

480 INTERACTIONS OF NICOTINE AND ALCOHOL AT NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS.

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A positive correlation between alcohol drinking and tobacco smoking is well established, heavy drinkers tending to be heavy smokers. However, the mechanism underlying this correlation remains largely to be seen. Through patch clamp experiments using rat cortical neurons in primary culture, we obtained a clue to the mechanism. At least two types of currents were generated in response to the application of acetylcholine (ACh), a rapidly desensitizing ((7-like), (-bungarotoxin ((-BuTX)-sensitive current, and a non-desensitizing ((4-like), (-BuTX-insensitive current. The latter current was potentiated by the application of 3-300 mM ethanol, and desensitized without stimulation by nicotine at 200 nM, a concentration found in smoker's blood. This nicotine-induced desensitization may be related to antianxiety effects of smoking. Nicotine markedly desensitized the ACh-induced current potentiated by ethanol. Thus, ethanol-induced potentiation of current may lead to a craving for smoke. In conclusion, ethanol potentiation and nicotinic desensitization of ACh-induced currents may form the basis for the heavy drinking-heavy smoking correlation.

481 NICOTINIC RECEPTOR GENE FAMILY MEMBERS TARGETED BY ANTHELMINTICS AND INSECT CONTROL AGENTS.

D B Sattelle, E Culetto, N Mongan, J C Freeman and K Matsuda. *The Babraham Institute Laboratory of Molecular Signalling, Department of Zoology, Cambridge, UK.* Sponsor: T Narahashi.

Nicotine acetylcholine receptors (nAChRs) of the nematode C. elegans are the largest gene family of nAChRs currently known Molecular diversity is evident in regions of nAChR (subunits that contribute to the ACh binding site and the channel lining second transmembrane (M2) region. Functional expression studies on homomeric and heteromeric nAChRs provide evidence for multiple nAChR subtypes with differing sensitivities to anthelmintic drugs such as levamisole (1, 2). A complex family of nAChR genes is also present in the insect D. melanogaster. Recombinant expressed nAChRs containing a D. melanogaster (subunit SAD have enabled us to show that at certain nAChRs, Imidacloprid, a potent insecticide, is a partial agonist (3). Thus the study of nAChRs in model organisms which permit advanced genetic, genomic and functional analysis not only enhances our understanding of the full physiological spectrum of actions of ACh mediated by nAChRs, but also adds to out knowledge of the actions of anthelmintics and insect control agents.

1. Ballivet et al (1996) J Mol Biol 258, 261-269.
2. Fleming, J.T. et al., (1997) J. Neurosci. 17,5843-5857.
3. Matsuda, K et al. (1998) Br. J. Pharmacol. 123, 518-524.

482 PHARMACOLOGICAL PROPERTIES OF CENTRAL NICOTINIC RECEPTORS.

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There are multiple nicotine cholinergic receptors in the central nervous system that are either heterologous or homologous pentameric ion channels. Evidence is emerging that pain perception as well as seizure activity may be mediated through (4(2 and (7 receptors, respectively. Nicotine is effective in a wide range of antinociceptive assays in mice and rate when administered peripherally and centrally. These effects are blocked by mecamylamine but only weakly by methyllycaconitine (MLA, and (7 antagonist). Structure-activity relationship studies reveal that 3H-nicotine binding in rat brain (predominately (4(2) correlated well with both antinociceptive and motor depressant potencies. However, some nicotine derivatives, such as metanicotine, exhibit potent antinociception while being devoid of depressant properties. Nicotine also dose-dependently induced seizures in mice that are effectively antagonized by the centrally active antagonist mecamylamine (AD50 = 0.1 mg/kg), but not by hexamethonium. In addition, methyllycaconitine (MLA), an (7 antagonist, blocks the convulsive effect of nicotine (AD50 = 1.9 mg/kg). MLA fails to block other pharmacological effects of nicotine such as hypothermia, hypomotility and antinociception. While antinociception and seizure activity appear to be mediated via different receptor subtypes, increase in intracellular calcium levels appears to be involved in both effects. Calcium channel blockers as well as a calcium channel activator modulate nicotine-induced antinociception and seizure activity. These findings demonstrate that the multiplicity of nicotine's effects on the central nervous system can now be explored in a systematic manner. (Supported by NIDA Grant #DA-05274.)

483 CHRONIC NICOTINE INFUSION EFFECTS ON MEMORY: A VENTRAL HIPPOCAMPAL MECHANISM.

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Chronic nicotine infusion has been found to improve cognitive performance in humans and experimental animals. In humans, nicotine administration via skin patch has been found in our studies to significantly improve attentional performance in patients with Alzheimer's disease, schizophrenics, adults with attention deficit/hyperactivity disorder and normal nonsmoking adults. In an experimental rat model, we have found that chronic 4-week nicotine infusion with osmotic minipumps significantly improves working memory performance in the radial-arm maze. Since we found local infusions of nicotinic antagonists into the ventral hippocampus to cause significant working memory impairments, we hypothesized that this area is a critical locus for chronic nicotine infusion induced memory improvements. To test this hypothesis, female Sprague-Dawley rats were pretrained on an 8-arm radial maze and then given small ibotenic acid lesions in the ventral hippocampus or sham lesions. Half of the rats with each lesion treatment were given subcutaneous infusions of nicotine with osmotic minipumps (5 mg/kg/day) or saline ($N = 8 - 10$ in each of the four groups). As seen previously, chronic nicotine infusion significantly ($p < 0.05$) improved working memory performance in the sham lesioned group. The ventral hippocampal lesion blocked this effect even though by itself this small lesion did not impair working memory performance. These data show that the ventral hippocampus is critical for chronic nicotine-induced memory improvements. This mechanism further demonstrates the importance of hippocampal nicotinic systems for cognitive function.

484 DRUG HYPERSENSITIVITY: MECHANISMS OF IMMUNE-MEDIATED REACTIONS.

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Drug hypersensitivity (DH) is a common side effect of several classes of drugs. Though these reactions are considered to be immune mediated, there is a lack of basic mechanistic information regarding the immunology of DH reactions. Current information regarding the role of the immune system in DH will be presented. In many cases of DH, the antigen or hapten responsible for inducing an immune response is not known. In several cases, reactive drugs or drug metabolites have been shown to be the hapten. However, studies are presented demonstrating that chemically non-reactive drugs can be antigens in a manner independent of drug metabolism. Other studies suggest that the antigens are self proteins that have been altered by the drug resulting in the formation of neoantigens that are recognized by the immune system. These self peptides may either be altered by covalently bound haptens or simpy be made available to the immune system (cryptic self peptides). In studies with mouse globin, both types of neoantigens appear to exist. Once the immune system recognizes these drug antigens as foreign, it is not clear how a particular response (Th1 versus Th2) is generated. The role of cytokines in the development and manifestation of Th1 and Th2 responses will be discussed. Interestingly, a hypothesis is presented stating that the normal immune response against haptens is the development of immunological tolerance. The pathway suggested for this to occur is by oral tolerance. Recent studies have shown that drugs which are very diverse in structure form protein adducts in the gut-associated lymphoid tissue, where tolerance against protein antigens is initiated. DH is an important issue as it can present major hurdles in the development of drugs; DH is generally observed in clinical trials and is not detected in preclinical testing. New revelations in the understanding of DH may lead to new approaches for predicting DH potential.

485 METABOLIC BIOACTIVATION IN DRUG HYPERSENSITIVITY.

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It has been estimated that hypersensitivity (allergic) idiosyncratic reactions

account for between 3-25% of all drug toxicities. In order for a drug to lead to an immune-mediated response, most evidence indicates that the first step involves the covalent attachment of the drug to one or more endogenous carrier proteins. Since most drugs are unreactive, it is usually a reactive metabolite that forms these covalent adducts. With the use of specifically designed antibodies, numerous carrier proteins of reactive metabolites of drugs associated with allergic reactions, such as halothane, tienilic acid, dihydralazine, ethanol, and sulfonamides have been identified. It has been found that patients may develop antibodies against the covalently bound reactive metabolite, covalently altered carrier proteins, and/or native carrier proteins. Moreover, the antibodies may be directed against linear or conformational epitopes. It is not clear, however, why more people do not develop immune reactions against protein adducts of drugs. Perhaps, the normal response against covalently altered carrier proteins is the development of immunological tolerance. One pathway this may occur is by oral tolerance, a hyporesponsive immunological state resulting from the oral administration of antigens. Indeed, recent studies have shown that drugs as diverse in structure as diclofenac, acetaminophen, halothane, and penicillin form protein adducts in the gut-associated lymphoid tissue, where tolerance against protein antigens is initiated.

486 INVOLVEMENT OF T CELLS IN DRUG ALLERGY.

W J Pichler, S V Greyerz, and B Schnyder. *Institute of Immunology and Allergology, Inselspital, Bern, Switzerland.* Sponsor: E E Sikorski.

To understand the role of T cells in drug allergy, we generated T cell clones (TCC), which specificallly interact with drugs like sulfamethoxazole (SMX), lidocaine or various β -lactams. We used these TCC to analyze the recognition of drugs by T cells and to evaluate their functional potential. Recognition: While chemically reactive drugs or drug metabolites were shown to bind in a stable way to the MHC-peptide complexes (covalent binding to proteins, followed by processing), certain chemically non-reactive drugs like SMX or lidocaine were shown to interact directly, in a non-covalent way with MHC/peptide complexes and TCRs. This concept is based on the findings that (a) gluaraldehyde-fixed APC were able to present drugs to specific CD4+ and CD8+ T cell clones; (b) pulsing of APC abrogated the stimulatory capacity, indicating a labile, non-covalent binding; and (c) kinetics of TCR expression after incubation of TCC with APC in the presence or absence of drugs revealed a rapid, drug dependent TCR-down-regulation and an immediate increment of intracytoplasmatic Ca++ in T cells, an early sign of T cell activation. Our data let us develop a new concept of drug recognition by activated T cells, namely a direct, metabolism and processing independent way of drug recognition. Functional analysis of drug specific CD4+ and CD8+ TCCs obtained from peripheral blood and skin revealed IL5 production, which might explain eosinophilia frequently observed in drug allergy. Moreover, some TCC showed a drug specific cytotoxicity against B-LCL and autologous keratinocytes incubated with the drug, which was mediated by perforin as revealed by blocking experiments and immunohistochemistry. Analysis of morbilliforme-like drug eruptions by immunohistochemistry revealed CD4 and CD8 cell infiltrations, some of them perforin containing. We conclude that the frequent morbilliforme drug eruption is a T cell mediated process with active involvement of cytotoxic CD4+ T cells. Dependent on the drug presentation on MHC class I or II, perforin mediated cytotoxicity may lead to more bullous (CD8) or maculopapular exanthema (CD4).

487 TH1/TH2 CYTOKINES AND REGULATION OF SPECIFIC IMMUNE RESPONSE TO DRUGS.

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Elicitation of CD4+ and CD8+ T cell responses play a central role in allergic reactions to drugs that behave as haptenic compounds (β-lactamins, lidocaine, sulfamethoxazole). Thus, drug-specific T lymphocytes can be isolated from cutaneous lesions and/or peripheral blood in allergic patients. Recent studies allowed us to demonstrate that immediate-type hypersensitivity to β-lactams (urticaria, shock, Quincke oedema) is not associated with a clear Th2-like response as assessed by quantitative RT-PCR. Indeed, lymphocytes from patients with a clear history of β-lactam-induced immediate allergic reaction can be specifically triggered to express high levels of interferon- (IFN-γ mRNA, combined or not with an interleukin-4 (IL-4) response. However, only patients with immediate reactions can elicit an IL-4 response since subjects with delayed hypersensitivity to penicillins (maculopapular rash, exanthema, contact dermatitis) only show an IFN-γ response. Other investigators have shown that drug-specific (especially lidocaine-specific) CD4+ lymphocytes producing Th2 cytokines (IL-4 and IL-5) can be isolated from patients with delayed cutaneous reactions. These cytokines play critical roles in controlling drug-specific cellular and humoral immune responses. We and others have shown that IL-4 production strongly modulates penicillin-specific IgG and IgE production in the mouse during primary or secondary immune responses to the drug. Another Th2 cytokine, IL-10, also strongly modulates penicillin-specific IgG responses in the mouse. In addition, IL-4 regulates penicillin-specific cellular responses by modulating CD4+ and CD8+ drug-specific cell growth and apoptosis, as assessed by using human penicillin cell lines and clones in vitro.

488 WHAT DO T CELLS RECOGNIZE IN ADVERSE IMMUNE REACTIONS TO CHEMICALS?

E Gleichmann, T Pape, M Wulferink and P Griem. *Med. Inst. Environmental Hygiene, Heinrich Heine University, Düsseldorf, Germany.* Sponsor: E Sikorski.

Adverse immune reactions to chemicals are initiated by T lymphocytes. To be activated, T cells need to receive signal 1 together with signal 2. Signal 1 consists of T cell recognition of MHC-embeded peptides. While the self-peptides normally presented induce T cell tolerance, chemical alteration of self-proteins may lead to a spectrum of altered self-peptides, i.e., neoantigens, presented by APC. The altered self-peptides presented either may carry covalently bound haptens, i.e., reactive metabolites, or consist of cryptic self-peptides. Signal 2 consists of costimulatory signals which can be provided by professional APC. Our studies addressed the potential role of macrophages in the formation of neoantigens induced by xenobiotics and recognized by T cells. Several parent compounds were studied including among others aniline, benzene, gold(I), and procainamide (PA). Whereas the parent compounds mentioned failed to elicit T cell reactions when tested in the murine popliteal lymph node (PLN) assay, their protein-reactive metabolites were able to do so. Hence, for these compounds there is insufficient enzymatic capacity for bioconversion of prohapten into hapten in the PLN assay. In the case of aniline, gold(I), and the SLE-inducing drug PA, it was formally shown that macrophages can generate the immunogenic metates, gold(III) and hydroxylamino-PA, respectively. Whereas myeloperoxidase plus H_2O_2 seems to be the drug-oxidizing enzyme system in the case of gold(I), in the case of PA the macrophage enzyme involved in the oxidations step appears to be prostaglandin-H synthase-2 (PGHS-2 or COX-2).

In studies assessing T cell recognition, T cell clones, obtained from mice that were immunized with mouse globin haptenated with benzoquinone, specifically recognized peptides with benzoquinone coupled to cysteine in position 96. Preliminary data indicate that other CD4+ T cell clones, obtained from the same immunization, recognized cryptic peptides of globin.

489 MECHANISMS OF ACTION OF NATURALLY OCCURRING ANTICARCINOGENS - INTRODUCTION.

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The human diet contains a host of natural products that have been associated with both carcinogenic and anticarcinogenic activities. Cooking proteinaceous food results in formation of highly mutagenic aromatic amines that also exhibit carcinogenic effects in multiple animal models and target organs. The potential role of these compounds in the etiology of human cancers is being extensively investigated. Fruits, vegetables and high fiber diets are known to protect from development of several cancers, and some specific classes of chemicals in these foods have been strongly associated with their anticarcinogenic activities. In this symposium, the mechanisms of action of several different classes of naturally-occurring anticarcinogens and their derivatives will be discussed and these include isoflavones such as genistein, indole-3-carbinol and diinolylmethane, components of green tea and various vegetable-derived organosulfur compounds. These presentations will not only identify sources and mechanisms of action of natural anticarcinogens but also discuss some analogs that may have potential for clinic applications.

490 ANTIESTROGENIC AND ANTITUMORIGENIC ACTIVITIES OF DIINDOLYLMETHANE.

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Diindolylmethane (DIM) is formed from acid-catalyzed dimerization of indole-3-carbinol, a major anticarcinogenic compound found in cruciferous vegetables. DIM competitively binds to the aryl hydrocarbon receptor (AhR) but is a weak inducer of CYP1A1 gene expression in MCF-7 human breast cancer cells. In contrast, DIM inhibits 17(-estradiol (E2)-induced proliferation of MCF-7 cells, reporter gene activity in cells transiently transfected with an E2-responsive plasmid (containing a frog vitellogenin A2 gene promoter insert), and downregulates the nuclear estrogen receptor (ER). Moreover, DIM (5 mg/kg every other day) also inhibits DMBA-induced mammary tumor growth in Sprague-Dawley rats and this is not accompanied by induction of hepatic CYP1A1-dependent activity. Several new substituted DIMs also bind the AhR and exhibit antiestrogenic and antitumorigenic activities, and mammary tumor growth inhibition is observed at doses as low as 1 mg/kg (administered every 48 h). Thus, DIM represents a new class of relatively non-toxic AhR-based antiestrogens that inhibit E2-dependent tumor growth in rodents, and current studies are focused on development of specific analogs for clinical treatment of breast cancer.

491 ORGANOSULFUR COMPOUNDS IN ALLIUMS: MECHANISM OF CHEMOPREVENTIVE ACTION.

M J Wargovich. *Department of Pathology, University of South Carolina School of Medicine and South Carolina Cancer Center, Columbia, SC, USA.* Sponsor: S H Safe.

Our laboratory has shown that volatile organosulfur compounds from garlic and onion are strikingly inhibitory toward chemical carcinogens activated by CYP 2E1. Diallyl sulfide potently inhibits dimethylhydrazine and azoxymethane-induced colon cancer in mice, as well as nitrososmethylbenzylamine-induced esophageal cancer in rats. Administration of garlic or onion volatiles during initiation results in strong suppression of tumorigenesis but these compounds are inactive when given after initiation despite the fact that we have found them to active inducers of glutathione-s-transferase activity. Limited structure/function studies suggest that the degree of unsaturation in the organosulfur molecule correlates with chemopreventive activity in the animal models. Current studies are focusing on the impact of these agents on CYP 2E1 activity at the protein and gene level.

492 GENISTEIN: IN VIVO MECHANISMS OF ACTION AND CHEMOPREVENTION.

C A Lamartiniere. *Department of Pharmacology and Toxicology, University of Alabama at Birmingham, Birmingham, AL, USA.*

Genistein (5,7,4'-trihydroxyisoflavone) is a phytoestrogen component of soy that has been associated with reduced incidences of breast and prostate cancers. In vitro, genistein has been described as an inhibitor of protein tyrosine kinases, topoisomerase II, angiogenesis, and cell proliferation. It has been reported to have weak estrogenic and antiestrogenic properties, to be an antioxidant, and to induce cell differentiation. However, very little of this has been confirmed in vivo. In the rat uterus, genistein down-regulates estrogen receptor alpha mRNA and EGF-receptor protein, and does not alter progesterone receptor protein levels. In the mammary gland, genistein up-regulates the expression of the EGF-receptor and beta casein proteins, enhances mammary gland differentiation, decreases cell proliferation, and protects against chemically-induced mammary cancer. In the rat prostate genistein up-regulates the androgen receptor protein, and results in less estrogen receptor-alpha and -beta, and EGF-receptor mRNAs. The latter may explain the protective actions of soy against prostate cancer.

493 GREEN TEA IN CHEMOPREVENTION OF CANCER: MECHANISM OF ACTION.

H Mukhtar. *Department of Dermatology, Case Western Reserve University, Cleveland, OH, USA.*

Green tea, obtained from evergreen shrub Camellia sinesis, is a popularly consumed beverage in some parts of the world. Green tea contains several polyphenolic antioxidants known as catechins. The major polyphenolic antioxidants present in green tea are (-)-epicatechin, (-)-epicatechin-3-gallate, (-)-epigallocatechin, and (-)-epigallocatechin-3-gallate (EGCG). Large volumes of data, from several laboratories worldwide, accumulated in last several years has provided convincing evidence that green tea and/or the polyphenolic antioxidant present therein afford protection against cancer risk in a variety of animal tumor bioassay systems. In this presentation, the available information on experimental studies showing an association of reduced cancer development with the consumption of green tea or its constituents will be provided. Most of these studies have been conducted in the skin tumor model systems where oral consumption or topical application of green tea or its major polyphenolic constituent EGCG has shown significant protection against chemically- and ultraviolet B radiation-induced tumorigenesis. Green tea has also been shown to afford protection against carcinogen-induced lung, esophagus, forestomach, duodenum, pancreas, liver, breast and colon carcinogenesis in organ-specific tumor bioassay systems. Epidemiological studies in the human population, though inconclusive, suggest that green tea may prevent against the risk of cancer of some body sites. Understanding the molecular mechanism by which green tea imparts protective effects may provide opportunities to interfere with cancer development. Much of the chemopreventive effects of green tea are attributed to EGCG. A discussion regarding the molecular mechanism(s) responsible for the broad chemopreventive effects of EGCG will be presented.

494 CARCINOGENICITY OF CIGARETTE SMOKE : BRIDGING THE GAP BETWEEN COMPLEX MIXTURES AND INDIVIDUAL COMPONENTS.

S W Burchiel[1] and H R Witschi[2]. [1]*University of New Mexico, College of Pharmacy, Albuquerque, NM;* [2]*University of California, Davis, CA.*

While it is generally accepted that cigarette smoking increases the risk of lung and other cancers in humans, there is not widespread agreement on what specific chemicals in the volatile and particulate fractions might be responsible for these effects. Since the components of cigarette smoke have continued to change over the years, especially with regard to those derived from tar, it is important to utilize state-of-the-art molecular and cellular techniques to analyze potential mechanisms of carcinogenicity of cigarette smoke. It is also important to understand how to extrapolate data obtained from isolated components to complex mixtures. The overall purpose of this workshop will be to bring together scientists with established track records performing cigarette smoke inhalation studies with those focusing on isolated components and mechanisms of carcinogenicity. Early cellular and molecular markers and dosimeters of tissue injury need to be established and validated. The effects of various mainstream and sidestream smoke components, as well as specific chemicals and oxidants present in smoke, on DNA and cell signaling pathways will be the focus of this workshop.

495 FREE RADICALS AND OXIDANTS IN AQUEOUS EXTRACTS OF CIGARETTE SMOKE: DAMAGE TO LIPIDS, PROTEINS, AND DNA.

W A Pryor. *Biodynamics Institute, Louisiana State University, Baton Rouge, LA , USA.*

Cigarette smoke consists of gas and particulate ("tar") phases. Gas-phase smoke contains more than 1017 free radicals per puff, but these radicals are short-lived and disappear rapidly when the smoke dissolves in a liquid. The principal radical in tar consists of low molecular weight semiquinones (QH.) in equilibrium with the related quinones (Q) and hydroquinones (QH2), and this tar radical is virtually infinitely stable either adsorbed on a cigarette filter or as an extract in aqueous or organic solvents. Aqueous cigarette tar (ACT) extracts are potent oxidizing solutions; we believe these ACT solutions represent an excellent model of the oxidants present in a smoker's lungs. ACT solutions initiate lipid peroxidation and oxidize the antiproteinase, alpha-1-proteinase inhibitor (a1PI). The oxidation of a1PI occurs specifically at a methionine residue at the active site (converting it to methionine sulfoxide) as well as to other, possibly random sites causing generalized radical damage. ACT solutions can penetrate viable cells and cause nicks in nuclear DNA. Fractionation of ACT solutions demonstrates that almost all of the DNA nicking activity is present in those fractions that contain the QH./Q/QH2 radical system. (Supported by grants from NIH.)

496 CIGARETTE SMOKE AS A LUNG CARCINOGEN IN MAN AND ANIMALS.

H P Witschi. *Institute of Toxicology and Environmental Health, University of California, Davis, CA, USA.*

At the turn of the century, lung cancer was a rare disease. Nowadays it is in the USA the leading cause of death from cancer in men and women. Although it does occur occasionally in non-smokers, the overwhelming majority of lung cancers can be attributed to the smoking of tobacco products, mostly cigarettes. Other known human lung carcinogens include asbestos, radon, arsenic, silica and some metals and it is suspected that common air pollutants, most notably environmental tobacco smoke, are risk factors, too. At one time it was hoped that reduction of tar and nicotine content of cigarettes would mitigate the adverse effects of smoking, an expectation that remains unfilled. While the epidemiological evidence is overwhelming, attempts to study tobacco smoke carcinogenesis in animal models have been not very successful. For example out of more than 1700 mice exposed for life to tobacco smoke, only 6% developed lung tumors (4% in controls). In rat studies, lung tumor incidence is practically never higher than 10%. A recently developed tobacco smoke lung tumor model in strain A/J mice promises to overcome some of these difficulties and has made it possible to evaluate the effects of tobacco smoke gas phase in comparison with the effects. A recently developed tobacco smoke lung tumor model in strain A/J mice promises to overcome some of these difficulties and has made it possible to evaluate the effects of tobacco smoke gas phase in comparison with the effects of full tobacco smoke as well as to evaluate the effectiveness of putative chemopreventive agents.

497 CIGARETTE SMOKE CARCINOGENS: MECHANISMS OF DNA DAMAGE.

S A Belinsky. *Lovelace Respiratory Research Institute, Albuquerque, NM, USA.* Sponsor: S W Burchiel.

Lung cancer (LC) is the leading cause of cancer-related deaths in adults. Identifying the specific carcinogens within tobacco that are responsible for cell damage and the molecular mechanisms that underlie tumor development is critical for developing approaches to prevent and treat LC. Recent studies within our Institute demonstrated that lifetime exposure of female Fischer 344 rats to mainstream smoke results in a tumor incidence of about 8%. A synergistic interaction between cigarette smoke and 239Plutonium was also observed. Thus, the rat represents a model to study cigarette smoke-induced LC and interactions with other DNA damaging agents. Lung tumors induced by defined exposures indicative of the types of DNA damage from cigarette smoke (e.g., oxidative damage) were used to determine the prevalence for inactivation of the p16 tumor suppressor gene and the estrogen receptor (ER) by aberrant CpG island methylation. Inactivation of the p16 gene was frequent (> 65%) in tumors induced by the tobacco-specific carcinogen NNK, the particulate carcinogen beryllium metal, and by ionizing radiation. Moreover, methylation of p16 was an early event in the development of NNK-induced tumors. Both the timing and frequency for inactivation were recapitulated in human squamous cell carcinoma of the lung. In contrast, inactivation of the ER varied as a function of environmental exposure, occurring at the highest frequency (87%) in tumors induced by ionizing radiation. These studies indicate that the rat may be an excellent model for identifying the critical pathways in cigarette smoke-induced LC and implicate inactivation of the p16 gene as an important event in the development of non-small cell LC. (DE-FC04-96AL76406)

498 DO CIGARETTE SMOKE OXIDANTS ALTER SIGNALING PATHWAYS ASSOCIATED WITH TUMOR PROMOTION AND PROGRESSION?

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Polycyclic aromatic hydrocarbons (PAHs) are a well known class of chemical carcinogens that are present in cigarette smoke. PAHs are complete carcinogens capable of both tumor initiation and tumor promotion. Previous studies in this lab have shown that certain PAHs, such as benzo(a)pyrene (BaP) and 7,12-dimethylbenz(a)anthracene (DMBA) alter antigen receptor signaling pathways in lymphocytes as well as growth factor signaling pathways in human mammary epithelial cells (HMEC) and the MCF-10A cell line. In the present studies, BaP (3μ M) was found to elevate intracellular Ca^{2+} in MCF-10A cells by two mechanisms: a P450-dependent process which could be inhibited by α -napthoflavone, and a P450-independent photochemical process. The photochemical process appeared to cause oxidative stress, was cytotoxic to MCF-10A when cells were exposed to BaP + hv for brief periods (5-30 min). Cells surviving BaP + hv were found to have dramatically altered morphology and increased cell adhesion. These results show that chemical oxidants alter Ca^{2+} signaling in MCF-10A cells, which may lead to tumor promotion, and may be relevant to oxidants in cigarette smoke. (Supported in part by NIH ES07259.)

499 CIGARETTE SMOKE CARCINOGENS AND LUNG CANCER.

S S Hecht. *University of Minnesota Cancer Center, Minneapolis, MN, USA.* Sponsor: S W Burchiel.

Lung cancer is the leading cause of cancer death in the United States, with over 160,000 deaths expected in 1998. At least 85% of lung cancer death is caused by cigarette smoking. Cigarette smoke is a complex mixture of approximately 4,000 compounds, of which at least 55 are recognized carcinogens. One can evaluate the role of specific carcinogens in lung cancer induction based on the levels of these compounds in cigarette smoke, their carcinogenicity in laboratory animals and comparative biochemical and molecular biological data in animals and humans. Based on these parameters, polycyclic aromatic hydrocarbons (PAH), typified by benzo[a]pyrene, and the tobacco-specific carcinogen 4-(methylnitrosamino) -1-(3-pyridyl)-1-butanone (NNK) are the most important compounds in cigarette smoke with respect to lung cancer induction in humans. Our goal is to understand uptake, metabolism and macromolecular adduct formation of PAH and NNK in humans. In recent studies, we have quantified urinary metabolites of NNK in smokers, non-smokers exposed to environmental tobacco smoke, newborns exposed transplacentally to cigarette smoke, and smokers who have quit. Other recent results of research in this area will be discussed. It is our view that insights on mechanisms of cancer etiology and strategies for prevention can be developed more readily by focusing on particular relevant carcinogens in a complex mixture, rather than on the whole mixture. (Our research is supported by Grants CA-44377 and CA-46535 from the National Cancer Institute.)

500 TELEMETRY, TOXICOLOGY, AND SAFETY ASSESSMENT.

L B Kinter[1] and D J Murphy[2]. [1]*Astra Pharmaceuticals LP, Wayne, PA and* [2]*SmithKline Beecham Pharmaceuticals, King of Prussia, PA, USA.*

Advances in radio-telemetry technology are providing new approaches for assessing the effects of toxicants in animals for purposes of risk assessment. Telemetry systems consist of miniaturized fully-implantable sensors and transmitters which detect and transmit physiological parameters (pressures, flows, temperatures, pH, electrical potentials, activity) to remote receivers. Currently, the technology can be applied in all commonly used laboratory species, from mice to monkeys. Using telemetry, physiological parameters may be continuously monitored in conscious unrestrained animals maintained in their natural or home environments. For toxicologists, telemetry permits parallel, sensitive, and dynamic assessments of critical organ system functions (blood pressure, respiratory parameters, ECG, etc.) using toxicological study designs, or simultaneous assessments with traditional toxicological endpoints in a single study. In addition to reducing the numbers of animals needed for risk assessment through combining physiological and toxicological endpoints, telemetry is a substitute for traditional anesthetized pharmacological preparations, and is consistent with the use of advanced statistical procedures (blocking and factorial analyses), supporting further reductions and refinements in animal use in safety assessment. Identification of effects of toxicants on critical organ system functions, including cardiac/cardiovascular, respiratory, and central nervous systems, provides useful markers for critical care physicians conducting subsequent studies in humans. Integration of effects of toxicants on physiological and toxicological endpoints contributes to elucidation of target organs and mechanisms of toxicity.

501 TELEMETRY: REAL REDUCTION AND REFINEMENT ALTERNATIVES IN RISK ASSESSMENT.

L B Kinter[1], D K Johnson[2], and J Ventre[2]. [2]*Nycomed Amersham and* [1]*Astra Pharmaceuticals LP, Wayne, PA, USA.*

Using radio-telemetry technology for assessing the effects of toxicants in animals offers significant opportunities to reduce and to refine animal use (Reduction and Refinement alternatives) in risk assessment. Telemetry systems consist of miniaturized fully-implantable sensors and transmitters which detect and transmit physiological parameters (pressures, flows, temperatures, pH, electrical potentials, activity) to remote receivers. Telemeterized animals can be studied in their 'home' environments. Using telemetry to monitor physiological parameters in near-natural conditions minimizes stress factors affecting both absolute values and/or inherent variability (both intra- and inter-subject types) of measured parameters, reducing the numbers of animal s needed to achieve any level of statistical power. Telemeterized animals can be reused indefinitely; using telemeterized animals to study additional doses and control treatments permits the use of blocked (vs. Randomized) study designs, which can reduce animal use by 75% without loss of statistical power. Using animals for 2 or more studies further reduces total animal use 50% per study. Carry-over effects inherent with resue of animals can be minimized by careful consideration of doses, dosing schedules, and pharmacokinetics, and through application of prospective requalification criteria and procedures. Finally, experimental groups of telemeterized animals can be further subdivided to include different ages, sexes, strains, or other variables and the effects of the additional variables evaluated using factorial statistical analyses, with little decrement in overall statistical power. In summary, incorporation of telemetry technology for monitoring effects of toxicants on physiological functions and increase both data quality while decreasing animal use (90%, compared to traditional techniques.

502 CARDIOVASCULAR TELEMETRY: FROM MICE TO MONKEYS.

C Hassler. *Battelle Memorial Inst., Columbus, OH, USA.* Sponsor: L B Kinter.

Modern assessments of effects of toxicants on cardiovascular functions include measurements of blood pressure, ECG, and vascular resistance (blood flow/blood pressure). Modern telemetry technology now permits remote monitoring of blood pressure, ECG, and, most recently, blood flow (and vascular resistance). Pressure catheters and transmitters have been miniaturized to permit chronic blood pressure measurements in all species including mice, while blood flow technology is limited to larger species (dogs and primates). Remote monitoring of cardiovascular parameters in laboratory species maintained in their home cages is redefining many facets of cardiovascular toxicology. Baseline systemic pressure values are significantly lower than in traditional preparations due to removal of experimental stress factors. Conversely, heart rates may be lower (dogs, primates) or higher (mice) in telemeterized preparations, underscoring the differential effects of acute procedures in different species. Reduction of stress factors permits detection of normal cardiovascular rhythms (e.g. circadian rhythms) and acute responses to dosing or husbandry procedures, which can complicate data analysis if not planned for in advance. Telemetry can be particularly useful to detect transient pseudo-allergic phenomena associated with some classes of toxicants, which are otherwise missed in animal preparations, but may be problematic in humans. Technology for measurements of two pressure sources are available, permitting simultaneous remote monitoring of systemic and pulmonary artery or left ventricular pressures. For toxicologists, cardiovascular telemetry permits dynamic assessments of critical cardiovascular functions using toxicological or clinical study designs, facilitating integration of physiological, toxicological, and pharmacokinetic endpoints, and contributing to identification of target organs and elucidation of mechanisms of cardiovascular toxicity.

503 TELEMETRY AS A METHOD FOR MONITORING RESPIRATORY FUNCTION CHRONICALLY IN CONSCIOUS ANIMALS.

D J Murphy, J P Renninger and K A Gossett. *Department of Toxicology, SmithKline Beecham Pharmaceuticals, King of Prussia, PA, USA.*

A novel procedure for chronically monitoring pleural pressure in conscious rats has been developed by surgically implanting the catheter of a pressure sensitive telemetry transmitter beneath the serosal layer of the esophagus within the thoracic cavity. This procedure can be used to monitor breathing rates or to assess (indirectly) airflow obstruction in conscious, non-restrained rats. When combined with lung airflow measurements, obtained using a plethysmograph chamber or a face mask and pneumotachometer, these pleural pressure measurements can be used to chronically measure changes in lung compliance and airflow resistance - two critical parameters for assessing lung function. The accuracy of these measurements was verified by comparison with direct pleural pressure measurements over the range of -3 to -34 cm H20. The pleural pressures remained constant for at least 14 weeks following surgery and there was no evidence of tissue damage or growth around the catheter. This novel method for measuring pleural pressure chronically in conscious rats will facilitate evaluation of the effects of drugs, environmental agents or disease on respiratory function by allowing repeated and simultaneous measurements of both ventilatory (breathing) patterns and lung function in conscious animals.

504 TELEMETRY AND ELECTROCARDIOGRAPHY: CARDIOTOXICITY AND ARRHYTHMOGENICITY.

J-C P Vincent, K M Robinson and G H Bode. *Lead Optimization, Hoechst Marion Roussel, Romainville, France.* Sponsor: L B Kinter.

For the registration of pharmaceuticals, animal toxicological and pharmacological studies need to be performed before the first administration in humans. In safety pharmacology, efforts are underway to harmonize the technical requests among Japanese, American and European regulatory agencies. Safety pharmacology focuses on all functions but vital functions such as cardiovascular, central nervous, and respiratory systems must be investigated before human administration. For the cardiovascular evaluation of novel compounds, the use of telemetry in freely moving dogs and monkeys demonstrates several advantages and provides reliable information on blood pressure, ECG, and heart rate. The QRS complex and the intervals of PR, QT and ST can be determined both by manual and automated measurements of the ECG obtained by telemetry. The accuracy and precision of different software systems are compared and the limitations of the use of this software are described, particularly in the case of arrhythmia. Based upon our experience, ECG measured by telemetry represents considerable progress for evaluating cardiotoxicity of compounds. To increase predictability and sensitivity, further improvements in signal quality (several leads) and data analysis (more sophisticated software) are needed.

505 FREE RADICAL/ANTIOXIDANT APPROACH TO CHEMO-PREVENTION OF ETOPOSIDE-INDUCED ACUTE MYELOID LEUKEMIA (AML).

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Etoposide (VP-16) is extensively used to treat cancer, yet its efficacy is calamitously associated with an increased risk of secondary AML. The mechanisms for the extremely high susceptibility of myeloid stem cells to the VP-16 leukemogenic effects have not been elucidated. We propose a mechanism to account for the VP-16-induced secondary AML and nutritional strategies to prevent this complication of VP-16 therapy. We hypothesize that VP-16•-phenoxyl radicals formed from VP-16 by myeloperoxidase (MPO) are responsible for its genotoxic effects in bone marrow, which contains constitutively high MPO activity. Here, we used purified human MPO, as well as, human leukemia HL60 cells with high MPO activity and provide evidence that: (i) VP-16 undergoes one-electron oxidation to VP-16•-phenoxyl radical catalyzed by both purified MPO and MPO activity in HL60 cells; formation of VP-16•-radicals is completely blocked by MPO inhibitors, cyanide and azide. (ii) Intracellular reductants - GSH and protein sulfhydryls (but not phospholipids) – are involved in MPO-catalyzed VP-16 redox-cycling that oxidizes endogenous thiols; pretreatment of HL60 cells with a thiol reagent ThioGlo™ prevents redox-cycling of VP-16•-radicals and permits their direct EPR detection in cell homogenates. VP-16 redox-cycling by purified MPO (in the presence of GSH) or by MPO-activity in HL60 cells is accompanied by generation of GS•-radicals (determined by HPLC-assay of DMPO-SG nitrone adducts). (iii) Ascorbate competitively inhibits VP-16•-radical-induced thiol oxidation. Ascorbate prevents VP-16-induced inhibition of topoisomerase II in MPO-rich HL60 (but not in HL60 cells with MPO activity depleted by pretreatment with succinyl-acetone). (iv) A vitamin E homologue 2,2,5,7,8-pentamethyl-6-hydroxychromane, whose phenoxyl radicals do not oxidize endogenous thiols, competes with

VP-16 as a substrate for MPO. We conclude that nutritional antioxidants can be used for chemoprevention of VP-16-induced AML. (Supported by AICR 97B128, Johns Hopkins CAAT 9829R3, NIH/NINDS/WHO F05 NS 10669 and NCI Oncol.ogy Res. Faculty Devel. Program.)

506 FREE FATTY ACID (FFA)-INDUCED MISHANDLING OF COPPER (Cu) BY SERUM ALBUMIN IN PREECLAMPSIA: CONTRIBUTION TO OXIDATIVE STRESS.

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Redox activity of Cu in plasma may be associated with oxidative stress in the pregnancy disorder, preeclampsia. Mechanisms by which free Cu or Cu-binding proteins are involved in this redox dysregulation are unknown. We hypothesize that preeclampsia is accompanied by alterations in the ability of specific Cu-binding proteins to handle Cu and render it redox inactive. We first assessed the ability of preeclamptic and control plasma to bind exogenous Cu by competition with the specific Cu (I) chelator, bathocuproine. We found that preeclamptic plasma samples had a 2-fold lower capacity to bind Cu than plasma from normal pregnant women. Similarly, we observed by EPR a 2-fold greater rate of ascorbate radical (ASC•) production in preeclamptic samples compared to controls using exogenously supplied Cu/ASC. A candidate protein to account for these effects is human serum albumin (hSA) due to its ability to bind various plasma constituents, including Cu. Since preeclampsia is associated with elevations in plasma FFAs, we hypothesized that enhanced interactions of hSA with FFAs could impair its ability to bind Cu. In a model system, we observed that the ability of hSA to bind Cu (and quench ASC• formation) was decreased by oleic acid. We next compared the effect of a Cu(II) chelator, cuprizone I on Cu-dependent t-BuOOH-induced ASC• production in plasma samples. Cuprizone I had no effect on the rate of ASC• production in any control plasma samples (n = 10). In contrast, preincubation with cuprizone I produced a remarkable decrease in ASC oxidation in half of preeclamptic samples, while the remainder were unchanged. Human ceruloplasmin or apo-transferrin added to control or preeclamptic plasma had no effect on ASC• formation. We conclude that FFA can induce improper handling of Cu by hSA and elevations in chelatable, redox-active Cu can be a significant component to oxidative stress in many preeclamptic women. (Supported by 2PO1 HD 30367-04A1, EPA STAR Grant Prog., NCI Oncology Res. Faculty.Devel. Program.)

507 OXIDATIVE STRESS AND PROTECTIVE SYSTEM AGAINST REACTIVE OXYGEN SPECIES (ROS) IN BLOOD OF WORKERS EXPOSED TO ORGANIC SOLVENTS AND OTHER CHEMICALS.

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It is now believed that generation of ROS by many occupational and environmental agents might lead to some pathological conditions, mainly of the respiratory tract. Few studies on humans have demonstrated that exposure to organic solvents, heavy metals, and other organic substances may affect the oxidant/antioxidant potential in the body. In this study we examined selenium (Se), glutathione peroxidase (GSH-Px) activity and thiobarbituric acid reactive substances (TBARS) as a marker of lipid peroxidation in the blood of workers in a refining and petrochemical plant (n=131), rubber factory (n=50), in tannery workers (n=34) and in workers exposed to high concentrations of lead (Pb) and cadmium (Cd) (battery plant; n=65). These parameters were used to determine the "oxidative stress status" of individuals, which reflects the state of balance between the numerous exogenous or endogenous prooxidants and antioxidants. It was found that Se levels in blood and excreted in urine were significantly lower in all selected groups of workers as compared to appropriate reference groups. The GSH-Px activity in red blood cells and in plasma of industrial workers was evidently lower than in the reference group, except for tannery workers. TBARS concentrations in blood plasma were significantly increased in petrochemical workers and tannery workers. The concentration of TBARS in plasma of rubber factory workers was higher by 16%, but without statistical significance. In a pilot study some workers exposed to lead and Cd with low blood Se levels were supplemented by 100 μ g Se/day for 3 months. Statistically significant changes in GSH-Px activity in erythrocytes and plasma were observed. We hypothesize that occupational exposure to organic substances and heavy metals may influence the protective antioxidant potential in the blood of exposed workers.

508 MARKERS OF ANTIOXIDANT STATUS IN BRONCHO-ALVEOLAR LAVAGE FLUID AND BLOOD OF RATS FED DIET WITH DIFFERENT AMOUNT OF SELENIUM AND EXPOSED TO NO_x.

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The aim of the study was to examine whether appropriate concentration of antioxidants in the diet may modify toxic effects of environmental oxidants on the lungs. Three groups of rats were fed a diet with different Se content: low-Se (1.3 μ g Se/day), adequate (3.0 μ g Se/day) and high-Se (6.0 μ g Se/day). Erythrocyte glutathione peroxidase (GSH-Px) activity was monitored weekly during the study. After 12 weeks of feeding, 25% of the animals from each of the groups were whole body exposed in inhalation chambers to 0.5, 1.5 or 5.0 ppm NO_x for 4 week (6 hours/day, 5 days/week). The control group consisted of animals which were not exposed to NO_x and consumed appropriate amounts of Se. Plasma Se levels in rats exposed to 5.0 ppm NO_x were lower than in controls: 473± 73 μ g/l vs. 548± 24 μ g/l ($p<0.01$) for high Se rats, 364± 29 μ g/l vs. 507± 86 μ g/l, ($p<0.001$) for animals on adequate Se diet, and 216± 38 μ g/l vs. 250± 60 μ g/l for low Se rats. Erythrocyte GSH-Px activity was significantly lower in all groups of animals exposed to 0.5 and 1.5 ppm NO_x. In the group exposed to 5.0 ppm NO_x, GSH-Px activity was significantly lower only in low-Se rats. Erythrocyte GSH-Px activity in low-Se rats was inversly proportional to the extent of exposure: 15.4± 3.7 u/g Hb, after exposure to 0.5 ppm NO_x, 24.5± 6.6 u/g Hb after exposure to 1.5 ppm NO_x and 26.6± 6.4 u/g Hb after exposure to 5.0 ppm NO_x vs. 39.2± 10.0 u/g Hb in controls. Brochoalveolar lavage fluid (BAL) GSH-Px activity in rats exposed to 0.5 and 1.5 ppm NO_x was higher than in the corresponding control group, irrespective of the amount of supplied Se. Only in low-Se rats exposed to 5.0 ppm NO_x, GSH-Px BAL was by 40% lower than in the corresponding control group. Low Se-diet causes an exposure-dependent decrease of GSH concentration and total reactive antioxidant potential. A two-fold increase of BAL thiobarbituric acid reactive substances concentration was observed in animals exposed to 0.5 ppm NO_x and fed a low Se diet. Results of our study indicated that different Se levels in the diet may influence the antioxidant potential of animals exposed to NO_x.

509 ENDOGENOUS N^2,3-ETHENOGUANINE IS FORMED BY LIPID PEROXIDATION.

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Recently a number of endogenous DNA adducts have been attributed to the reaction of DNA with products of lipid peroxidation. Here we demonstrate that N^2,3-ethenodeoxyguanosine (εG) is formed from the reaction of either deoxyguanosine (dGuo) or calf thymus DNA (CT-DNA) with either ethyl linoleate (LA) or 4-hydroxynonenal (HNE) under peroxidizing conditions. HNE (100 μg) or LA (10 mg) were reacted with 0.2 μmol of deoxyguanosine in the presence of t-butylhydroperoxide (t-BuOOH) for 72 hr at 50°C. εG was analyzed by immunoaffinity chromatography-gas chromatography-high resolution mass spectrometry (IA/GC/HRMS) of the pentafluorobenzyl derivative of its base. This reaction resulted in a 38- and 82-fold increase in εG for LA and HNE, respectively, as compared to control reactions containing only t-BuOOH and solvent. Similarly, HNE and LA, in the presence of t-BuOOH, were reacted with 300 μg DNA at 37°C for 89 hr. Samples were filtered to separate intact DNA from depurinated bases. The amount of εG in intact DNA was analyzed by IA/GC/HRMS. Under these conditions, 114 ± 32 and 52.9 ± 16.7 pmol εG/μmol dGuo were formed for LA and HNE, respectively. This compared to 0.26 ± 0.05 pmol εG/μmol dGuo in control DNA which was not subjected to incubation or t-BuOOH exposure. Control DNA incubated with t-BuOOH and solvent resulted in 2.02 ± 0.17 and 2.05 ± 0.47 pmol εG/μmol dGuo and controls subjected to incubation with solvents for LA and HNE, but no t-BuOOH resulted in 0.62 ± 0.13 and 1.00 ± 0.86 pmol εG/μmol dGuo for LA and HNE, respectively. These data demonstrate εG is formed from lipid peroxidation and HNE under peroxidizing conditions. These data also suggest εG is formed from an alternative mechanism since a 10-fold increase in εG was seen in control incubations containing t-BuOOH versus control DNA which was not incubated. (Supported by NIH grants ES05779 and ES05948.)

510 SITE SELECTIVE OXIDATION AND REGULATION OF MICROSOMAL RYANODINE-SENSITIVE CALCIUM RELEASE CHANNELS BY QUINONES.

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Quinones undergo redox cycling and/or arylation reactions with key biomolecules involved with cellular Ca^{2+} regulation. We utilized 10nM of the fluorogenic maleimide CPM to assess changes in the oxidation state of hyperreactive sulfhydryl moieties localized within ryanodine-sensitive Ca^{2+} channel complexes (RyR) found in skeletal and cardiac microsomes (Liu and Pessah, 1994), in the presence or absence of 1,4-naphthoquinone (NQ) or 1,4-benzoquinone (BQ). Kinetic and SDS-PAGE analyses revealed that NQ and BQ rapidly and selectively reduced the rate of forming fluorescent CPM-thioether adducts with hyperreactive thiols residing on the RyR complex (IC_{50}=0.3 and 1.8μM). The reduced forms of quinone, *e.g. tert*-butylhydroquinone, failed to alter the pattern or kinetics of CPM labeling of RyR thiols, suggesting that NQ and BQ site-selectively oxidize hyperreactive channel thiols. The functional consequence of this mechanism was directly assessed by measuring (1) macroscopic microsomal Ca^{2+} fluxes using a metallochromic indicator and (2) open probability of single channels reconstituted in bilayer lipid membranes under voltage clamp. Results obtained from both measurements showed that 200nM NQ or BQ activate microsomal Ca^{2+} efflux by directly interacting with RyR in a reversible manner. NQ $\geq$2μM sequentially activated then irreversibly inactivated RyR. These results suggest that hyperreactive thiols associated with RyR constitute an exquisitely sensitive microsomal "sensor" of redox active quinones. The Ca^{2+}-dependent cytotoxicities observed with quinone metabolites formed at the microsomal surface may be related to their ability to selectively modify hyperreactive thiols regulating normal functioning of microsomal Ca^{2+} release channels. (Supported by NIH ES05002.)

511 MITOCHONDRIA AND THE ENDOPLASMIC RETICULUM ARE SOURCES OF REACTIVE OXYGEN SPECIES IN 2-Br-*bis*-(GLUTATHION-S-YL)HYDOQUINONE TREATED LLC-PK1 CELLS.

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2-Br-*bis*-(glutathion-*S*-yl)hydroquinone (BBGHQ) causes DNA single strand breaks, growth arrest and cell death in renal proximal tubular epithelial cells (LLC-PK1), all of which are inhibitable by catalase, suggesting a role for reactive oxygen species (ROS) in these processes. We therefore investigated the potential source of ROS in LLC-PK1 cells, upon treatment with BBGHQ (50 - 400 μM). Fluorescence imaging with 2,7-dichlorofluorescein (DCF) was used to assess ROS generation using confocal fluorescence microscopy. A rapid concentration- and time-dependent increase of ROS was observed. The antioxidants 1,10-phenanthroline, deferoxamine and 2-mercaptopropionyl glycine, and catalase, all blocked BBGHQ-induced increases in ROS. Rotenone and thenoyltrifluoro-acetone, inhibitors of the mitochondrial electron transport chain, and cimetidine, a nonisoform-specific inhibitor of cytochrome P-450, also inhibited BBGHQ-mediated increases in ROS. These data suggest that both the mitochondrial electron transport chain, and cytochrome P-450 participate in BBGHQ-mediated generation of ROS. Changes in mitochondrial morphology were followed utilizing MitoTrackerfi green staining. Incubation of LLC-PK1 cells with 200 μM BBGHQ caused mitochondrial swelling subsequent to the initial production of ROS, and 400 μM BBGHQ caused a substantial reduction in the mitochondrial membrane potential (Ψ). Our data indicate that mitochondria and the endoplasmic reticulum are potential sites of ROS production in BBGHQ treated LLC-PK1 cells, and that ROS generation precedes BBGHQ-induced changes in mitochondrial morphology, Ψ, and cytotoxicity. (ES07359)

512 SEX DIFFERENCES IN DIQUAT-MEDIATED HEPATIC NECROSIS IN VIVO.

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Redox cycling metabolism of diquat catalyzes generation of reactive oxygen species and the acute hepatic necrosis caused in male Fischer-344 rats by diquat has been studied as a model of oxidant mechanisms of cell killing in vivo. At equal doses of diquat, female F344 rats sustained lower levels of hepatic damage than did male rats, as estimated by plasma ALT activities after 6 h. The differences in hepatic injury are observed at equal rates of diquat-stimulated biliary excretion of GSSG, which although requiring higher doses of diquat in female rats than in males, nevertheless indicates that the greater resistance of the female rats is not explained by drug distribution and rates of redox cycling metabolism. Hepatic activities of glutathione peroxidase and superoxide dismutase were similar and glutathione reductase and glutathione S-transferase activities were higher in the more susceptible male rats. Previous studies in male rats have implicated formation of DNPH-reactive "protein carbonyls" and related iron chelate-catalyzed redox reactions as mechanisms critical to the expression of acute cell death in vivo. However, diquat-treated female rats showed higher levels of DNPH-reactive proteins in hepatic homogenates and in bile than did males, both at identical doses and at similar plasma ALT activities. Differences in the specific proteins affected in male and female rats were observed. Fragmentation of hepatic DNA was increased within 2 h, before elevation of plasma ALT activities, and by doses of diquat that did not increase plasma ALT activities in female rats. However, TUNEL analyses showed greater reactivities in diquat-treated female rats than in similarly treated males. In the present studies, hepatic necrosis is most closely associated with DNA fragmentation, although additional studies are needed to determine the mechanisms and pathophysiological consequences of the DNA fragmentation. (Supported by GM44263 from the NIH.)

513 BCL-2 ENHANCES REDOX IMPACT OF NEOCARZINO-STATIN (NCS) IN PC12 RAT PHEOCHROMOCYTOMA CELLS.

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The product of *bcl-2* gene blocks apoptosis and protects cells from oxidative stress. In our experiments, Bcl-2 overexpression in PC12 cells prevented apoptosis and protected phospholipids against peroxidation by an azo-initiator, AMVN. Paradoxically, Bcl-2 potentiated apoptosis following a reduction-dependent enediyne prodrug, NCS. Our previous studies have identified oxidation of phosphatidylserine (PS) as a signaling pathway preceding PS externalization in apoptotic cells. Therefore, we compared the effects of NCS on phospholipid peroxidation and PS externalization in mock-transfected PC12 cells with those transfected with *bcl-2* gene. We found that 1 h exposure of cells to NCS (1.0-6.0 μg/10^6 cells) resulted in oxidation of all major membrane phospholipids: phosphatidylinositol, phosphatidylethanolamine (PE), phosphatidylcholine and PS in mock-transfected PC12 cells. Overproduction of Bcl-2 rendered a significant protection of all classes of phospholipids except PS against NCS-induced oxidation. PS oxidation preceded externalization of PS and PE as determined by direct fluorescamine staining of external membrane aminophospholipids and staining with Annexin V. PS externalization following NCS was approximately 2-fold greater in *bcl-2*-transfected than in mock-transfected cells. Thus Bcl-2 does not abrogate, but rather potentiates NCS-induced PS oxidation and externalization in PC12 cells. The increased sensitivity of *bcl-2* transfected PC12 cells to NCS may result from increased activation of NCS by GSH. Therefore, enhanced formation of GS• might be expected in *bcl-2*-transfected cells. Indeed, by using our novel HPLC assay of GS•-adducts with DMPO we found a 2-fold greater accumulation of DMPO-GS nitrone in *bcl-2*- than in mock-transfected cells. Thus, enhanced redox activation of NCS by GSH is accompanied by site-specific oxidation of PS and its externalization in *bcl-2* transfected PC12 cells experiencing apoptosis. (Supported by CA74289, ES-09387, DAMD17-97-1-7247, Johns Hopkins CAAT 9829 R3, NIH/ NINDS/WHO F05 NS 10669 and NCI Oncology Res. Faculty Devel. Program.)

514 GLUCOSE DEPRIVATION-INDUCED OXIDATIVE STRESS.

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The mechanism responsible for glucose deprivation-induced cytotoxicity, activation of signal transduction and gene expression was examined in MCF-7/ADR human breast carcinoma cells. Glucose deprivation (0-4 hrs) caused clonogenic cell death and oxidative stress as evidenced by increased

oxidized glutathione content (GSSG) and intracellular prooxidant production (presumably hydroperoxides). In addition glucose deprivation increased the activities of mitogen activated protein kinases (ERK1/ERK2), c-Jun N-terminal kinase and Lyn kinase as well as led to the accumulation of basic fibroblast growth factor (bFGF) and c-Myc mRNA. Treatment with the thiol antioxidant N-acetylcysteine (1-10 mM) during glucose deprivation suppressed cytotoxicity, prooxidant production and GSSG accumulation as well as inhibited kinase activation and bFGF/c-Myc gene expression. These results show that glucose deprivation-induced cytotoxicity, activation of signal transduction, and expression of two genes thought to govern the malignant phenotype appear to be mediated by oxidative stress in MCF-7/ADR cells. This work supports the hypothesis that a common mechanism of glucose deprivation-induced cytotoxicity in tumor cells may involve metabolic oxidative stress as well as the hypothesis that alterations in intracellular oxidation/reduction reactions link changes in glucose metabolism to signal transduction and gene expression. (Supported by NIH HL51469, CA48000, CA44550, CA75556.)

515 A SYSTEM FOR THE DETECTION AND MEASUREMENT OF THE EFFECTS OF OXIDANTS (INCLUDING EXPOSURE TO METHYLENE BLUE PLUS LIGHT), AND ANTIOXIDANTS ON PRODUCTIVE INFECTIONS OF HeLa CD4LTRßGal CELLS WITH HUMAN IMMUNODEFICIENCY VIRUS (HIV).

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The relationship between oxidative stress and virus-infected cells is an area of medical importance that is incompletely understood. A form of oxidative stress in present use is the purging of viruses, especially the human immunodeficiency virus (HIV), from blood and blood products by photodynamic action based upon the sensitizer methylene blue (MB). Photoinactivation of the RNA bacteriophage Qβ with MB is associated with formation of 8-oxoGuanine, protein carbonyls, RNA strand breaks and RNA-protein crosslinks. HeLaCD4LTRβ Gal cells yield blue nuclei upon infection with HIV-1 (due to tat activation of an LTR region joined to the full length β - galactosidase gene) and histochemical staining, and the cells produce new virions. We show that exposure of suspensions of HIV-1 to MB plus light, rose bengal plus light or UV light inactivates the ability of the virus both to infect HeLaCD4LTRβ Gal cells and to produce new infectious virions. In contrast, and as a control, selected protease inhibitors do not prevent infection by mature virions but do inhibit production of new infectious virions, demonstrating that this system mimics a portion of the normal HIV-1 life cycle. Toxicity of agents toward infected cells can be monitored by staining for the accumulation of ß-galactosidase in situ. It is also demonstrated that selected antioxidants added to the host cells immediately following exposure to virus, do not significantly effect the proportion of cells that are infected. (This research is supported by grant number HL 53585.)

516 THE ROLE OF CYTOCHROME P450 IN THE METABOLISM OF ACRYLAMIDE.

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Acrylamide (AM) is used as a grouting agent, in the production of polyacrylamide, and as an intermediate in the synthesis of plastics and polymers. Previous studies showed that AM is metabolized in rodents to produce the epoxide glycidamide (GA). GA is subsequently metabolized via hydrolysis or glutathione (GSH) conjugation. AM is also metabolized via direct conjugation with GSH. The goal of this study was to determine the role of cytochrome P450 in the in vivo metabolism of AM. Wild-type mice (CYP2E1+/+), aminobenzotriazole (ABT) pretreated (50 mg/kg ip, 2 hr pre-exposure) wild-type mice, and cytochrome P450 2E1 knockout mice (CYP2E1-/-) were administered 50 mg/kg 13C-AM by gavage. Urine was collected for 24 hr. Urinary metabolites were characterized using 13C-NMR, enabling the detection of AM-derived metabolites, as well as detection of possible new urinary metabolites produced by changes in cytochrome P450 activities. Metabolites derived from GA and from direct GSH conjugation with AM were observed after administration of 13C-AM to wild-type mice. Only metabolites derived from direct GSH conjugation with AM were observed in the ABT pretreated wild-type mice and the CYP2E1-/- mice. Based on evaluation of urinary metabolites, this study suggests that CYP2E1 is the major and possibly only cytochrome P450 involved in the metabolism of AM in mice, that inhibiting (via ABT) total P450 activity does not result in new pathways of non-P450 metabolism of AM, and that mice devoid of CYP2E1 do not excrete metabolites of AM that would be produced by other specific cytochromes P450.

517 THE ROLE OF CYTOCHROME P4502E1 (CYP2E1) IN ACRYLONITRILE METABOLISM.

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Acrylonitrile (AN) is widely used in the manufacturing of many products including acrylic fibers and plastics. AN is a multisite carcinogen after oral or inhalation exposure of rats and is a suspected human carcinogen. AN is metabolized by direct conjugation with glutathione (GSH) and oxidation to 2-cyanoethylene oxide (CEO). CEO conjugates with GSH or hydrolyzes to yield cyanide and glycoaldehyde. Subsequent metabolism of the GSH conjugates of AN and CEO results in the excretion of a variety of urinary metabolites. Current studies were undertaken to characterize the role of CYP2E1 in the metabolism of 1,2,3-13C-AN in CYP2E1-null (KO) or wild-type (WT) mice. 13C-AN was administered at 2.5 or 10 mg/kg by gavage, mice were placed in plastic metabolism cages, and urine was collected over a 24 hr period. AN urinary metabolites were quantitated using 13C NMR spectroscopy. Metabolites originating from the oxidative metabolism of AN and excreted in the urine of WT mice accounted for approximately 24-37% of dose. In contrast, no AN metabolites formed via this pathway were detectable in the urine of KO mice. Further, metabolites formed via the non-oxidative metabolism of AN accounted for approximately 6 and 35% of dose in WT and KO mice, respectively. These data clearly indicate that CYP2E1 is the principal enzyme responsible for the oxidative metabolism of AN in mice. CYP2E1-null mice may be an appropriate model for the investigation of the role of oxidative metabolism in AN toxicity and carcinogenicity.

518 ASSESSING THE ROLE OF CYP2E1 IN THE METABOLISM AND GENOTOXICITY OF 1,3-BUTADIENE.

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1,3-Butadiene (BD), a multiple site carcinogen in mice, is metabolized to several DNA-reactive epoxides *in vivo* and *in vitro*. These epoxides are the putative genotoxic compounds. We hypothesized from previous studies that cytochrome P450 2E1 (CYP2E1) is responsible for BD metabolism. To test this hypothesis, we utilized CYP2E1 homozygous null mice (cyp2e1-/-) and the parental strain (SV/129) in closed-chamber gas uptake studies. Mice (5/exposure) were exposed to 0, 1100, or 5000 ppm initial BD concentration for a maximum of 6 hr. The decline in BD concentration in the inhalation chamber with time due to uptake and metabolism was monitored using gas chromatography. The gas-uptake data were analyzed using a previously published physiologically based pharmacokinetic (PBPK) model to evaluate rates of BD oxidation. Preliminary simulations using the PBPK model indicate that BD metabolism is decreased but not eliminated in the cyp2e1-/- mice. To assess genotoxicity, the mouse bone marrow micronucleus assay was utilized. The frequency of micronucleated polychromatic erythrocytes (MN-PCE) per 1000 PCE for the 0, 1100, and 5000 ppm BD exposure groups were 21.4 ± 3.8, 39.4 ± 4.8, 61.5 ± 5.8 for the cyp2e1-/- mice and 19.2 ± 3.7, 51.2 ± 4.9 and 83.1 ± 6.7 for the SV/129 mice, respectively. Preliminary analysis indicates that a correlation exists between the amount of BD metabolized and the micronuclei frequencies. Further, since the cyp2e1-/- mice did not demonstrate the complete elimination of BD metabolism or BD-mediated genotoxicity, enyzmes other than CYP2E1 may be important in the metabolism of BD.

519 THE RATE OF CHLOROFORM METABOLISM CORRELATES WITH THE DEGREE OF TARGET TISSUE TOXICITY.

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Chloroform is a nongenotoxic-cytotoxic carcinogen that has been shown to

induce liver and kidney tumors in various rodent strains. Tumor formation appears to be secondary to events associated with cytotoxicity and regenerative cell proliferation in the target organ. The toxicity of chloroform, which varies among rodent strains and often between sexes of the same strain, appears to depend on the rate of conversion of chloroform to toxic metabolites. The purpose of these experiments was to determine if precision-cut kidney slices could be used to measure the rate of chloroform metabolism in target tissues and if the resulting rates of chloroform metabolism correlated with toxicity previously measured by induced pathological lesions and regenerative cell proliferation. A Krumdieck Tissue Slicer was used to generate precision-cut kidney slices from male and female B6C3F1 and BDF1 mice and F344 rats. Chloroform uptake by the slices from the headspace of sealed flasks was measured by gas chromatography. Substantial variations in the rates of chloroform metabolism were observed between the kidney slices that correlated well with species, strain, and sex differences in chloroform-induced renal toxicity in vivo. These results are in agreement with data from CYP2E1 knockout (cyp2e1-/-) mice indicating that chloroform-induced cytotoxicity is obligatory to the production of toxic metabolites. Thus, this approach may be extended to examine the relative metabolic rates in rodent and human tissues as one part of a toxicodynamic model to better define quantitative, target organ-specific, chloroform toxicity and cancer risk assessments.

520 ROLE OF CYP1A2 IN UROPORPHYRIA CAUSED BY POLYHALOGENATED AROMATIC HYDROCARBONS USING CYP1A2 KNOCKOUT MICE.

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Uroporphyria, characterized by hepatic accumulation of uroporphyrin (URO), is produced in experimental animals by a number of Ah receptor ligands such as TCDD and hexachlorobenzene (HCB). CYP1A2 catalyzes the oxidation of uroporphyrinogen (UROX). Here we have investigated the requirement for CYP1A2 in mouse uroporphyria, by comparing ***Cyp1a2(-/-)*** knockout mice with ***Cyp1a2(+/+)*** wild-type mice. Uroporphyria was produced by injecting animals with iron dextran and giving the porphyrin precursor, 5-aminolevulinic acid, in the drinking water. Some animals also received 3-methylcholanthrene (MC) or HCB to induce hepatic CYP1A2. Uroporphyria was produced by these treatments in the wild-type mice, but not in the null mice. Uroporphyrinogen oxidation activity in hepatic microsomes from untreated wild type mice was 3-fold higher than in the null mice. Hepatic CYP1A2 in the wild type mice was increased by MC, and to a lesser extent, by HCB. Following MC-treatment, microsomal MROD to EROD ratios in wild type mice were twice those of null mice, reflecting the presence of CYP1A2. In wild type mice, treatment with MC increased hepatic microsomal UROX by 9 and 2-fold, and HCB by 2-fold, respectively, whereas there were no increases in the null mice. These results indicate the absolute requirement for hepatic CYP1A2 in causing experimental uroporphyria in mice treated with MC or HCB. (Supported by NIH grants ES 06321 and 06263, and by the Department of Veterans Affairs.)

521 INCREASED SUSCEPTIBILITY TO HYPEROXIC LUNG INJURY OF MICE LACKING THE CYP1A2 GENE.

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We reported earlier that 48 h of hyperoxia induces activities and contents of hepatic cytochromes P450 (CYP)1A1 and 1A2 in male Sprague-Dawley rats. In the current study, we tested the hypothesis that liver CYP1A enzymes play a role in lung injury induced by hyperoxia. Twelve week-old wild type (WT) (+/+) (C57BL/6J) male mice or mice lacking the gene for CYP1A2 (-/-), which is normally expressed only in the liver, were exposed to hyperoxia or room air for 48 or 72 h. Activities of ethoxyresorufin O-deethylase (EROD) and methoxyresorufin O-demethylase (MROD), which reflect primarily CYP1A1 and CYP1A2 enzymes, respectively, were determined in livers of these animals. Lung injury was evaluated by histological analysis of formalin-fixed lung tissues. While 11/12 of the (+/+) animals survived 72 h of hyperoxia, 4only 7/12 of the CYP1A2 (-/-) animals survived. The lungs of the surviving CYP1A2 (-/-) mice exposed to 48 or 72 h of > 95% O_2 displayed greater lung damage than similarly exposed wild type mice, suggesting that hepatic CYP1A2 serves protective functions in defenses against hyperoxic lung injury. Hepatic EROD activities, but not MROD activities, were lower (56 %) in WT mice exposed to hyperoxia for 48 h than in air-breathing controls, as determined by two-way ANOVA. The EROD activities returned to control levels after 72 h of hyperoxia. EROD activities in the CYP1A2 (-/-) animals were drastically lowered, by 96% and 85%, after 48 and 72 h of hyperoxia, respectively, than in air-breathing animals. The increased sensitivities of CYP1A2 (-/-) mice to lung injury and the decrease of hepatic EROD activities by hyperoxic exposure suggest an important role for liver CYP1A enzymes in the manifestation of lung injury induced by oxygen. (Supported in part by NIH grant ES09132 and by ALA of Texas.)

522 HEPATIC CYTOCHROME P450 EXPRESSION IN IL-6 GENE KNOCKOUT MICE FOLLOWING ENDOTOXIN ADMINISTRATION.

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Hepatic cytochrome P450 (CYP) expression has been shown to decrease following endotoxin (LPS) or pro-inflammatory cytokine (interleukin-1 [IL-1], IL-6, tumor necrosis factor α) administration. Using mice defecient in IL-6, the role of IL-6 in the regulation of various CYP isoforms following LPS administration was evaluated. Mice were given LPS (2 mg/kg i.p.) or saline (control) and euthanatized 24 hours following treatment. Constitutive EROD activity significantly increased in IL-6 knockouts (to 155.0 % of B6x129 mice). Administration of LPS (2 mg/kg i.p.) decreased ethoxyresorufin-O-dealkylase (EROD) activity and 6β hydroxytestosterone (OHT) formation in both normal B6x129 mice (to 67.6 % of control for EROD and 46.6 % of control for 6β OHT) and IL-6 knockout mice (to 48.0 % of control for EROD and 50.1 % of control for 6β OHT). However, no decrease was observed for pentoxyresorufin-O-dealkylase activity, 7α OHT formation, 16α OHT formation, or 6-hydroxychlorzoxazone formation following LPS administration in either B6x129 or IL-6 knockout mice. Hepatic lauric acid hydroxylase (LAH) activity significantly increased following LPS administration in B6x129 mice (to 223.0 % of control) with no effect in IL-6 knockout mice (108.4 % of control). These results support differences in species specific responses of CYP expression following LPS administration. In addition, LAH activity following LPS administration as well as constitutive EROD activity appears dependent upon IL-6.

523 A NEUROBEHAVIORAL DEVELOPMENTAL STUDY OF AMMONIUM PERCHLORATE ADMINISTERED ORALLY IN RINKING WATER TO RATS.

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Ammonium Perchlorate [AP], an oxidizer for solid rocket fuel, has recently been recognized as a persistent and pervasive contami-nant of water supplies. This GLP compliant study followed the 1996 US EPA Developmental Neurotoxicity guideline. Female Sprague-Dawley rats [25/group] were given continual access to 0 (Carrier), 0.1, 1.0, 3.0 and 10.0 mg/kg/day [MKD] AP in drinking water begin-ning on gestation day 0 and continuing through lactation day (DL) 10. On DL 12, 1_/1_ pup/litter/group were assigned to four subsets: 1) brain weights & juvenile neurohistopathology; 2) passive avoid-ance & watermaze testing; 3) motor activity & auditory startle habit-uation; and 4) regional brain weight & adult neurohistopathology. TSH, T_3 and T_4 serum levels were increased on DL 10 at all exposure levels in the Fo generation dams; thyroid weights and morphology were not affected. AP did not cause any neurobehav-ioral effects in the offspring when administered to dams during gestation and lactation at target exposures as high as 10.0 MKD. However, there was a significant increase in follicular epithelium hypertrophy and a significant decrease in the thyroid follicle size from the F1 generation male pups in the 10.0 MKD exposure group culled on DL 5. The 10.0 MKG F1 serum levels for TSH, T_3 and T_4 (pooled by litter) were significantly different than controls. No other effects in the F1 generation rats were observed. Cognitive functions (learning, memory, habituation) were not impaired in the pups at any time at any exposure level. These data demonstrate that AP is not a selective developmental neurotoxicant in the rat. Due to uncertainty in any toxicity associated to thyroid hormone alterations, the NOAEL for maternal toxicity in this study was 10.0 MKD (based on organ weight and histopathology). The NOAEL for pup toxicity was 3.0 MKD (based on the histopathology findings in the thyroid).

524 KINETICS OF RADIOLABELED IODIDE IN THE MALE SPRAGUE DAWLEY RAT.

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Iodide is actively transported by the sodium/iodide (Na^+/I^-) symporter, a carrier protein located in the basolateral membrane of the thyroid follicles. The objective of this project was to investigate the kinetics of iodide uptake, organification in the rat thyroid, tissue distribution and elimination in urine. A physiologically based kinetic model (PBPK) for iodide will be developed for examining the competitive inhibition of iodide uptake in the thyroid by perchlorate. Male Sprague Dawley rats were given two doses (2.6 pg and 33μ g/300 g rat) of ^{125}I via tail vein (6 rats per group). The latter dosing solution was prepared with cold and hot iodide. These doses do not inhibit iodide uptake in the rat thyroid (autoregulation). Thyroid gland and blood were collected at 5, 15, and 30 min, 1, 2, 6, 9 and 24 hours post dosing. Rats exposed for 24 h were placed in the metabolism cages to collect urine, and water and powdered food were freely available. Free and bound iodide was determined by precipitating bound iodide by trichloroacetic acid, and free and bound iodide was determined by a γ -counter. In the thyroid gland more than 90% of the radioactivity was bound within 2h and remained same till 24 h. In serum 70% of the radioactivity was free upto 6h and decreased to less than 10% at 24h. About 80% of the dose was excreted as free iodide in the urine in 24h. All the results were same in both doses.

525 TWO-GENERATION REPRODUCTION STUDY OF AMMONIUM PERCHLORATE IN RATS.

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Sprague-Dawley rats (30/sex/dose) were randomly assigned to either 0.0, 0.3, 3.0 or 30.0 mg ammonium perchlorate (AP)/kg/day in drinking water in a two-generation reproduction study. Concentrations were adjusted based on consumption data to approach the targeted dose. No deaths, abortions or premature deliveries in the P1 generation were attributed to the test substance, nor were clinical observations observed in the P1 generation. Mating and fertility parameters were unaffected by dosages as high as 30.0 mg AP/kg/day. No necropsy observations were related to the test substance in the P1 generation. Absolute thyroid weights for P1 male rats were increased in all three treated groups in a dosage-dependent manner and were significant at 3.0 and 30.0 mg/kg/day and in female rats at 30.0 mg/kg/day. Relative thyroid weight was increased at 30.0 mg/kg/day in both males and females. No significant results were found on sperm parameters or male reproductive function, nor were delivery observations or clinical and necropsy observations seen in the F1 generation pups. The weight of the thyroid and parathyroid glands was significantly increased for both male and female F1 generation pups at 30.0 mg/kg/day. Thus, the paternal rat NOAEL for AP is 0.3 mg/kg/day and the maternal rat NOAEL for AP is 3.0 mg/kg/day based on the significant increase in absolute thyroid weights. The reproductive NOAEL is greater than 30.0 mg/kg/day; no effects on mating or fertility occurred. The NOAEL for viability and growth in F1generation pups is 3.0 mg/kg/day based on the significant increase of thyroid and parathyroid weights in both male and female F1 generation pups. Based on these data, AP should not be identified as a reproductive toxicant.

526 DEVELOPMENTAL TOXICITY OF AMMONIUM PER CHLORATE IN RABBITS.

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Naturally-bred female New Zealand White rabbits were randomly assigned (25/dose) to six dosage groups of ammonium perchlorate (AP). Targeted doses of 0.0, 0.1, 1.0, 10.0, 30.0 and 100.0 mg AP/kg/day were continuously administered in the drinking water on gestational days 6 through 28. Concentrations were adjusted based on consumption data to approach the targeted dose. On day 29, blood was collected and analyzed for TSH, T3 and T4 levels. Gross necropsy was performed and the thyroids and parathyroids evaluated histologically. Pregnancy parameters were evaluated and fetuses were examined. There were no deaths attributed to the test substance. All clinical observations were considered unrelated to the test substance. Microscopic examination of the thyroid glands revealed hypertrophy of the follicular epithelium at 10.0, 30.0 and 100 mg/kg/day which was considered treatment-related. In the affected thyroids, the hypertrophy of the follicular epithelium was occasionally accompanied by a decrease in the lumen of the follicles. Serum T4 levels appeared to decrease in an exposure-related pattern with statistically significant decreases at the 30.0 and 100.0 mg/kg/day target doses. Dosages as high as 100.0 mg/kg/day did not affect day 29 Caesarean-sectioning or litter parameters. No fetal alterations were attributable to exposures of AP as high as 100.0 mg/kg/day. The maternal NOAEL for AP is 1.0 mg/kg/day based on a critical effect of hypertrophy of the follicular epithelium of the thyroid gland of the rabbit. The developmental NOAEL is greater than 100.0 mg/kg/day. Based on these data, AP should not be identified as a developmental toxicant.

527 ACUTE AND CHRONIC EFFECTS OF LOW DOSE PERCHLORATE ON HUMAN THYROID FUNCTION.

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Pre-shift and post-shift thyroid function profiles of subjects with occupational exposure to soluble perchlorate dust were obtained and compared with a larger number of control workers without occupational perchlorate exposure. Workplace perchlorate exposure measurements were made and analyzed using highly sensitive ion chromatography methodology. Equivalency of oral and respiratory doses could be assumed because of the high solubility of perchlorate salts at body temperature and because the target organ effects related to systemic absorption. Doses were estimated based on airborne concentrations and standard breathing rates. Calculated single shift doses ranging from 0.2 to 436 μ g/kg (average 36 μ g/kg) had no effect on thyroid stimulating hormone levels or the free thyroxine index at the end of the shift. Working lifetime cumulative doses ranging from 8,000 to 88,000 μ g/kg (average 38,000 μ g/kg) did not show any effect relative to control subjects on these same hormone levels. Average daily doses among exposed subjects were 1,000 times higher than doses attainable from drinking two liters of water from Lake Mead.

528 FETAX ASSAY OF AMMONIUM PERCHLORATE.

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Amphibian metamorphosis is thyroid driven. Addition of thyroid hormone accelerates development. Methods for inhibiting metamorphosis include hypophysectomy, thyroidectomy, and exposure to chemicals such as dinitophenol, thiocynate, thiouracil, and perchlorate salts. Perchloric acid and its salts have been used in a number of commercial applications ranging from wet digestions, organic synthesis, pyrotechnics, and missile propellants. Perchlorate has recently been found in the ground and surface water in several western states. The largest surface water concentrations of up to 1700 ppb have been found in a Lake Mead inlet. To investigate possible ecotoxicity ammonium perchlorate was tested using FETAX (Frog Embryo Teratogenesis Assay Xenopus) in accordance with ASTM guidelines. 100% embryo mortality is attained by 600 mg/L. The LC-50 for all tests is 496.1 mg/L. Malformations appear over a broader concentration range and the average EC-50 is 396.1 mg/L. The Tertatogenic Index or (TI) is 1.25 which suggest that ammonium perchlorate is a significant threat . Instead embryotoxicity is apparently the most significant threat. The NOEC (No Observed Effect Concentration) and LOEC (Lowest Observed Effect Concentration) concentrations for mortality are 271.8 and 356 mg/L respectively. Those for malformations are 203.3 and 247.2mg/L. The most dramatic malformation observed is the dorsal curvature of the tail which suggest abnormalities of in the notochord. Additionally malformations of the head and face were commonly observed. The head is reduced in size and appears flattened in anterior-posterior axis. These malformations are dose dependent in that more severe developmental consequences are found in embryos exposed to higher concentrations. In summary, the FETAX tests indicate that although severe malformations are caused, the TI and growth criteria suggest that perchlorate is embryotoxic and not teratogenic.

529 THYROID STATUS OF PERCHLORATE WORKERS.

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Thyroid examinations and thyroid function tests were conducted on workers at an ammonium perchlorate manufacturing plant to determine whether perchlorate exposure has adversely affected the thyroid status. Perchlorate is known to affect the thyroid as an inhibitor of iodine uptake. Thirty-seven perchlorate workers and twenty-one comparison workers were examined. Both respirable and total particle perchlorate inhalation during a 12-hour work shift were measured, as was urinary perchlorate excretion. Perchlorate concentrations were measured in urine samples obtained both before and after a 12-hour work shift. Total particle perchlorate was a better measure of perchlorate exposure than was respirable perchlorate. The exposure groups (high, medium, and low) averaged 30 mg/day, 4 mg/day and 0.3 mg/day, respectively. The workers in the comparison group averaged an exposure of 0.01 mg/day. Blood was drawn at the end of the shift for a blood chemistry panel, a complete blood count, and thyroid function studies. No differences in the distribution of thyroid function test results (including thyroid stimulating hormone and free thyroxine index) existed across the exposure groups or with respect to the comparison group. Further, no evidence of hematotoxicity, nephrotoxicity, or hepatotoxicity was found. A three-day post-exposure study of two highly exposed workers indicated an excretion half-time of about eight hours. Long-term occupational exposure to perchlorate at levels at or exceeding 30 mg/day did not demonstrate an adverse effect on the thyroid.

530 PERCHLORATE IN DRINKING WATER AND RISK OF CONGENITAL HYPOTHYROIDISM.

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Perchlorate has been detected in the public water supply for much of Southern California and Southern Nevada at levels of 4-16 μ g/l (ppb). Since perchlorate is a known inhibitor of iodine uptake by the thyroid, an investigation has been carried out to examine whether maternal exposure to perchlorate through the drinking water supply has increased the risk of congenital hypothyroidism. The US Environmental Protection Agency (EPA) has identified one county in Nevada and six counties in California with perchlorate in the water supply. The water source for 96% of the supply for Clark County, Nevada (Las Vegas) has been found to contain perchlorate, as does the Colorado River which is an intermittent source of water for much of Southern California. This investigation has sought to determine whether the congenital hypothyroidism rate is increased in the counties with perchlorate-containing water. The state health departments maintain a neonatal screening program for congenital hypothyroidism using heel-stick blood samples. The county-specific 1996 and 1997 congenital hypothyroidism screening data for California and Nevada have been obtained from the respective health departments. The congenital hypothyroidism birth prevalence rate for Clark County did not differ from that for the rest of the state. The California data also showed that the rates did not differ. The California data have been adjusted for ethnicity. The observed to expected ratios for the six counties together was 1.0 (95% confidence limits, 0.9-1.2). The ratios for the individual counties ranged between 0.6 and 1.1. These data do not support the hypothesis that these levels of perchlorate in the drinking water have increased the rate of congenital hypothyroidism. (Funding was provided by American Pacific Corp.)

531 PERCHLORATE ENVIRONMENTAL CONTAMINATION: TESTING STRATEGY BASED ON MODE OF ACTION.

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Perchlorate (ClO_4^-) is an anion that originates as a contaminant in ground and surface waters from its readily dissolved salts of ammonium, potassium or sodium. The anion is exceedingly mobile and stable under environmental conditions. A recent increase in detection capabilities led to discovery of ClO_4^- at various sites in CA, NV, UT, AZ, TX, AK, NY and MD. The existing clinical and toxicity database on ClO_4^- in March 1997 was determined by an expert peer review panel to be inadequate as the basis for an oral reference dose (RfD). No data on its carcinogenicity were available. Consideration of the known mode-of-action (MOA) for ClO_4^- at the time, competitive inhibition of iodide uptake into the thyroid gland, was used to construct a testing strategy that targeted potential endpoints indicated by disruption of the thyroid-pituitary-hypothalamic axis as well as provided data critical for health assessment approaches. Specific concerns included the potential for developmental toxicity, notably neurological development. Tests were also included to determine the genotoxic effects of ClO_4^-, with a recovery satellite to evaluate the reversibility of thyroid hyperplasia after cessation of exposure, in order to characterize its potential for thyroid neoplasia. Interspecies aspects were also a design factor. The MOA strategy allowed an expedited evaluation of a widespread contaminant without a 2-year assay.

532 HARMONIZED HEALTH RISK ASSESSMENT FOR PERCHLORATE BASED ON MAPPING ITS MODE OF ACTION(MOA).

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Perchlorate anion (ClO_4^-) competitively inhibits iodide uptake into the thyroid. A testing strategy that utilized this information confirmed that the primary target tissue for ClO_4^- is the thyroid gland in rats, rabbits, and mice. Perturbations of thyroid hormone economy were measured by monitoring T3, T4 and TSH levels at various time points. The challenge to the health risk assessment was to integrate this information to map toxicity, in adult versus developing organisms, against T3, T4 and TSH levels as internal biological markers of response. Thyroid hyperplasia in the adult rats can be mapped along a pathogenesis continuum that could include neoplasia. The lack of evidence for direct action on DNA from a genotoxicity testing battery, together with demonstration of recovery of hyperplasia after cessation of exposure, supported proposal of an indirect mechanism for potential carcinogenicity. The lack of any other target toxicity in adult animals allowed harmonization of the noncancer and cancer effects based on the adverse T3, T4 and TSH levels correlated with hyperplasia. Consideration of developmental toxicity, however, required that hormone levels be mapped against lesions observed in the neonatal thyroid and neurobehavioral effects. Pharmacokinetic data will be used to refine the MOA approach as well as influence the magnitude of the uncertainty factors for intra- and interspecies extrapolation.

533 DOSE-RESPONSE AND PHYSIOLOGY PERTINENT TO DEVELOPMENT OF A REFERENCE DOSE FOR PERCHLORATE.

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Recent application of a sensitive analytical method led to detection of perchlorate in major drinking-water supplies in Nevada, California, and Arizona. Using standard assumptions for drinking-water consumption and body weight for an adult male, the highest level of perchlorate measured, 16 ppb, leads to an estimated perchlorate intake of 0.00046 mg/kg-d. The current provisional RfD for perchlorate is based on an acute exposure study in Graves'-disease patients pretreated with an inhibitor of iodide organification (Stanbury and Wyngaarden, 1952). The dose-response for the effect observed in that study (acute discharge of iodide from the organification-blocked thyroid) is unlikely to be predictive of the dose-response for the critical health effect defined by US EPA (functional disturbance of the thyroid-pituitary axis) under conditions of chronic exposure. US EPA is in the process of reevaluating the RfD, taking into account new toxicity data from four drinking-water-exposure studies of ammonium perchlorate in experimental animals: subchronic, developmental, neurobehavioral developmental, and reproductive. The lowest ammonium perchlorate dose tested in each of these four studies was 0.01, 0.1, 0.1, and 0.3 mg/kg-d, respectively. The dose spacing was such that a factor of 5 or 10 between doses was common. Such large gaps between dose levels can lead to several-fold underestimation of the no-observed-adverse-effect level (NOAEL). Analysis and comparison of uncertainties associated with NOAEL-based and benchmark-dose (BMD) approaches to derivation of possible RfD values, given the dose levels employed, demonstrates the superiority of the BMD approach. Another consideration in the derivation of a perchlorate RfD is thyroid homeostasis, *i.e.*, the maintenance of normal thyroid function over a broad range of thyroid-hormone and thyrotropin levels in serum. Treatment-related changes in these

hormone levels should not be considered adverse as long as the levels remain within normal physiological bounds. Consideration of the underlying physiology, and not just the statistical significance of changes, should be incorporated consistently into risk assessment methodology.

534 A 90-DAY DRINKING WATER TOXICITY STUDY IN RATS WITH AMMONIUM PERCHLORATE.

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Ammonium Perchlorate (AP) is an oxidizer that has been used as a component of solid rocket fuel. Perchlorate, the dissociated ion of AP, has been recently recognized as a persistent and pervasive contaminant of drinking water supplies in a number of major metropolitan areas. Current efforts at assessing the health risks of perchlorate have been hampered by a lack of relevant toxicity data and by a lack of understanding of the potential toxic mechanism(s) of action. The purpose of this study was to evaluate the potential toxic effects of AP when administered to rats in the drinking water for 14 or 90 days at dosage levels of 0.01, 0.05, 0.2, 1.0 and 10.0 mg/kg/day (10 rats/sex/group/exposure period). Additional animals in the control, 0.05, 1.0 and 10.0 mg/kg/day groups (10/sex/group) were scheduled for euthanasia following a 30 day nontreated recovery period to assess the reversibility or persistence of effects. No toxicologically meaningful differences were observed among the groups with respect to clinical observations, body weights, food and water consumption, hematology and clinical chemistry parameters, ophthalmology, male sperm parameters, female estrous cyclicity, or bone marrow micronuclei formation. Increased thyroid weights and corresponding follicular cell hyperplasia were observed after 14 and 90 days at the 10.0 mg/kg/day level. These changes were reversible after the 30 day recovery period. Significant changes in thyroid hormones (primarily increased TSH and decreased T_3 and T_4) were observed at levels of 0.01 mg/kg/day and above; however, no thyroid organ weight or histopathological effects were noted at levels of 1.0 mg/kg/day and lower. Based on the absence of organ weight and histopathological effects, 1.0 mg/kg/day was considered the no-observed-adverse-effect level (NOAEL) for the study. In the absence of other effects, the toxicological significance of thyroid hormone alterations remains to be determined.

535 EFFECTS OF AMMONIUM PERCHLORATE ON THYROID, HEMATOLOGICAL, AND IMMUNOTOXICOLOGICAL PARAMETERS.

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The use of AP as an oxidant in solid propellants has resulted in soil and ground water contamination and the potential for human exposure via ingestion. As part of an effort to create a toxicological database that could serve as the basis for regulatory decision making, this study was undertaken to assess thyroid, hematological, and immunological effects of AP. Adult, female $B6C3F_1$ mice were administered AP in their drinking water (0, 0.1, 1.0, 3.0, or 30 mg/kg/day) for 14 or 90 days, after which several toxicological endpoints were examined. These same endpoints were also examined 30 days after a 90-day drinking water exposure to assess the reversibility of any observed effects. Preliminary results indicate that the AP concentrations examined had no significant effect on body and organ weights, red blood counts and indices, hemoglobin levels, hematocrit, white blood counts and differentials, cytotoxic T cell activity, and splenocyte CD4/CD8 subpopulations. In addition, there was no change in nitrite production by peritoneal macrophages after 14-day exposure to AP. This is in contrast to increased nitrite production observed after 90-day exposure to 3 and 30 mg/kg/day AP. Phagocytosis of *Listeria monocytogenes* by peritoneal macrophages was decreased by as much a 50% after 14 and 90-day exposure to AP. This effect was reversible when measured 30 days post-exposure. No changes were detected in serum T_4 levels after 14 days at any dosage level. Exposure for 90-days to 30 mg/kg/day AP significantly affected T_4 levels by 30%, however, these levels returned to normal when measured 30-days post-exposure. Thyroid stimulating hormone levels (TSH) were not significantly affected in any of the treatment groups after exposure to AP for 90-days. To identify histological changes after AP exposure, thyroid from mice exposed to AP have also been submitted for pathology. While preliminary, results suggest that in mice, AP might have immunotoxic effects at dosage levels at or below those inducing changes in thyroid hormone levels. (Funded by DSWA01-97-0008.)

536 COMPARISON OF END-POINTS TO DETECT PULMONARY PHOSPHOLIPIDOSIS FOLLOWING INHALATION EXPOSURE OF RATS AND DOGS.

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The pulmonary response of Wistar rats and beagle dogs to single and repeated nose-/head-only inhalation exposures to an aerosolized dicationic amphiphilic drug (DCAD) was examined using either a high (hydrochloride) or low (sulfate) water soluble salt of the drug. The objective of this study was to test the hypothesis whether DCAD-aerosols interact directly with surfactant by formation of surfactant-DCAD precipitates and whether high-/low soluble particles differ in response. Bronchoalveolar lavage (BAL) was used to evaluate the following end-points: neutrophil and macrophage numbers, protein, phosphatidylcholine in BAL-fluid and -cells, lactate dehydrogenase (LDH), alkaline and acid phosphatase, and β -N-acetylglucosaminidase (β -NAG). At higher exposure doses the results indicate that the most salient finding in both species was a pulmonary lipoproteinosis elicited by increased loading of phospholipids in alveolar macrophages. This response was associated with increased activities of β -NAG, alkaline and acid phosphatase, and LDH. In regard to the relative magnitude of response, pulmonary lipoproteinosis appears to precede the other effects. Appreciable differences in response to low or high water soluble particles were not observed. It appears, however, that higher exposure doses of low-soluble dust particles might stimulate more effectively their phagocytosis by alveolar macrophages and, subsequently, the test agent is leached from this cell in a more protracted manner. Toxicokinetic evidence demonstrated a concentration x time - dependent accumulation of DCAD in lung tissue and BAL-cells. Nevertheless, concomitant adverse effects occurring in lung parenchyma were not observed. In summary, after dosimetric adjustment, the data obtained in rats and dogs demonstrate similar response.

537 A NEW RESPIRATORY MONITORING SYSTEM FOR CONSCIOUS PRIMATES DURING INHALATION EXPOSURES POSSIBLY DEMONSTRATING A CORRELATION BETWEEN RESPIRATORY RATES AND HISTOPATHOLOGICAL CHANGES IN THE LUNG.

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A technique for respiratory monitoring of conscious primates has been developed for use during inhalation exposures to a gas/vapour, liquid aerosol, or solid aerosol of chemicals or drugs. This respiratory monitoring can include measurements of the respiratory rate, duration of inspiration, duration of expiration, duration of end inspiratory pause, and the number of discontinuous flow events. A review of data from two studies was conducted to determine if respiratory rates could be indicative of other toxicological findings. During a 7-day primate study, the respiratory rate was monitored at pre-exposure and on days 1 and 7. The percentage of the pre-exposure respiratory rates on day 1 (mean ± s.d.) were 101 ± 11%, 153 ± 24%, and 199 ± 28%; and on day 7, these values were 102 ± 11%, 134 ± 18%, and 173 ± 20% for the low, mid, and high groups, respectively. The histological examination demonstrated the presence of bronchiolitis involving terminal and alveolar bronchioles in 1 low group male, 1 intermediate group female, and all high group animals. For a 26-week primate study of a protein, respiratory rate was monitored. No statistically significant exposure-related respiratory changes were observed, however, a slight increase in the respiration rate at the high group was noted at the end of the study. Histopathological evaluation of the lungs revealed no statistically significant changes. Consequently, a new procedure has been established for measuring respiratory parameters during inhalation exposures, permitting an immediate assessment of the pulmonary responses to exposures of drugs/chemicals. Additionally, data produced during two studies suggested that changes in respiratory rates might indicate some histopathological changes in the lung.

538 SUBCHRONIC EXPOSURES OF RATS TO AN AEROSOL OF GENERIC COMMERCIAL ENGINE OIL.

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A 13-week inhalation study was performed with aerosolized Generic Commercial Engine Oil (GCEO). Rats (15/sex) were exposed 6 hr/d, 5 d/wk to 0 (both untreated and sham-exposed controls), 50, 150, or 400 mg/m³. An additional 10 males were in each group for pulmonary function tests at the end of exposures. No treatment-related effects were noted during the course

of exposures and no changes were found in body weights, clinical observations, serum chemistry, or the number and morphology of sperm at the terminal sacrifice. In the blood, the percentage of segmented neutrophils increased and the percentage of lymphocytes decreased in a concentration-related manner at 13 weeks of exposure. Otherwise, the lung was the primary organ affected by exposure. Increased lung weight and grossly visible plaques occurred in a concentration-related manner. Histologically, foamy alveolar macrophages accumulated in the alveoli. Lung volumes, pressure-volume curves, and maximal forced exhalations were not significantly different from the control groups. The lack of a significant tissue reaction in the lung, other than increased macrophages, after exposure to aerosol concentrations as high as 400 mg/m³ indicates a relatively low order of toxicity for GCEO.

539 INHALATION TOXICITY STUDIES OF METHYLVINYL KETONE IN RATS AND MICE.

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Methylvinyl ketone (MVK) is a prototype of the straight-chain aliphatic α,β-unsaturated ketones and is used in the production of pesticides, perfumes, plastics and resins and as a pharmaceutical intermediate. Exposure to MVK is widespread due to its presence in cigarette smoke and its production by γ-irradiation of sugars in fruit. Although occupational and consumer exposure to MVK has been documented, health effects testing has been inadequate. Prechronic inhalation studies were conducted to provide data required to design chronic toxicity and carcinogenicity studies of MVK. In initial studies, rats and mice were exposed to 0, 0.25, 0.5, 1, 2, 4, or 8 ppm MVK 6 hr/day, 5 days/week for 12 days. No toxicity was observed in animals exposed to up to 1 ppm. Morbidity and early deaths occurred in male and female rats (day 1) and male mice (day 10) exposed to 8 ppm. Rats exhibited nasal cavity toxicity and lung necrosis at 4 ppm. Decreased body and liver weights were observed in male rats exposed to 2 ppm. Mice appeared to be less sensitive than rats to the toxic effects of MVK. Based on these results a 13-week study in mice and rats was conducted at 0, 0.5, 1, and 2 ppm MVK. As in the first study, the respiratory tract was the main target organ and mice were less sensitive than rats to inhaled MVK.

540 TWO-WEEK (TEN-DAY) INHALATION TOXICITY AND TWO-WEEK RECOVERY STUDY OF PHENOL VAPOR IN THE RAT.

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The toxicity of phenol vapor was evaluated in male and female Fischer 344 rats (20/sex/group) via flow-past nose-only inhalation exposure. The test animals were exposed to target concentrations of 0 (air control), 0.5, 5 or 25 parts per million (ppm) of phenol in air for 6 hours/day, 5 days/week for two weeks. HPLC (high pressure liquid chromatography) measurement of phenol test atmospheres determined mean (±standard deviation) analytical concentrations of 0.0±0.0, 0.52±0.078, 4.9±0.57 and 25±2.2 ppm, respectively. Test animals were examined daily for signs of toxicity. Body weights and food consumption were measured weekly. After two weeks of exposure, ten test animals/sex/group were sampled for clinical chemistry and hematology parameters. The test animals were then sacrificed, organs weighed and tissues collected. Histopathological examination included the nasopharyngeal tissues, larynx, trachea, lungs with mainstem bronchi, kidney, liver and spleen. The remaining ten animals/sex/group were retained for a two-week recovery period. Recovery groups of animals were then sampled and sacrificed as described previously. No signs of toxicity in clinical observations, body weights, food consumption, clinical pathology, organ weights, macroscopic pathology or microscopic pathology were seen during the exposures or at either sacrifice interval. In conclusion, two-week inhalation exposures to phenol vapor at concentrations up to and including 25 ppm did not produce any adverse effects. (The Phenol Panel of the Chemical Manufacturers Association sponsored this study.)

541 TISSUE AND SERUM MARKERS OF JP-8 EXPOSURE: TWO-DIMENSIONAL PROTEIN MAPPING.

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We analyzed protein expression in the cytosolic fractions of cerebellum, cerebrum, kidney, liver and lung tissues and serum samples from male Swiss-Webster mice exposed 1 h/day for 7 days to aerosolized JP-8 jet fuel at 1000 and 2500 mg/m³, simulating occupational exposure. Cytosol/serum samples were solubilized and separated via large scale, high resolution two-dimensional electrophoresis (2-DE) and gel patterns scanned, digitized and processed for statistical analysis. Quantitative changes in tissue cytosol proteins resulting from 2500 mg/m³ exposure were significant (P<.001) for 12 cerebellar, 10 cerebral, 65 kidney, 12 liver, 42 lung and 4 serum proteins. In comparison, the 1000 mg/m³ exposure altered 5 cerebellar, 5 cerebral, 7 kidney, 2 lung, 3 kidney and 0 serum proteins. At the 2500 mg/m³ exposure, of all the proteins altered, only 19 kidney, 2 liver, 14 lung and 3 serum proteins changed quantitatively by 2-fold or more. None of the proteins underwent >3-fold alterations in abundance. These results demonstrate a significant but comparatively moderate JP-8 effect on protein expression, particularly in kidney and lung, and corroborate previous morphological and biochemical evidence. Further molecular biomarker development and mechanistic inferences from these observations await proteomic analysis of whole tissue homogenates and cell compartments, *i.e.* mitochondria, microsomes, and nuclei of these and other targets. (Supported by AFOSR Grants F49620-96-1-0156 and F49620-96-1-0074.)

542 AN INHALATION ONCOGENICITY AND CHRONIC TOXICITY STUDY OF PHOSPHINE IN RATS.

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Phosphine is a highly toxic gas used as a fumigant, a dopant in semiconductor manufacturing, and in the production of organophosphines. In a chronic toxicity and oncogenicity study of phosphine, 60 male and female F344 rats per group were exposed via whole-body inhalation for 6 hours per day, 5 days per week for up to 104 weeks to mean concentrations of 0, 0.3, 1, or 3 ppm of phosphine. Ten per sex per group were sacrificed after 52 weeks of exposure. Survivors were sacrificed after 104 weeks of exposure. There were no test article-related effects seen on clinical observations, body weight, food consumption, hematology, clinical chemistry, urinalysis, or ophthalmology. There were no test article-related macroscopic findings or effect on absolute or relative organ weights. No histomorphologic alterations attributable to test article exposure were seen. In conclusion, under the conditions of this study, there were no treatment related changes suggestive of a toxic or carcinogenic effect seen in rats following 52 weeks and 2 years of whole-body inhalation exposure to 0.3, 1, or 3 ppm of phosphine. (This research was supported by the 1996 Phosphine Task Force.)

543 FOURTEEN-DAY INHALATION TOXICITY STUDY OF N-ETHYL-M-TOLUIDINE IN RATS.

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N-Ethyl-m-toluidine (NEM) is a chemical intermediate used in the manufacture of photographic developers. To study the effects of repeated inhalation exposure, 5 male and 5 female albino rats were given whole-body exposures to either air, 5.6, 32.8, or 67.6 ppm NEM for 6 hours per day, 5 days per week over a 14-day period. A satellite group of 5 males at each exposure concentration was exposed similarly and used for methemoglobin determinations. Additional groups of 5 rats/sex were added to the air exposure and high exposure groups and observed for 14 days post-exposure to assess recovery. Core toxicology and recovery animals were bled for clinical pathology, euthanized and necropsied the day after final exposure and recovery period observation, respectively. There were no deaths during the study and no significant changes in mean body weight by comparison to controls. The 5.6 ppm group had decreased methemoglobin and hemoglobin, erythro-

poiesis in the bone marrow, liver, and spleen, and congestion of the spleen. In addition to these effects, the 32.6 and 67.6 ppm core groups had increased spleen weight; increased leukocytes, lymphocytes, mean corpuscular volume and mean corpuscular hemoglobin; decreased erythrocytes and hematocrit, macrophage pigmentation in the spleen; and hyaline droplet formation and tubule cell pigmentation in the kidney. Males in the high exposure core group had increased incidence and severity of nephropathy. While the incidence and severity of findings were generally reduced, the 67.6 ppm recovery group still had decreased methemoglobin, increased mean corpuscular volume, enlarged spleens, erythropoiesis in the bone marrow, and macrophage pigmentation in the spleen; and, in addition, platelets were decreased in males. Findings were consistent with hemolytic anemia.

544 TOXICITY OF BREVETOXIN-2 IN RATS FOLLOWING ACUTE AND REPEATED ADMINISTRATION VIA THE RESPIRATORY TRACT.

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Brevetoxins are cyclic polyether neurotoxins produced by the marine dinoflagellate, *Ptychodiscus brevis*. Blooms of *P. Brevis* (red tides) can be lethal to fish and marine mammals and toxic to humans. Although a common route of human exposure is through inhalation of seaspray aerosols, the health effects associated with repeated inhalation of low levels of brevetoxin remain unknown. The purpose of these studies was to evaluate the toxicity of brevetoxin administered to rats via the respiratory tract. Acute toxicity was estimated by administering 100 - 200 μ g/kg brevetoxin 2 (PbTx-2) to male F344/Crl Br rats by intranasal (IN) and intratracheal instillation (IT; one rat per dose). Clinical signs of toxicity included gasping and dyspnea; no rats died. The median lethal concentration (LC50) for PbTx-2 administered IT is 100 - 150 μ g/kg. This is approximately twice the estimated LC50 for PbTx-2 administered iv to rats (Templeton *et al*, 1989). For repeated exposures, groups of 5 male F344/Crl Br rats were administered saline (controls) or 100 μ g/kg PbTx-2 IN for 5 consecutive days. PbTx-2-exposed rats lost weight. Clinical signs included ataxia, gasping, and dyspnea. Total cells recovered in bronchoalveolar lavage fluid were not different from control values. Numbers of neutrophils and lymphocytes were increased. No DNA damage to the lavage cells was detected using the COMET single cell gel electrophoresis assay. Histopathological evaluation of the respiratory tract is being conducted. Results of these studies indicate that PbTx-2 is less acutely toxic when administered IT or IN than when administered iv and that repeated inhalation may cause pulmonary inflammatory and immune responses.

545 DEVELOPMENT OF A RECIRCULATING, NOSE-ONLY EXPOSURE SYSTEM TO STUDY UPTAKE OF [14C]-STYRENE.

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An exposure of rats and mice to [14C]-styrene was conducted to study the disposition of styrene. An exposure system for conducting nose-only exposures was adapted to recirculate a portion of the flow. The purpose of the recirculating portion was to reduce the total amount of [14C]-styrene required from a single-pass dynamic system operating at an equivalent air flow. A condenser was used to remove moisture from the system, and soda lime was used to remove carbon dioxide. Oxygen was also added to the system to maintain levels above 19%. The labeled styrene was allowed to evaporate in a stainless steel pressure vessel and then pressurized with nitrogen to a predetermined level. The styrene mixture was added to the recirculated atmosphere to maintain 160 ppm. A group of 12 rats and two groups of 30 mice were exposed separately to 160 ppm styrene over a 6-hr period. The amount of styrene used for the rat exposure was approximately 60% of what would be required for a single-pass nose-only exposure. The amount was approximately 90% for the mouse exposures. Styrene uptake was calculated from the rate of styrene addition to the recirculating system. Uptake was calculated to be 79 μmol/kg/hr for the rats and 135 μmol/kg/hr (average of two experiments) for the mice. The rate of styrene uptake was relatively stable over the 6-hr exposure for the rats, while the rate decreased steadily over the 6-hr period for the mice. (This study was supported by the Styrene Information and Research Center.)

546 ASSESSMENT OF OCCUPATIONAL EXPOSURE OF WELDERS TO MANGANESE.

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Exposure to high levels of Mn can lead to toxic effects, notably on the respiratory and central nervous systems. In the workplace, welding operations represent a potential source of exposure to Mn. Recently, ACGIH adopted a new Threshold Limit Value (TLV) of 200 μg/m³ (TWA) for Mn. This study aims to assessing the occupational exposure of welders to Mn. We measured the exposure of 10 welders during a 5-day work period using personal sampling pumps. Total Mn (Mn_T) and respirable Mn (Mn_R, 5 μm) were quantified on filters by neutron activation. Values obtained ranged from 27.4 to 496.5 μg/m³ with a mean of 191.1μg/m³ for Mn_T and from 19.4 to 257.5 μg/m³ with a mean of 114.6 μg/m³ for Mn_R. The mean Mn_R/Mn_T ratio was 60%. These concentrations are lower than the Quebec permissible exposure level of 5000 μg/m³ (Ceiling). However some are higher than, 1) the ACGIH level of 200 μg/m³ and, 2) recent reported Mn concentrations which are associated with observable health effects. This on going research project will provide additional information that will be used eventually in the review of Quebec permissible level.

547 A SMALL ANIMAL PLETHYSMOGRAPH/EXPOSURE TUBE FOR DETERMINATION OF RESPIRATORY MECHANICS DURING EXPOSURE, USING NON-INVASIVE METHODS TO MEASURE INTRAPLEURAL PRESSURE.

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The real-time measurement of changes in respiratory mechanics, primarily dynamic compliance (C_{dyn}) and airway resistance (R_L), is often used to assess the acute pulmonary toxicity of inhaled irritants; particularly those thought to cause an airways hyperreactivity response. Simple volume displacement plethysmography for determining ventilation in spontaneously breathing animals can be adapted for the determination of C_{dyn} and R_L by including the simultaneous measurement of intrapleural pressure (Ip). Accurate estimates of Ip can be obtained by measurement of esophageal pressure (Pes) using transoral insertion of a water filled cannula . Measurement of Pes does not require surgical insertion of a cannula through the chest wall as is required for measurement of Ip. However, the use of conventional head-out plethysmography to measure ventilation and respiratory mechanics during exposure, often precludes the use of transoral insertion of an esophageal cannula to measure Pes. Thus invasive methods must be used to measure Ip. We have developed a combination head-out plethysmograph/nose-only exposure tube (PET) suitable for measurement of R_L and C_{dyn} using transoral cannulation for determination of Pes. The cannula neither obstructs the nose of the animals nor prevents insertion of the PET into a nose-only exposure chamber port. Experiments conducted in 25 rats demonstrated that the insertion of the esophageal cannula did not significantly alter ventilation (with exception of an increase in peak inspiratory pause time). R_L and C_{dyn} in measured in normal rats using the PET did not differ from RL and Cdyn determined using more conventional plethysmographic methods.

548 A SIMPLE, INEXPENSIVE SOLID FUEL FURNACE FOR SMALL SCALE COMBUSTION TOXICITY STUDIES.

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Red oak combustion atmospheres produced by in an inexpensive furnace designed for use in combustion toxicity studies were compared to similar atmospheres produced by more conventional combustion furnaces. The furnace is based on a simple 1.5 ohm resistance wire heater which achieves temperatures ranging from ambient to 640 °C. The heater temperature produced was linear with input voltage over this working range. Combustion occurred in three phases (initial non-flaming, flaming, and post flaming). In the non-flaming phase, the sample charred and smoked, produced carbon monoxide (CO) concentrations ranging from 2600-4600 ppm, and carbon dioxide (CO_2) ranging from 3300 to 3700 ppm. Carbon monoxide to CO_2 ratios were from 0.8 to 1.3 for the non-flaming phase. In the flaming phase

CO_2 concentrations ranged between 6-11% and CO concentrations ranged from 200-300 ppm. In the final smoldering phase CO levels rose to 2000 ppm and CO_2 levels dropped to 1%. Aerosol size distribution was examined using a time of flight, laser optical, aerodynamic particle sizer. Typical aerosol concentration during the non-flaming phase was 1.70×10^7 particles/cm^3 (1.5 g/m3). Aerosol mass median aerodynamic diameter was 0.84 μ m.

549 THE GENERATION OF RESPIRABLE PARTICULATE AND FIBERS FROM THE PARTIAL PYROLIZATION OF ADVANCED COMPOSITE MATERIALS.

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The increasing use of advanced composite materials (ACM) in weapons platforms and commercial applications has stimulated interest in their potential combustion toxicity. A system based on a modified UPITT II mini-cone calorimeter was used to characterize ACM combustion atmospheres. Partial pyrolysis of ACM (26 g at 625 C°) generated aerosols composed of small, nearly spherical particles, agglomerates of these particles and respirable fibers. Vapor and gas phase chemistry from this ACM generated pyrolysate atmosphere has been reported previously (Kuhlmann et. al. 1997). Aerosol particles were collected using inertial impaction and electrostatic precipitation methods. SEM samples revealed 3 populations of particles: agglomerates, small spherical particles and fibers. Agglomerate chains ranged in size from (approximately 500 nm in length up to non-respirable sizes). Individual particles were (approximately 100 nm diameter up to 450 nm diameter). Fibers were found to be relatively homogenous (approximately 200 nm x 500 nm up to approximately 500 nm x 10 μ m). Energy dispersive x-ray (EDX) of fibers showed them to be predominantly silica, carbon, titanium, copper and aluminum. The combination of these collection techniques coupled with scanning electron microscopic examination and spectral processing and analysis of particle morphometry have provided conclusive evidence for the presence of respirable fibers, though their formation/liberation from pyrolysate ACM generated atmospheres.

550 A 13-WEEK INHALATION TOXICITY STUDY (WITH RECOVERY) OF AMMONIUM PERSULFATE IN ALBINO RATS.

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The subchronic inhalation toxicity of ammonium persulfate was characterized using Sprague-Dawley rats (20/sex/group) at respirable dust concentrations of 0, 5.0, 10.3 and 25 mg/m3. Whole-body exposures were conducted 6 hours/day, 5 days/week for 13 weeks. Gravimetric airborne test material samples were taken daily from each exposure chamber. Particle size samples were taken weekly from each exposure chamber. Ten animals/sex/group were necropsied after 13 weeks of exposure and 5 animals/sex/group were held for either a 6- or 13-week non-exposure recovery period. Animals were observed for clinical signs of toxicity, effects on body weight, food consumption, clinical pathology, ophthalmologic parameters, organ weights and macroscopic and microscopic pathology. There were no exposure-related deaths during the study. Rales and increased respiration rate were noted in both males and females in the 25 mg/m3 group, and in a few animals in the 10.3 mg/m3 group. The incidence of these clinical signs decreased to zero during the first few weeks of the recovery period. Body weights for both males and females in the 25 mg/m3 group were significantly depressed during most of the exposure period when compared to the control group. Body weights completely recovered during the recovery period. Lung weights were elevated in the 25 mg/m3 group after 13 weeks of exposure, but were similar to controls at 6 weeks post-exposure. Irritation of the trachea and bronchi/bronchiole was noted microscopically after 13 weeks of exposure to 25 mg/m3. These lesions had recovered by 6 weeks post-exposure. Based on the results of this study, the NOEL for exposure of rats to a dust aerosol of ammonium persulfate was 5.0 mg/m3, while the NOAEL was 10.3 mg/m3.

551 ACUTE INHALATION TOXICITY OF ETHYLAMINE, DIETHYLAMINE AND TRIETHYLAMINE - EFFECTS ON RODENT NASAL PASSAGES.

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To address occupational toxicology needs, the acute inhalation toxicity of ethylamine (EA), diethylamine (DEA) and triethylamine (TEA) was investigated using F344 rats, and B6C3F1 mice (DEA only). Groups of 4-5 animals were exposed via whole body inhalation to each amine at 250 or 1000 ppm for 6 hrs/day for a total of 10 exposure days. Body weights and histopathological evaluations of major organs including the nasal passages were conducted. In general, mean body weights were unchanged or decreased slightly in the groups exposed at 250 ppm. In contrast, mean body weights were significantly decreased in both sexes from all groups exposed at 1000 ppm, regardless of amine. No deaths occurred in rats exposed to EA or to the other amines at 250 ppm. Two of five male and 1/5 female rats exposed to 1000 ppm TEA died. Four of five male and female rats and 1/5 male and female mice exposed at 1000 ppm DEA also died. Histopathology of the major organs was unremarkable except for the upper respiratory tract and nares. Treatment-related effects seen in the nasal passages of animals exposed at 1000 ppm ranged from moderate necrotizing inflammation (EA, TEA) to squamous metaplasia and turbinate atrophy (DEA, both species). Effects seen in the nasal passages of animals exposed at 250 ppm ranged from slight necrotizing inflammation (EA) to squamous metaplasia and localized turbinate atrophy (DEA, both species). No effects were seen in the nasal passages of rats exposed at 250 ppm TEA. Squamous metaplasia of the tracheal mucosa was found in rats exposed to 1000 ppm TEA and 1000 ppm DEA. Ulcerative tracheitis was also found in rats exposed to 1000 ppm DEA. Squamous metaplasia of the trachea and the mainstem bronchi was found in mice exposed to 1000 ppm DEA. Based on acute toxicity to the nasal passages, the ethylamine series is ranked as follows: DEA > EA > TEA.

552 EFFECTS OF DIFFERENT CELLULOSE-CONTAINING RESPIRABLE SAMPLES IN THE LUNG OF FISCHER 344 RATS.

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Persistent inflammatory effects of cellulose containing materials, such as cellulose insulation and microcrystalline cotton cellulose, in the lung have been reported previously. Also, prior studies have used high doses likely to overwhelm lung clearance mechanisms. The objectives of the present study were to compare effects of respirable fractions of bleached cellulose (BC), cellulose insulation (CI), and microcrystalline cotton cellulose (MC), following intratracheal instillation. Also, to examine lung effects at low dose to avoid confounding effects of potential lung overload. Male Fischer 344 rats were given four consecutive daily instillations of pre-sized particulate for total doses of 0, 0.25, 1.0 and 4.0 mg/animal. Interim sacrifices were conducted at days 3, 7, 14, 28 and 90 for bronchoalveolar lavage fluid analysis for biochemical markers and differential cell counts, and for histopathology at days 28 and 90. Sample characterization indicated significant differences in size and shape of the three materials influenced by the type of bulk material. Also CI exhibited significant enrichment for borax content with 13% in bulk material and 48% in the respirable fraction. BC caused no significant effects at any dose in the lung. Significant cytotoxicity was observed in the high dose of CI and MC, with greater severity and persistence seen with CI. Evidence of inflammation was also observed with the high dose of MC and CI, exhibited as neutrophyl and leukocyte infiltration. Therefore, there are differences in severity and duration of response to instilled respirable particulate, depending on the type of cellulose-containing material. Differences in response may be due to differences in particulate characteristics and/or different locations of instilled materials in the respiratory tract. Also the high borax content may influence the effects seen for CI. Finally, responses were only observed at the highest dose where lung overload may be an influencing factor.

553 13 WEEK INHALATION TOXICITY STUDIES OF 2-CYCLOHEXENE-1-ONE.

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2-Cyclohexene-1-one (CHX) is a cyclic α,β-unsaturated ketone with broad human exposure. CHX is an environmental pollutant and is present in tobacco smoke and soft drinks sweetened with cyclamate. Prechronic toxicity studies were conducted to provide data required to design chronic toxicity and carcinogenicity studies of CHX. Groups of 10 male and female F344 rats and B6C3F1 mice were exposed to 0, 2.5, 5, or 10 ppm CHX for 6 hr/day for 13 weeks. All animals survived until sacrifice. Microscopic lesions included hyperplasia, and squamous metaplasia in the nasal cavity in rats and mice of both sexes at all doses. Erosion and suppurative inflammation also occurred in high dose mice. Larynx and lung were not affected in either sex-species. Dose related centrilobular cytoplasmic vacuolation was seen in male rats only. Body weights were not significantly different from controls after 13 weeks of exposure. Liver/body weights were increased in male and female mice exposed to 5 and 10 ppm, and in male and female rats exposed to 10 ppm CHX. No adverse effects on sperm motility or vaginal cytology were observed. These data will be used in the design of a 2-year inhalation bioassay of CHX.

554 CYTOTOXICITY OF ABRASIVE BLASTING SUBSTITUTES.

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Increased morbidity and mortality from sandblasting with crystalline silica continues to occur even when respiratory protection is used. Because of the high risk of acute silicosis and increased mortality associated with the use of silica in sand blasting, it is banned in many industrialized countries and many substitutes are now being commercially used. We studied few of the most frequently used substitutes for *in vitro* cytotoxicity and tested aerosolized dusts within two hours of blasting and after aging for four weeks. Treated sand, sand, garnet, coal slag, specular hematite, and staurolite were collected by drawing air from a blasting area through a cyclone precipitator and then onto a 47 mm, 0.8 μm FWS-B filter within one meter of the operator's breathing zone. Samples of the pre-blasted and post-blasted materials were used for chemical and electron microprobe analyzes and compared. Samples prepared on polycarbonate filters were analyzed by scanning electron microscopy, x-ray spectrometry, and plasma emission spectrometry. Cytotoxicity studies using rat alveolar macrophage monitored cell viability, and leakage of lactate dehydrogenase (LDH) and N-acetyl-β-D glucosaminidase (NAG). Measurements of hydroxyl radical (•OH) generation and lipid peroxidation potential were also made on blasted dusts. The blasting process changed the trace metal content of all the blasted particles. Blasting generally increased the relative proportion of iron in blasted materials. This iron was apparently derived from the steel plates that were blasted. Blasting also resulted in the generation of particles with an average diameter of 1 μm. Both fresh and aged blasted particles decreased rat alveolar macrophage viability and increased enzyme release to varying degrees. Toxicity was generally more distinct for staurolite, coal slag, and garnet compared to sand. The •OH generation from all freshly blasted particles was generally higher than from aged particles. Lipid peroxidation potential was greatest for garnet and staurolite. This study demonstrates that silica sand blasting substitutes are not without biological effects and in some instances the cytotoxicity exceeds that of sand and may provoke lung injury.

555 GAMMA SCINTIGRAPHY OF RADIOLABELED VIRUS FOR IN VIVO DETERMINATION OF THE DOSE DELIVERED TO TARGET ORGANS IN GENE THERAPY: APPLICATION TO ADENOVIRUS-CFTR ADMINISTERED AS AEROSOL IN BABOONS.

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Scintigraphy enables visualization and quantification of pharmaceutical products in the body, both accelerating exploratory research and validating toxicology protocols. For cystic fibrosis therapy via inhalation of CFTR gene vectorized by adenovirus, optimization of aerosol is a prerequisite to obtain a functionnal CFTR in central lung. In preclinical efficacy and safety evaluations, imaging of virus is the only non invasive technique to quantify the lung deposition and baboon is the most relevant species for predictive studies on lung aerosol deposition in humans. Therefore adenovirus was administered to baboons as an aerosol, technetium 99m being used to label adenovirus in such conditions that bioactivity was preserved. Particle size (MMAD) and dispersion (σ g) of the aerosol generated with a breath-activated jet nebulizer were determined using a cascade impactor connected to a pump modelizing baboon respiratory conditions. DTPA-99mTc scintigraphy was used as a reference prior to validation with 99mTc-adenovirus. Three baboons were submitted to scintigraphy, receiving 100 puffes of 99mTc-DTPA. A week later, 99mTc-adenovirus (7.7 10^9 pfu, 814-925 MBq, 2 ml) was administered. Lung regional deposition was assessed from Regions Of Interest corresponding to central and peripheral regions. Granulometry corresponded to a 1.6μ m MMAD with a 1.8 geometric dispersion.Nebulized fraction was 15-17% of the initial dose and total lung deposition 2.7 to 7.5% of the nebulized fraction. Regional deposition to the central lung was 1.0 to 3.5%. These results confirm that this technique allows to determine the actual dose delivered to the target organ. Furthermore, similarities observed between DTPA and virus deposition validates the use of DTPA scintigraphy as a pretherapeutic test for each patient.

556 AMIODARONE-INDUCED PULMONARY TOXICITY (AIPT) IN F344 RATS.

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Amiodarone (AD) is a very effective antiarrhythmic drug, but its side effects limit its clinical application. AIPT involves both pulmonary inflammation and fibrosis in humans. The goal of this study was to develop an animal model of AIPT and examine the possible mechanisms leading to its development. F344 rats were intratracheally instilled with AD (6.25 mg/kg with a 3.125 mg/ml solution) in sterile water or the sterile water vehicle on the first day and then again 48 hours later. Animals were killed 5 days after the initial treatment and bronchoalveolar lavage (BAL) was performed. AD-treated rats had increased neutrophils and eosinophils recovered in the BAL fluid. BAL cells from AD-treated rats produced more integrated phorbol-myristate-acetate-stimulated luminol-dependent chemiluminescent (LDCL) counts over 20 minutes than BAL cells from control rats. Experiments using specific inhibitors implicate peroxynitrite in at least part of the LDCL response. When rats were examined 28 days after the initial AD or vehicle treatments, lung fibrosis was present both biochemically and histopathologically in only the AD-treated animals. Hydroxyproline, an amino acid found in collagen and used as a biochemical indicator of fibrosis, was elevated in AD-treated rats compared to controls. Examination of sections of lung tissue revealed minimal to mild, multifocal, interstitial fibrosis in the AD-treated animals. These findings indicate this model exhibits the pulmonary inflammation and fibrosis similar to that occurring in human patients and that elevated immune and oxidative processes may be involved in the development of AIPT in this model. (Supported by NIH 5 T32 GM07039.)

557 POTENTIAL HEALTH EFFECTS OF DBPs USING QUANTITATIVE STRUCTURE TOXICITY RELATIONSHIP.

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Disinfection by-products (DBPs) are produced as a result of disinfecting water using various treatment methods. Over the years, chlorine has remained the most popular disinfecting agent due to it's ability to kill pathogens. However, in 1974, it was discovered that the superchlorination of drinking water resulted in the production of chloroform and other trihalomethanes. Since then hundreds of additional DBPs have been identified, including haloacetic acids and haloacetonitriles. Quantitative Structure Toxicity Relationship (QSTR) utilizes a computer based technology to assess the toxicity of a chemical solely from it's molecular structure. The current research was conducted utilizing the TOPKAT® / QSTR software package which is comprised of robust, cross-validated QSTR models, for assessing specific toxic endpoints. A total of 253 DBPs were analyzed using the carcinogenicity and developmental toxicity submodels of TOPKAT®.

The model predictions were evaluated for any correlation between the functional groups (e.g. alcohols, acids, etc) and specific toxic endpoints. Developmental toxicity was identified as an endpoint common to the majority of aliphatic mono- and dicarboxylic acids. Developmental toxicity was also associated with aliphatic halogenated and non-halogenated ketones, and aliphatic haloacetonitriles. In the case of the NTP carcinogenicity submodels, most aliphatic aldehydes were identified as carcinogens only in the female mice submodel. The majority of the aliphatic and aromatic dicarboxylic acids were identified as carcinogens in the female rats submodel. All other functional groups were largely predicted as non-carcinogens in all the NTP cancer submodels, i.e., male/female rats and mice. The QSTR results should aid in the prioritization for evaluation of toxic endpoints in the absence of *in vivo* bioassays. (This abstract does not necessarily reflect U.S. EPA policy.)

558 RATS AND HAMSTERS BOTH EXHIBIT A 9% LUNG DEPOSITION EFFICIENCY FOR INHALATION OF A DISTRIBUTION OF FIBERS GREATER THAN 5 MICRON IN LENGTH.

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Previous work on the comparison of pulmonary and pleural responses of rats and hamsters to inhaled refractory ceramic fibers has indicated that rats and hamsters may have similar lung deposition efficiencies for selected size intervals of fiber length with diameters near 1 µm. A quantitative comparison has not been done of the deposition efficiency between species for that portion of a fiber aerosol having lengths greater than 5 µm. In previously reported research on the comparison of pulmonary and pleural responses of 250- to 275 g male rats and 140- to 150 g male hamsters to inhaled refractory ceramic fibers, detailed size analysis was given for both the exposure atmosphere and lung deposition. A count of 296 fibers per cc (Å 45%) of the exposure atmosphere fiber distribution consisted of fibers having length > 5 µm and diameters < 1 µm. Rats and hamsters were exposed nose-only, 4 h a day, 5 days a week for 4 or 12 weeks. The average count in 6 each rat and hamster lungs following exposures of 4, 12, and 12 weeks plus 12-week recovery were respectively, in millions of fibers, 19.0, 49.2, and 60.9 for rats and 8.9, 17.1, and 18.6 for hamsters. An estimate of the deposition fraction was calculated by combining these parameters with the best available information on the minute ventilation of rats and hamsters (158 and 51 ml/min respectively for the size of animals). There was no difference between species, and the deposition efficiency for inhalation of a distribution of fibers > 5 µm length was 9 ± 2 %.

559 NINETY-DAY INHALATION TOXICITY OF TRANS-1,2-DICHLOROETHYLENE IN RATS.

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trans-1,2-Dichloroethylene (t-DCE) is used as an intermediate in the production of chlorinated chemicals and as a solvent. The toxicity of t-DCE (>99.4% pure) was evaluated in Crl:CD®BR male and female rats exposed to analytically determined mean concentrations of 0, 200, 1000, or 4000 ppm for 6 hrs/day, 5 days/wk over a 90-day period. Body weights and food consumption were measured weekly, liver cell proliferation was evaluated in 5 M and 5 F/group after 7, 45, and 90 days; clinical pathology in 10 M and 10 F/group at 45 and 90 days and anatomical pathology in 10 M and 10 F/group at 90 days and in 5 M and 5 F/group after a one-month post-exposure period. There were no adverse clinical signs noted during or following the exposures. There were no adverse, exposure-related effects on body weight, food consumption, clinical or anatomic pathology parameters, or liver cell proliferation. This contrasts with previous reports of liver and lung changes at 200 and 1000 ppm and cardiac changes at 3000 ppm in single 8 hr rat exposures conducted in the 1970s. The NOEL for this study was 4000 ppm in both males and female rats, the highest concentration tested, suggesting that t-DCE may be appreciably less toxic in rats than previously reported.

560 EFFECTS OF EXPOSURE TO ASPHALT FUME CONDENSATE (AFC) ON ACUTE INFLAMMATORY RESPONSES.

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Asphalt fumes are complex mixtures of particulate and organic compounds. Field studies report an increased incidence of mucus membrane irritation and workshift decline of peak flow for road pavers. The objective of the present study was to characterize the acute pulmonary responses in rats after AFC exposure. AFC was collected from a paving asphalt storage tank. Rats received a single intratracheal instillation (IT) of saline, 0.1 or 0.5 mg AFC, and were sacrificed 1- or 3-day later. Another set of rats were exposed by IT to saline, 0.1, 0.5, or 2 mg AFC for 3 consecutive days and were sacrificed the following day. Differential cell counts, acellular protein and lactate dehydrogenase (LDH) were measured in bronchoalveolar lavage fluid (BALF) to monitor inflammation and damage. Chemiluminescence (CL) generated from alveolar macrophages (AM) was measured and tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) in AM-conditioned media were assayed to monitor AM function. The results show that AFC exposure did not induce significant neutrophil infiltration or alter LDH and protein content in BALF. CL and nitric oxide-dependent CL generated from AFC-exposed AM were not different from control at rest or in response to zymosan stimulation. Likewise, AFC exposure did not induce increased TNF-α or IL-1 secretion from AM at rest or in response to LPS. These results suggest that exposure of rats to AFC did not cause acute pulmonary inflammation or injury. Furthermore, AFC exposure did not alter AM functions such as the release of oxidants or inflammatory cytokines by AM at rest or in response to stimulants.

561 TARGETED DELIVERY OF INHALED PHARMACOLOGIC DRUGS FOR THE TREATMENT OF LUNG DISEASES CAUSED OR EXACERBATED BY AIR POLLUTANTS.

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We have developed a supercomputer code which describes the position of individual airways in the complex branching structure of the human lung. The code is designed to be used in a complementary manner with SPECT (Single Photon Emission Computed Tomography) protocols. Specifically, the code will aid clinicians and research scientists working with gamma camera images that are divided into discrete volumes (voxels). The code permits a comprehensive mapping of airways, assigning each to an unambiguous location. It itemizes the composition of a three-dimensional (3-D) voxel matrix superimposed over the human lung in terms of the number and type of airways present (e.g., Weibel generation I). The 3-D simulations are integral components of: (1) morphological models for improved gamma camera imaging analyses used to measure particle deposition patterns within human lungs; and, (2) particle dosimetry models used to predict and target the delivery of inhaled pharmacologic drugs used in the treatment of respiratory diseases caused (e.g., cigarette smoke and lung cancer) or exacerbated (e.g., sulfuric acid aerosols and asthma) by air pollutants of health effects concern to the EPA. DISCLAIMER: This is an abstract of a proposed presentation and does not necessarily represent EPA policy. (C J Musante was funded by the EPA/UNC Toxicology Research Program, Training Agreement CT902908.)

562 HUMAN SMOKING BEHAVIOR STUDY: ECLIPSE CIGARETTE COMPARED TO USUAL BRAND.

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Human smoking behavior measures from several studies conducted in our laboratory have shown significant differences when smokers smoked Eclipse (a cigarette that primarily heats tobacco) compared to their usual brand (UB) of tobacco burning cigarette. Two studies (conducted about 6 months apart) are presented comparing puffing profiles, cigarette sensory attribute ratings, blood carboxyhemoglobin (%COHb), and serum nicotine concentrations in smokers smoking their UB of tobacco burning cigarette to these same measures after smoking Eclipse cigarettes for two weeks. Two groups of smokers (n = 26 for each group) participated in two laboratory smoking tests, each between the hours of 1:30 and 4:30 p.m. Subjects

smoked one of their UB cigarettes in the first test session after reporting the number of cigarettes they had already smoked that day. Smoking profiles were recorded and blood samples were collected for measurement of %COHb and serum nicotine concentrations. Smokers were then sent home with a supply of Eclipse cigarettes which had a hollow filter design (EHF). Subjects smoked these cigarettes exclusively and as desired for two weeks, then returned to the laboratory for their next test session. Identical procedures were used for both laboratory visits. Puffing and absorbed CO measures varied widely as seen in our previous work, indicating a range of smoking behavior. On average, in both groups, smokers took significantly more puffs, closer together in time, resulting in significantly greater total puff volume when smoking Eclipse cigarettes than when smoking their UB. Average %COHb concentrations were: in the first test, UB = 9.6 and EHF = 10.2, and in the second test, UB = 8.3 and EHF = 9.0. Average absorbed CO measures showed no statistically significant difference from UB in either test of the Eclipse cigarette.

563 INHALATION TOLERANCE STUDY FOR P-ARAMID RESPIRABLE FIBER-SHAPED PARTICULATES (RFP) IN RATS.

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The aim of the study was to assess the maximum functionally tolerated dose (MFTD) for p-aramid respirable fiber-shaped particulates (RFP), and to study inflammatory and proliferative effects of this synthetic organic material. Male Wistar rats were exposed 6 hrs/day, 5 days/week for 3 months to RFPs at concentrations of 25, 100, and 400 RFP/ml. Upon cessation of exposure, recovery effects were followed up during a 9 month postexposure observation period. After 3 months of exposure the retention of RFP (WHO fraction) was 25 x 10^6 RFPs per lung in the low dose group. The corresponding values in the medium and high dose group were 122 x 10^6 and 576 x 10^6 RFPs per lung, respectively. A decrease of the length of the RFPs over the 9 months recovery period was observed. The kinetics are indicative of fragmentation of longer fibers into shorter ones and subsequent removal of the latter. The results of the alveolar clearance (measured by using short-term exposure to γ -labeled particles) indicate a "dust overloading of lungs" for the high dose group (400 RFP/ml). For the medium dose group (100 RFP/ml) the 3-month exposure induced only slight lung overloading. For the low dose no change in tracer clearance was detected compared to controls. BAL parameters (differential cell count and biochemical parameters) revealed that p-aramid RFPs induced pronounced effects in the medium dose group, which were reversible within 3 months postexposure. Effects were stronger in the high dose group; they recovered after 9 months postexposure for biochemical parameters. Also for PMNs and lymphocytes a recovery was observed, but to a lesser extent. No effects were seen in the low dose group. Histopathological examination showed very slight to slight fibrotic and hyperplastic lesions in the medium and high dose groups up to 9 months postexposure. This study demonstrated transient effects of a p-aramid exposure in rats. The MFTD was reached at 400 RFP/ml. Earlier reports demonstrating biodegradation by fragmentation of longer fibers into shorter ones were confirmed. A NOAEL of 25 RFP/ml was found.

564 PULMONARY FUNCTION IN UNTREATED RATS.

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Pulmonary function tests (pfts) often are used in both humans and small animal species as physiologic biomarkers of pulmonary disease caused by the inhalation of toxic atmospheres. Physiologic biomarkers, used in conjunction with histopathological and biochemical biomarkers can be used to diagnose disease, characterize dose/response relationships, assess disease pathogenesis, and indicate the degree of debilitation following pulmonary insult. We have developed methods to perform a battery of pfts which will be used to assess acute and chronic pulmonary toxicity in small animals exposed to inhaled toxins. The pfts developed can be used to assess physiologic responses in real-time during exposure, post exposure progress of induced pulmonary lesions, or both. Included are measures of ventilation (frequency, tidal volume and minute ventilation), breath waveform analysis, dynamic pulmonary mechanics (compliance and resistance), sub-divisions of lung volume, lung pressure-volume relationships and quasistatic compliance, pulmonary dynamics (forced maneuvers), distribution of ventilation (single breath Nitrogen washout), gas exchange (carbon monoxide - single breath diffusing capacity, microcapnometry), and measurement of metabolic activity (microcapnometry). Over 200 untreated Long Evans rats were used to develop the assays and to form a historical data base for normal animals. Values for a variety of indices of pulmonary function measured using the methods developed in our laboratory were comparable to those reported by several other investigators.

565 BIOPERSISTENCE ASSAYS FOR SAFETY EVALUATION OF MAN-MADE VITREOUS FIBRES.

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There is clear evidence from chronic inhalation studies that fibres longer than 20μm and are persistent in the lung have the potential to cause lung cancer, mesothelioma, and pulmonary fibrosis. This concept is the basis for the recent European Communities Council Directive (67/548/EEC) related to the classification, packaging and labelling of dangerous substances. Exoneration from the carcinogen labelling is based on the results of chronic inhalation (CI), short-term inhalation biopersistence (SI), intratracheal instillation (IT), or intraperitoneal injection (IP) assays conducted with a defined fibre aerosol or suspension. These approaches have limitations in terms of cost, time, or route of administration. RCC has conducted many of the bioassays on which the European Directive (ED) is based and continues to conduct such studies that afford a comparison between the various approaches. CI studies are costly, however they provide evidence of long term effects and fibres that produce no relevant pathology can exceed the weighted half-life (WT½) in SI assays stipulated by the ED. However, fibres with a lower than 10 day WT½ have to date not been shown to be fibrogenic or carcinogenic. The fibre length requirements for the ED (20% greater than 20μm) can cause fibre agglomerations in the conducting airways that adversely affect clearance and biopersistence in IT assays. IP assays are not recommended because of the high number of fibres (1 x 10^9) make this abnormal route of administration inappropriately sensitive compared to inhalation and intratracheal assays. The SI appears to be a valid screen to identify potentially safe MMVFs. In Germany a Federal scheme for classifying fibres is in force that does not include CI and SI tests, but relies on chemical composition, IP, or IT assays with unspecified fibre solutions apart from the WHO size classification. This allows the preparation of fine and short fibre preparations that avoid the agglomeration problem but may not include sufficient fibres longer than 20μm that are thought to be important in the induction of pulmonary disease. The chemical composition index excludes most forms of MMVFs other than glass wools. The German and European regulations are currently incompatible and require separate testing strategies except when an IT assay passes the ED criteria.

566 HEAD-ONLY AND MASK INHALATION EXPOSURE IN THE CYNOMOLGUS MONKEY.

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The cynomolgus monkey is a standard species used in inhalation toxicity studies. Two methods of exposure can be used in these studies: via inhalation mask or head-only. The first exposure system consists of a pediatric anesthetic-type oronasal face mask fitted with inlet and outlet tubes and a breathing loop. During treatment, animals are placed in a restraint sling. This method limits the exposure to the oronasal route and reduces the amount of material needed for dosing. The procedure is well tolerated by the animals for daily exposures of up to 60 minutes (2 sessions of 30 minutes each). The head-only system consists of an inhalation chamber equipped with individual animal restraint boxes which are fitted onto the sides of the chamber, so that each monkey can sit comfortably in the box with its head projecting into the chamber through a neck-yoke. The head-only method of dosing is ideal for prolonged exposure durations (up to 3 hours) as it minimizes stress on the animals. Method development has provided a goggle and head cover system to minimize ocular and dermal exposures associated with head-only inhalation dosing for primates. This system confines the exposure to the oronasal region. Both methods of exposure are viable routes of administration and have been routinely used on preclinical acute and chronic safety studies for submission to FDA, EC and MHW.

567 COMPARISON OF COMPUTER SIMULATIONS AND HUMAN DATA FOR PARTICLE DEPOSITION IN HEALTHY SUBJECTS AND COPD PATIENTS.

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A mathematical model was used to predict the deposition fractions of particulate matter (PM) within human lungs. The US EPA has identified COPD (chronic obstructive pulmonary disease) patients as a critical subpopulation to be addressed in regulatory standards. Therefore, we have compared the deposition of monodisperse aerosols in COPD patients and healthy subjects. Simulations using the Weibel lung morphology were previously validated with human subject data, so this geometry was used as a control case. Human subject variation was subsequently accounted for by scaling Weibel lung dimensions based on measured FRC (functional residual capacity) values. COPD was modeled via reductions of airway diameters based on airway resistance measurements. Simulations were performed for VT (tidal volumes) of 360, 500, and 1000ml, respiratory times (T) from 2 to 8s and particle sizes of 1, 2, 3 and 5µm. Deposition patterns depended on particular ventilatory parameters, particle sizes and COPD conditions; for example, when V_T =500ml and T=2s, the deposition of 1µm particles was 100% greater for COPD patients. This may be attributed to increased efficiencies of specific deposition processes in different regions of the lung. The model is used in risk assessment efforts to estimate the inhalation hazards of airborne pollutants. (This is an abstract of a proposed presentation and does not necessarily represent EPA policy. Acknowledgements: RAS is funded by the NCSU/EPA Cooperative Training Program in Environmental Sciences Research, Training Agreement CT826512010, NCSU.)

568 A 28-DAY INTRANASAL STUDY IN RATS WITH AVICEL® RC-591.

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A repeated-dose intranasal administration study was conducted to evaluate the toxicity of Avicel® RC-591, a pharmaceutical excipient consisting of coprocessed microcrystalline cellulose (MCC) and carboxymethyl cellulose. Two groups of Sprague Dawley rats (10/sex/group) were dosed for 28 consecutive days with either 1 dose/day (100 uL/day) or 3 doses/day (300 uL/day) of a 1.5% solution of Avicel RC-591 in distilled water. A similar group of animals received 3 doses/day (300 uL/day) of distilled water for 28 days and served as the control. Material was administered to the nasal passages of each animal via a microliter pipettor. Each dose was split into two equal 50-uL doses (50 uL per nostril) and administered once per day for the low dose group and 3 times per day for the control and high dose groups. Clinical observations, body weights and food consumption were recorded weekly. After 28 days, blood was collected for hematological and clinical chemistry evaluations and brain, liver, lungs, kidneys and testes were weighed. Histopathological evaluation was conducted on all organs and tissues. Respiratory tract sections were also examined using polarized light for the presence of birefrigent MCC particles. No deaths or treatment-related effects were noted for clinical signs, body weight, food consumption, hematology, clinical chemistry, organ weights or macroscopic findings. During microscopic examination of the nasal cavity, MCC particles were found embedded in neutrophilic exudate. These particles were associated with neutrophilic inflammation and indicate that Avicel may cause mild irritation to the nasal cavity. Because these findings were attributed directly to the introduction of Avicel particles into the nasal cavity and were found in locations indicating proper biological filtration, these effects were not considered adverse effects of treatment. The no observed adverse effect level (NOAEL) for 1.5% Avicel RC-591 in distilled water is in excess of 300 uL/day (17.7 and 25.2 mg/kg/day Avicel RC-591 for males and females, respectively).

569 DNA-PROTEIN CROSSLINKS (DNAPC) AND DNA STRAND BREAKS (DNASB) IN HL-60 CELLS TREATED WITH THETOXIC BENZENE METABOLITES TRANS,TRANS-MUCONALDEHYE AND HYDROQUINONE.

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Benzene hematotoxicity is mediated, in part, by ring-hydroxylated metabolites including hydroquinone (HQ), and ring-opened metabolites including trans,trans-muconaldehyde(MUC). Previous studies from our laboratory indicate that MUC induces DNAPC in HL-60 cells and mouse bone marrow cells as determined by a K^+/SDS precipitation assay. DNAPC are toxic lesions which have been reported by other laboratories to correlate with increased DNASB. Hydroquinone has been reported by other investigators to induce DNASB. The aim of the present studies was to determine DNAPC levels and DNASB in HL-60 cells in response to HQ and MUC, respectively, and to determine effects of co-exposure to MUC and HQ on both endpoints. The present studies indicate that treatment of HL-60 cells with 25-100 uM HQ followed by incubation for 4 hr results in 1.3-2.8 fold increases in DNAPC levels compared with controls. At 25 and 100uM, MUC is 1.8 and 4.9 fold more effective in inducing DNAPC than HQ. Treatment of HL-60 cells with 1-50 uM MUC followed by incubation for 1 or 2 hr resulted in concentration and time-dependent increases in DNASB as measured by a fluorescence-based DNA unwinding (FADU) assay. Exposure to 10 and 25 uM MUC gave Qd values (quantity of strand breaks) of 8 and 34, respectively, at the 1 hr time point and 33 and 67, respectively, at the 2 hr time point. Exposure of HL-60 cells to binary mixtures of 25 and 50uM MUC and HQ was found to lead to additive increases in DNAPC and DNASB levels. (Supported by NIH grants ESO2558 and ESO9676 from the National Institute of Environmental Health Sciences, and NIEHS Center grant ESO5022.)

570 USE OF A CELL-BASED ASSAY FOR THE DETECTION OF NON-COVALENT DNA/DRUG INTERACTIONS.

R D Snyder. *Abbott Laboratories, Abbott Park, IL, USA.* Sponsor: W M Bracken.

Covalent chemical/DNA interactions may be detected and quantified by many methods including direct detection of DNA strand breaks, induction of repair, and adduct formation. Specific chemical moieties conferring DNA reactivity have been identified and the mutagenicity or clastogenicity of a compound can often be predicted based on the presence of these structural alerts alone. However, it is proposed that a largely overlooked component to genotoxicity arises via intercalative and other non-covalent chemical interactions with DNA; interactions which are difficult to study in cellular systems. We present findings showing that DNA intercalation may be detected and quantified in Chinese hamster V79 cells by a bleomycin amplification assay. The antibiotic bleomycin binds DNA in the minor groove and makes DNA double strand breaks which manifest as micronuclei. Intercalation of chemicals into cellular DNA enhances the binding of bleomycin and amplifies the formation of micronuclei. We show that the ability of a chemical to amplify bleomycin clastogenicity is governed by its structural class and that many classes of aromatic compounds beyond the classical intercalative fused planar tricyclics have this capacity. Intercalative activity is ranked: fused tricyclic≥ unfused tricyclic> unfused bicyclic> fused bicyclic, aliphatic and single ring structures. As a specific example of the utility of this approach, it is demonstrated that intercalation may account for the reported genotoxicity of marketed antihistamines. It is proposed that a cell-based bleomycin amplification assay may provide an important new model for the assessment of non-covalent DNA interaction of novel chemical entities.

571 COMPARISON OF HISTOPATHOLOGY AND DNA ADDUCTS IN RATS SUBCHRONICALLY EXPOSED TO SMOKE FROM CIGARETTES THAT BURN OR PRIMARILY HEAT TOBACCO.

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A subchronic nose-only inhalation study comparing the effects of smoke from a cigarette that primarily heats tobacco (Eclipse™ prototype 7-026A), to that of smoke from a Kentucky 1R4F reference cigarette was conducted using Sprague-Dawley rats. Rats of each sex were exposed 1 hour a day, 5 days per week, for 13 weeks to mainstream smoke at 0, 0.16, 0.32, or 0.64 mg/liter of air. Endpoints were carboxyhemoglobin (HbCO), serum nicotine, clinical signs, body and organ weights, serum chemistry, hematology, histopathology, and DNA adducts in heart and lungs using the P^{32} post-labeling procedure. Neither cigarette produced adverse changes in clinical signs, body and organ weights, serum chemistry, or hematology. HbCO and serum nicotine increased with both cigarettes in an exposure-dependent manner. Smoke related histologic changes were nasal chronic/active inflammation and epithelial hyperplasia, chronic/active inflammation of the larynx, squamous metaplasia and acute inflammation of the ventral larynx, tracheal epithelial hyperplasia and increased goblet cells, and increased non-pig-

mented and brown/gold pigmented lung macrophages. Inflammatory changes of the larynx and ventral larynx, and non-pigmented lung macrophages were similar in rats exposed to the 1R4F or Eclipse cigarette. Increased tracheal goblet cells and brown/gold pigmented macrophages were absent in the Eclipse exposed rats. All other smoke related histologic changes were reduced in Eclipse exposed rats when compared to the 1R4F exposed rats. DNA adducts of heart and lungs of Eclipse exposed rats were not different from sham control animals but were reduced compared to 1R4F responses. The reduction or absence of histologic changes and DNA adducts in lungs and heart of rats exposed to smoke of Eclipse cigarettes indicates a reduction of biological activity compared to the smoke of the tobacco burning 1R4F cigarette.

572 CROSSLINKING INTERFERES WITH ASSESSING SULFUR MUSTARD-INDUCED DNA DAMAGE IN HUMAN LYMPHOCYTES USING THE COMET ASSAY.

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Sulfur mustard (SM) is a vesicating agent that alkylates cellular DNA and produces DNA strand breaks. To detect SM-induced DNA single strand breaks in human peripheral blood lymphocytes (PBL), cells were exposed to various concentrations of SM (1 to 1000 μ M), and the comet assay (single-cell gel electrophoresis) was performed at selected times after exposure. At 4 hours, strand breaks were detected at SM concentrations ≥ 100 μ M. At 8 hours, strand breaks were detected at ≥ 6 μ M SM. Previous data suggest SM produces DNA crosslinks that are removed within 8 hours after exposure. To test the hypothesis that crosslinking inhibits DNA migration in the comet assay at < 8 hours after SM exposure, PBL were exposed to a) H_2O_2 (0.001%), which produces DNA single strand breaks with no crosslinks, b) SM alone (1 to 1000 μ M), or c) SM followed at 2 hours by H_2O_2. With H_2O_2 alone, a large amount of strand breakage was detected. With H_2O_2 and SM, detection of H_2O_2-induced strand breaks decreased as SM concentration increased up to 60 μ M; above 60 μ M, the response with H_2O_2 and SM was similar to that with SM alone. Detection of H_2O_2-induced DNA strand breaks appears to be inhibited by SM in a SM concentration-dependent manner, presumably due to SM-induced crosslinking. It follows that crosslinking in DNA of SM-exposed PBL also interferes with DNA migration and detection of DNA strand breaks when cells are exposed to SM alone.

573 IN VITRO PHOTOGENOTOXIC ACTIVITY OF CLINAFLOXACIN: A PARADIGM PREDICTING PHOTOCARCINOGENICITY.

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Fluoroquinolone antiinfective drugs exhibit phototoxic, photogenotoxic, and photocarcinogenic activities in experimental systems which may be interrelated. Clinafloxacin (CLX), a new fluoroquinolone, is a potent antiinfective agent being developed for use in life-threatening infections. While this drug has previously been demonstrated to be phototoxic, this report evaluated the photogenotoxic and photocarcinogenic potential of CLX. When Skh-1 mice were administered CLX in the presence of ultraviolet light (UVA) at the maximum tolerated dose expected for a photocarcinogenicity bioassay, induction of DNA strand breakage was noted in keratinocytes isolated from these animals. When compared with other well studied fluoroquinolones *in vitro*, CLX and Lomefloxacin (LMX) were equally effective in producing chromosome damage and DNA strand breakage in CHO cells exposed to UVA. Treatment of CHO cells with CLX in the presence of UVA also resulted in hydroxyl radical formation. However, coincubation of CHO cells with CLX and various antioxidants markedly reduced hydroxyl radical formation, but inhibited photogenotoxicity only to a limited extent. Thus, while reactive oxygen species contribute to the photogenotoxic activity of CLX, other factors may be involved. Since CLX exhibits both phototoxic and photogenotoxic activity, we predict that CLX would be photocarcinogenic *in vivo*. The present study establishes that under conditions of human exposure, the potential risk for CLX-induced photocarcinogenicity is small.

574 GENOTOXICITY ASSESSMENT OF SURAMIN – A POTENTIAL DRUG FOR PROSTATE CANCER.

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Suramin is an aminonapthalene-sulfonic azo dye under development for treatment of prostate cancer and attendant pain. The genotoxic potential of suramin was investigated in this study using a a battery of tests including bacterial mutagenicity, in vitro aberration and in vivo micronucleus. Bacterial mutagenicity was evaluated in *S. typhimurium* strains TA-98, TA-100, TA-1535, or TA-1537 and in *E. coli* strain WP2*uvr*A at 0.5 to 5000 μ g/mL. Results were negative in all strains with the greatest increase in revertant frequency being ≤ 1.7-times solvent control. In vitro aberrations were evaluated in CHO cells using 3 and/or 20 h exposure at 125 to 13000 μ g/mL. Suramin did not significantly increase aberration frequency in either the absence or the presence of S9 at concentrations up to 4000 μ g/mL. However, in the 3-h exposure, higher concentrations (≥ 6000 μ g/mL in S9+ and ≥ 10000 μ g/mL in S9-) caused aberrations 1 to 3-times the upper historical control value (6.3%). This response was not considered biologically significant based on International Conference on Harmonization guidelines because it was not concentration-related, not reproducible in the 20-h exposure, was seen at > 5000 μ g/mL, and these concentrations affected cell survival, osmolality, and pH. In vivo micronuclei were assessed using rats given single doses of 137.5, 275, and 550 (males) or 440 (females) mg/kg. The top dose represented a maximum tolerated dose. Bone marrow micronucleated polychromatic erythrocyte (MNPCE) frequency in suramin-treated groups at 24 or 48-h sampling times was within historical control range of 0.8 to 4.9 MNPCE/1000 cells and thus, was considered negative in this assay. Cmax plasma drug concentration in this assay was ~ 8 to 10 times higher than the human therapeutic range of 150 to 250 μ g/mL. Based on these data, it was concluded that suramin does not have any significant genotoxic potential.

575 ANALYSIS OF TAMOXIFEN-DNA ADDUCTS IN HUMANS AND RATS BY ACCELERATOR MASS SPECTROMETRY.

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Tamoxifen is widely used in the treatment of breast cancer and is currently being evaluated for its use as a chemopreventative agent for the same disease. However, tamoxifen causes liver tumours in rats and an increased incidence of endometrial tumours in women. In rats, liver tumour development is associated with the formation of large numbers of DNA adducts. In women it is not yet clear whether endometrial tumour development is associated with a genotoxic or hormonal mechanism. In breast cancer patients, tamoxifen-DNA adducts are absent or at low levels in the uterus making detection a problem. We have used [^{14}C] labelled tamoxifen and the sensitive technique of accelerator mass spectrometry (AMS) to detect adducts in human uterus DNA and compared this with the formation of adducts in rats. Six female volunteers were given a [^{14}C]tamoxifen capsule containing the therapeutic dose (20mg, 1.85MBq) approximately 18 hours prior to surgery (hysterectomy). Similar studies performed in 6 week old female rats used a single dose of tamoxifen equivalent to the human therapeutic dose (0.3mg/kg). Uterine tissue was removed and DNA, protein and tissue samples were measured for their [^{14}C] content by AMS. Radiolabelled carbon was detected in uterine tissues from the treated patients and rats, indicating that tamoxifen and its metabolites reached the uterus. DNA adducts and protein binding were detected in the uterus. These data show that tamoxifen administration (at comparable dose levels) results in low levels of DNA adducts in the uterus of humans and the reproductive tracts of rats (10-400 adducts per 10^{12} nucleotides). This is at least an order of magnitude lower than adducts levels in rat liver. It is still unclear what the relevance of these low levels of DNA damage is in the development of endometrial tumours in women treated with tamoxifen.

576 REGULABLE REPAIR OF OXIDISED DNA IN STABLE CELL LINES USING TETRACYCLINE-REGULATED EXPRESSION OF *FPG*.

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Cell lines with extra repair of oxidised DNA, specifically oxidised purines, were generated by stably transfecting chinese hamster ovary (CHO) AA8 cells with the bacterial DNA repair gene formamidopyrimidine DNA-glycosylase (*fpg*). Expression of *fpg* was controlled by a tetracycline-regulated system. Cells were co-transfected with: (a) a plasmid containing the tetracycline-controlled transactivator (tTA) protein driven by the CMV promoter and,(b) a plasmid containing the tetracycline operator sequence (tetO) placed upstream of the *fpg* gene. The presence of an intact fpg gene was confirmed by sequencing. In this system, regulation of expression was achieved by addition of tetracycline, which binds to tTA protein to prevent tetO-mediated expression. Cells were either transiently transfected, or stably transfected using puromycin resistance selection. Presence of *fpg* in stable lines was confirmed by Southern blotting. In cell lysates, repair of 8-oxoguanine was measured by cleavage of a 32P-labelled oligonucleotide containing an internal oxidised guanine. Radioactivity was visualized after acrylamide gel electrophoresis. Repair activities in transiently transfected cells were 100-fold higher than controls. Activities in individual stable lines were up to 10-fold higher than controls, and were returned to near-baseline levels by 72 h culture in 1 ug/mL tetracycline. This is the first report of cell lines with regulable activities of DNA repair. Since DNA repair activities can vary 100-fold between individuals, these cell lines will be useful in the study of cancer and other diseases thought to involve reactive oxygen species. (Support: Medical Research Council of Canada.)

577 FORMATION AND REPAIR KINETICS OF FURAN-MEDIATED DNA DOUBLE-STRAND BREAKS IN ISOLATED RAT HEPATOCTYES.

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Furan is a nonmutagenic, hepatotoxic compound that induces hepatocellular carcinomas in rats and mice through poorly understood mechanisms involving cell death and proliferation. Previous studies in our laboratory have shown that exposure of isolated F-344 rat hepatocytes to toxic concentrations of furan produced concentration- and time-dependent increases in DNA double-strand breaks (dsb), as analyzed by pulsed field gel electrophoresis, prior to cell death. DNA dsb have been linked with cell death and mutation induction. In this study, the repair kinetics of DNA dsb were determined after treatment of F-344 rat hepatocytes with 10 μ M furan for 4 hr in suspension followed by 24 hr in monolayer culture without furan. Hepatocyte viability (lactate dehydrogenase leakage) and DNA dsb (assayed using the fluorescent double-stranded DNA binding reagent PicoGreen®) were measured after 4 hr incubation in suspension (prior to placing in culture), after 90 min in the presence of 5% fetal calf serum (attachment period), and at 1, 2, and 24 hr postattachment in monolayer culture. DNA dsb in hepatocytes exposed to 10 μ M furan for 4 hr were significantly elevated after 4 hr suspension incubation, remained elevated for at least 2 hr in culture, and returned to near control levels at 24 hr without a significant decrease in cell viability. These results indicate that the surviving cells repair a significant portion of the DNA dsb within 24 hr. Erroneous repair of DNA dsb may be involved in the induction of mutations leading to furan-induced liver cancer.

578 THE GENOTOXICITY OF METHYLEUGENOL: A POSSIBLE MECHANISM OF ACTIVATION.

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Methyleugenol is a component of essential oils and is found in many teas, cosmetics, fragrances, soaps, shampoos, and foods. Like safrole, a structurally similar compound, methyleugenol has been found to be a rodent carcinogen in a recent NTP bioassay. We have, therefore, undertaken studies to determine the genotoxicity of methyleugenol over a non-cytotoxic range of doses in hepatocytes isolated from male Fischer 344 rats and female $B6C3F_1$ mice, and to investigate possible mechanisms by which the compound may exert its toxic effects. Cytotoxicity to hepatocytes was measured after 18 hr exposure to the compounds by measuring % lactate dehydrogenase (LDH) release from the cells. Genotoxicity was measured using the unscheduled DNA synthesis (UDS) assay that measures DNA repair by incorporation of [^{3}H]thymidine. Results for rats and mice showed similar patterns for all endpoints examined. Cytotoxicity results indicate that methyleugenol is relatively nontoxic to hepatocytes, showing control levels of LDH leakage up to 1000 μM. Cytotoxicity was increased by both pentachlorophenol (PCP), an inhibitor of sulfation, and cyclohexene oxide (CHO), an inhibitor of epoxide hydrolase. Methyleugenol induced UDS was observed at concentrations between 10 and 1000 μM. UDS levels were reduced to control levels by the presence of PCP but were unchanged by the presence of CHO. These results suggest that the genotoxicity of methyleugenol may be dependent upon the hydroxylation and subsequent sulfation pathway of activation. An epoxide dependent pathway of activation for genotoxicity is not suggested by these data. (This research was supported by NIEHS (NO1-ES-35367, P30-ES-06694 and T32-ES-07091-17).)

579 USE OF COMET ASSAY TO IDENTIFY DNA DAMAGE INDUCED BY *IN VIVO* ADMINISTRATION OF CYCLOPHOSPHAMIDE IN MICE.

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The COMET assay is a relatively new molecular biological assay that detects, in the nuclei of individual cells, DNA damage leading to strand breaks. As part of a project to identify antimutagenic soybean fractions, we are evaluating the effects of *in vivo* exposure of CD-1 mice to known mutagens on the tail moment (the integrated value of DNA density multiplied by the migration distance) of DNA in bone marrow and liver. Following single intraperitoneal doses of cyclophosphamide (CP) ranging from 75 to 300 mg/kg, tail moments in CP-exposed nuclei were as much as 2.3 times higher than tail moments in control liver nuclei. Tail moments in CP-exposed bone marrow cells were up to 5.0 times those in control bone marrow cells. Five daily doses of 150 mg/kg resulted in sharply decreased survival of bone marrow cells, and tail moments for CP-exposed nuclei were approximately twice those of control nuclei. Both the dose and the interval between exposure and death affected tail moments in CP-exposed cells. We are now evaluating the effects of *in vivo* exposure to 4-aminobiphenyl and benzidine in different dosing regimens.

580 PHARMACODYNAMICS OF FORMALDEHYDE: ARREST OF DNA REPLICATION BY DNA PROTEIN CROSS LINKS IS THE PROXIMAL CAUSE OF MUTATIONS.

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A variety of evidence suggests that DNA protein cross-links (DPX) are potentially mutagenic, but the mechanism of mutagenesis is unknown. A possible mechanism is suggested by the following results: (1) DNA replication in the nasal mucosa of rats exposed to formaldehyde (FA) was inhibited in portions of the DNA that contained DPX; (2) aldehydes including FA that form DPX arrested DNA replication *in vitro*, whereas aldehydes that lack the ability to form DPX did not inhibit DNA synthesis. Together these data suggest that DPX can block DNA replication. Failure to remove the blockage prior to cell division or excision followed by incomplete repair could result in a muta-tion. To determine the dose and time dependence of this mechanism, a model was developed based on the assumptions that DPX are formed randomly in the DNA, and that a replication fork can advance up to but not past a DPX. Incorporation of [14C]thymidine ([14C]TdR) derived from [14C]FA into DNA was used as a measure of DNA synthesis. [14C]TdR incorporation is expected to increase linearly with concentration and time at low values of these parameters, but the rate of increase is predicted to decline at higher values. Using the model, the average length of a replication bubble was estimated to be about 6 x 104 bp, which agrees with published data. The results suggest that DPX should not be regarded merely as dosimeters (pharmacokinetic factors useful for interspecies extrapolation), but they are also important pharmacodynamic factors, as they can block DNA replication and thereby induce a genotoxic response. Appreciation of both roles for DPX is necessary to understand the mechanism of FA carcinogenesis and to develop a sound basis for a biologically-based risk assessment at low concentrations of FA.

581 HUMAN CYTOCHROME P450 SPECIFCITY AND GENOTOXICITY INDUCED BY STYRENE.

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1,3-Butadiene (BD) and styrene (ST) are metabolized by cytochrome P450 (CYP450) enzymes to epoxide metabolites. Certain styrene metabolites such as styrene 7,8-oxide (SO) are DNA-reactive and have previously been shown to induce micronuclei *in vitro* and *in vivo*. To investigate the role CYP450 enzymes in the genotoxicity of ST, we quantified the induction of micronuclei in cell lines which contain differing amounts and types of CYP450 activity: AHH-1 (CYP1A1 activity), AHH-1/h2E1 (CYP1A1 and CYP2E1), and TK6 (no CYP450 activity). The cell lines were exposed for 24 hours to ST (1 or 5 mM) or its metabolite, SO, (0.125 or 0.250 mM). Two metabolites of BD, epoxybutene (EB, 200 or 400 mM) and diepoxybutane (DEB, 2 or 4 mM),were used as positive controls. Cytochalasin B (2 μg/ml) was added after 24 hr to inhibit cytokinesis, and slides were prepared at 72 hr after initial exposure. The frequency of micronucleated binucleated cells (MN-BNC) per 1000 binucleated cells (BNC) was then determined for each sample. Data were analyzed using either a one-way analysis of variance followed by a Tukey-Kramer post hoc test or a Student's *t*-test where appropriate. Differences were considered statistically significant at $P < 0.05$. TK6 cells exposed to ST did not show any increase in MN-BNC induction. AHH-1 and AHH-1/h2E1 cells exhibited similar background levels of micronucleus induction. ST induced micronuclei in both AHH-1 and AHH-1/h2E1 cells, but the response in AHH-1/h2E1 cells was greater. All cell lines responded with increasing micronuclei frequency to increasing levels of SO. Metabolism of ST is essential for genotoxicity. This is further evident from the increased frequency of MN in all cell lines following exposure to SO. Our data also demonstrate that CYP1A1 and/or CYP2E1 are capable of oxidizing ST to genotoxic metabolites.

582 ERYTHROCYTES (RBCs) AND GLUTATHIONE PEROXIDASE (GSH-Px): EFFECTS ON SPONTANEOUS AND DIEPOXYBUTANE (DEB)-INDUCED SISTER CHROMATID EXCHANGES (SCEs) IN TRANSGENIC BIG BLUE® MOUSE AND RAT PRIMARY FIBROBLASTS.

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The lack of the glutathione S-transferase theta gene is an enzymatic component associated with hypersensitivity to SCE induction by DEB in cultured lymphocytes of individuals diagnosed with Fanconi's anemia. Since GSH-Px is a major detoxification enzyme in RBCs, the present study examined the effects of either GSH-Px or species-specific RBCs on spontaneous and DEB-induced SCE frequencies in primary transgenic Big Blue® rat and mouse fibroblasts. Adherent monolayer cultures of 10^6 cells per culture flask per species were treated with either 0, 2 or 4 μ M DEB ± 0, 2 or 20 units of GSH-Px ± 2 x 10^8 species-specific RBCs for the final 70 h of a total 96 h culture period. Separately and together, GSH-Px and RBCs were effective in significantly reducing both the spontaneous and DEB-induced SCEs in mouse and rat cells. A species comparison of the SCE frequencies yielded no significant differences in regards to DEB-induced genotoxicity with GSH-Px alone or in combination with RBCs. Therefore, the present study demonstrates that GSH-Px is an important enzyme that is involved in the detoxification of DNA-damaging lesions that lead to SCEs in mammalian somatic cells.

583 GENETIC TOXICITY TESTING OF HFPO TRIMER AND HFPO TRIMER AMMONIUM SALT.

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Hexafluoroprppylene oxide trimer (HFPO trimer) is one of the intermediates that are generated during the fluoproduct synthesis. HFPO trimer and HFPO trimer ammonium salt were evaluated for clastogenic potential in human lymphocytes *in vitro*. Under the test condition, HFPO trimer is partially hydrolyzed to HFPO trimer carboxylate and fluoride anion. In order to evaluate the clastogenic potential of HFPO trimer without the effect of the fluoride ion, approximately equimolar concentrations of HFPO trimer ammonium salt were tested. Approximately equimolar concentrations of fluoride ion, comparable to the amount that would be generated upon complete hydrolysis of HFPO trimer, in the forms of NH_4F and NaF were also tested for comparison. Study was conducted in two independent trials at concentration range of 0 to 1mM. With HFPO trimer or HFPO trimer ammonium salt, extreme toxicity occurred at concentrations at or higher than 0.7 mM with or without metabolic activation. Statistically significant increases in the percent of aberrant cells were also observed with both compounds, HFPO trimer and HFPO trimer ammonium salt. Significant dose related increases in the percent abnormal cells were observed with and without activation with HFPO trimer and HFPO trimer ammonium salt. The statistically significant increases in the percent aberrant cells were only observed at the dose level that caused severe toxicity. Both HFPO trimer and HFPO trimer ammonium salt were negative for induction of micronuclei. No cytotoxicity was observed in cells tested with NH_4F or NaF. No statistically significant increases in the percent aberrant cells or dose related increases in the percent abnormal cells were observed at the concentrations tested. HFPO trimer carboxlyate was clastogenic with or without the presence of fluoride ion.

584 GENETIC VARIATION AND SUSCEPTIBILITY TO THE GENOTOXICITY OF 4-AMINOBIPHENYL (4-ABP).

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Biotransformation of 4-ABP to reactive products can occur via multiple pathways including N-hydroxylation by P450 1A2 and N- or O- acetylation catalyzed by N-acetyltransferases (*Nat*). In order to assess the contribution of *Nat*, inbred and congenic mice were used. C57Bl/6 (B6) and A/J (A) mice differ at many loci including *Nat* and are considered rapid and slow acetylators, respectively. B6.A.D have the *Nat* allele from the A strain and the Ah genotype of the DBA strain on the B6 background. Animals received 120 mg 4-ABP/kg or corn oil and livers were isolated after 24h. Hepatic 4-ABP-DNA adducts were assessed by an indirect immunofluorescent method. Negligible fluorescence was detected in control animals while there were specific nuclear signals in all animals receiving 4-ABP. No differences were seen in B6 compared to A, the strains with the greatest genetic heterogeneity, while B6.A.D was significantly lower. The mean relative nuclear fluorescent intensities in B6, A and B6.A.D were 22.1 ± 3.8, 21.4 ± 4.8 and 13.6 ± 2.3, respectively. When there was a large degree of genetic variation (A versus B6), there was no apparent association between genotoxicity and *Nat* genotype. However, comparison of the two most closely related strains, B6 and B6.A.D, suggest that the slow acetylator and Ah nonresponsive phenotypes decrease the genotoxicity of 4-ABP.

585 STRUTURE-ACTIVITY RELATIONSHIP STUDIES ON THE INDUCTION OF DNA-PROTEIN CROSSLINKS BY HEMATOTOXIC RING-OPENED BENZENE METABOLITES AND RELATED COMPOUNDS IN HL60 CELLS.

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Benzene, a bone marrow toxin and human leukemogen, induces increases in DNA-protein crosslink (DNAPC) levels in bone marrow cells of CD-1 mice. The DNAPC lesion is a potentially toxic lesion and it is likely that reactive benzene metabolites contribute to DNAPC formation.

Trans,trans-muconaldehyde (MUC) is an α,β-unsaturated six carbon diene dialdehyde well suited for the crosslinking of cellular macromolecules including protein and DNA. The present studies examined structure-activity relationships of ring-opened compounds including MUC, MUC metabolites, and related compounds with respect to induction of DNAPC in HL60 cells using a K+/SDS precipitation assay for DNAPC determination. When HL60 cells were treated with 100 μM MUC followed by incubation for 4 hr, DNAPC levels were 20 fold higher compared with control cells. No increases in DNAPC levels were measured in HL60 cells 4 hr after treatment with the MUC metabolites 6-hydroxy-trans,trans-2,4-hexadienal (CHO-M-OH), 6-oxo-trans,trans-2,4-hexadienoic acid (CHO-M-COOH),or muconic acid (COOH-M-COOH), each at 100 μM. At 500 and 1000 μM and the 4 hr timepoint, significant increases in DNAPC levels were observed for CHO-M-OH, but not CHO-M-COOH. At 100 μM and the 4hr timepoint, no effect on DNAPC lev-

els was observed for trans,trans-2,4-hexadienal, trans-2-hexenal,hexanal, trans,trans-2,4-hexadiene, glutaraldehyde, or acrolein. DNAPC induced by MUC and CHO-M-OH may be cytotoxic lesions, as increases in DNAPC levels by these compounds correlated with decreases in cell viability. Compounds not inducing DNAPC at 100 μM did also not affect cell viability. These studies indicate that both aldehydic carbons of MUC contribute to DNAPC induction, and that the presence of α,β-unsaturated double bonds increases the ability of MUC to induce DNAPC relative to the saturated dialdehyde glutaraldehyde. (Supported by NIH grant ES02558 from the National Institute of Environmental Health Sciences and NIEHS Center grant ES05022.)

586 HUMAN SULFOTRANSFERASE FORMS POTENT MUTAGENS FROM METABOLITES OF 2,4-DIAMINO-TOLUENE (2,4-DAT) AND 2,4-DINITROTOLUENE (2,4-DNT).

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2,4-DAT and 2,4-DNT, intermediates in the production of polyurethane foam, are carcinogenic in rodents and mutagenic in various test systems. Possible metabolic activation pathways include the formation of hydroxylamines or benzylic alcohols, followed by introduction of a good leaving group, such as acetate or sulfate. We have investigated the role of various human sulfotransferases in their activation using Chinese hamster V79 cell lines and *Salmonella typhimurium* TA1538 bacterial strains engineered for the expression of human xenobiotic-metabolizing enzymes. 2,4-DAT induced gene mutations in a V79-derived cell line expressing cytochrome P450 1A2 and human phenol-sulfating phenolsulfotransferase (P-PST, SULT1A1). Expression of P-PST (Arg-213 allel) in TA1538 strongly enhanced the mutagenic activity of several potential metabolites of 2,4-DAT and/or 2,4-DNT, that of 2-acetylamino-4-hydroxylaminotoluene from 0.1 to 1.5 revertants/nmol, that of 2,4-dinitrobenzylalcohol from 0.8 to 250 revertants/nmol, and that of 4-amino-2-nitrobenzylalcohol from 0.8 to 600 revertants/nmol. Only minmal activation was observed when the His-213 allel of P-PST was expressed. Expression of other human sulfotransferases (1A2, 1B2, 1C1) also enhanced the mutagenicity of some of these compounds, but the effects were much weaker than when P-PST (Arg-213 allel) was used. The results indicate that a specific form of human sulfotransferase, the predominating allel of P-PST, is particularly active in the final toxification of intermediates in the metabolism of 2,4-DAT and 2,4-DNT. (Supported by DFG (Innovationskolleg 26) BMBF (0311243) and NATO Collaborative Research grant (CRG.971600).)

587 CIGARETTE PARAMETERS THAT INFLUENCE THE MUTA-GENICITY OF MAINSTREAM SMOKE CONDENSATE.

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The Salmonella reverse mutation assay has been used to determine the influence of various cigarette design parameters on the mutagenicity of mainstream smoke condensate. Cigarette prototypes that differ in blend composition, paper porosity, paper additive (citrate or phosphate), filtration efficiency, and filter ventilation were constructed. In a fractional, factorial study design, 30 cigarette types were selected from 486 possible combinations. Three levels of each parameter were represented by 10 different cigarette types. Cigarettes were smoked according to ISO standards, and 4 condensate batches per type were collected by impaction and dissolved in dimethyl sulfoxide. Mutagenicity determination was carried out in the plate incorporation version of the Ames assay with *S. typhimurium* strain TA98 in the presence of S9. Four equidistant doses from 0.04 to 0.16 mg dry condensate per plate were applied. The specific mutagenicity, i.e., revertants/mg dry condensate, of each batch was calculated using linear regression analysis with Poisson-weighted data. To determine the influence of the cigarette design parameters, a multiple linear regression analysis on the specific mutagenicity values was performed. All regression coefficients except those for the paper additives were statistically significant ($p < 0.001$). Increasing the amount of Burley for bright tobacco from 0 to 65 % resulted in a 2.4-fold increase; filter ventilation from 0 to 60 %, in a 1.2-fold increase; filtration efficiency from 35 to 55 %, in a 1.1-fold increase; and paper porosity from 10 to 45 cm/min, in a 0.89-fold decrease in specific mutagenicity.

588 THE CONFOUNDING ROLE OF IMPURITIES IN THE GENOTOXIC EVALUATION OF A NEW PHARMACEUTICAL.

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The mutagenic potential of S 15261-3, a new antidiabetic agent, was assessed on Ames test as part of its safety evaluation. Laboratory scale material was found devoid of mutagenic potential in a preliminary Ames test and the compound selected as a drug candidate. The first batch issued from scale up synthesis elicited a concentration-dependent response of TA1535 at high concentrations, with or without metabolic activation. The mutagenic response still increased at precipitating concentrations leading to the hypothesis that a soluble impurity/degradation product may have been responsible for the discrepancy in the mutagenic response. Several fractions of impurities were collected by semi preparative HPLC from the Ames positive batch and tested on TA1535. One fraction was found mutagenic. The impurity, subsequently identified as a residual intermediate, was then tested in a conventional Ames test where it elicited a concentration-dependent response of TA1535 from minute concentrations which roughly corresponded to those provided in the positive batch of S 15261-3. The two E. coli were also found sensitive but at higher concentrations. The manufacturing process was adjusted to avoid the presence of this highly mutagenic intermediate and S 15261-3 currently used in preclinical and clinical trials is devoid of genotoxic potential in all the core battery of in vitro and in vivo genotoxicity assays. These results confirm the usefulness of the Ames test for monitoring the presence of mutagenic or potentially carcinogenic impurities in otherwise nonmutagenic compounds. They also underscore the importance of careful evaluation of any positive result prior to discard compounds of potential interest.

589 DETECTION OF SPONTANEOUS AND INDUCED MUTATIONS IN TRANSGENIC FISH CARRYING A BACTERIOPHAGE LAMBDA CII TARGET.

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Transgenic animal models that are capable of measuring mutations are providing powerful new tools for rapidly assessing *in vivo* mutations following mutagen exposure. We describe the development and initial validation trials of transgenic fish (medaka, *Oryzias latipes*) that carry multiple copies per cell of a bacteriophage lambda vector. The lambda vector carries both the lac I and cII genes as mutation target genes which can be analyzed using different assay procedures. The general approach entails the introduction of the vector which carries the target genes into the fish genome, and following exposure to a mutagenic agent, recovering the target gene via indicator bacteria to quantify the frequency of mutations. Transgenic fish from several lineages were examined with regards to efficiency of vector recovery, spontaneous mutation frequency and induced mutation frequency (MF) of the target genes following exposure to the model mutagen ENU. The high number of copies of the vector carried by one fish lineage (>75 copies/haploid genome) facilitated superior packaging and highly efficient recovery of the bacteriophage from fish genomic DNA (8-1.5 X 10^5 phage recovered/packaging reaction). The spontaneous mutation frequencies of the target genes in the fish, although slightly lower, were comparable to the ranges observed in transgenic mice which carry the identical mutation targets (~2-8 X 10^{-5}). Dose response increases in MF were observed following short-term (1 h) ENU treatment and two-fold MF increases were observed five days after exposure to 0.5 mM ENU. The sequencing of selected mutations recovered from the fish indicated a predominance of point mutations detected in the target gene, and demonstrated similar overall mutation spectra compared to lambda transgenic mice. These results indicate that the fundamental requirements for the transgenic fish models for mutation detection have been met, and that fish have a significant promise for a wide number of applications in genotoxicity, with particular environmental relevance to aquatic systems.

590 MUTAGENIC AND CLASTOGENIC ACTIVITY WAS NOT FOUND WITH TRIBUTYLPHENYL CYCLICPHOSPHITE OF BUTYLETHYL PROPRANE DIOL IN A STANDARD GENETIC TOXICOLOGY BATTERY.

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2,4,6-Tri-t-butylphenyl cyclicphosphite of butylethyl propane diol (Ultranox® XR-2677, CAS No. 161717-32-4), an antioxidant/stabilizer used in the manufacture of polymers, was evaluated for mutagenic and clastogenic activity in a standard *in vitro* and *in vivo* genetic toxicology battery. The bacterial mutation assay was conducted with tester strains TA98, TA100, TA1535, TA1537, WP2 uvrA (pKM101) and WP2 (pKM101) in the presence and absence of S9 activation. Each strain was tested using the preincubation method over a dose range of 33 to 5000 μ g/plate. No toxicity and no mutagenic activity were observed in two independent trials. In the mutagenesis assay using L5178/TK$^{+/-}$ mouse lymphoma cells, no mutagenic activity was observed over a dose range of 300 to 500 μ g/mL, with and without metabolic activation. In the *in vitro* chromosome aberration assay using CHO cells, doses up to 877 μ g/mL were used. No significant increases in numerical or structural chromosome aberrations were observed with and without metabolic activation. In the *in vivo* mouse micronucleus assay, male and female mice were dosed with 1250, 2500 or 5000 mg/kg body weight and bone marrow cells were collected 24, 48 and 72 hours after treatment. There was no significant increase in the number of micronucleated polychromatic erythrocytes. Based on the results of these assays, Ultranox® XR-2677 was concluded to be non-genotoxic *in vitro* and *in vivo*.

591 MOLECULAR ANALYSIS OF *HPRT* MUTANTS FROM 1,3-BUTADIENE EXPOSED WORKERS.

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Butadiene, an industrial chemical, is carcinogenic in rodents and, probably, man. Previously, we reported a 3–fold increase in *hprt* mutant lymphocyte frequency in workers. We have analyzed 143 *hprt* mutant clones from butadiene-exposed workers and 197 clones from non-exposed controls to determine whether butadiene exposure is associated with specific changes in the spectrum of mutations. Process workers in a styrene-butadiene rubber plant were exposed to about 1 ppm butadiene. A multiplex PCR-based method was used for detection of exon deletions of the *hprt* gene. Of the mutant clones from BD exposed workers, 25/143 (18%) were identified as large deletion mutations, while 19/197 (10%) mutants in control subjects were deletion mutations, a significant difference ($p<0.05$). Direct sequencing of a PCR amplified target cDNA representing the entire *hprt* coding region was also used to analyze 76 mutants from butadiene-exposed workers and 85 mutants from control subjects. There was no significant difference in the frequency of small deletion mutations between BD exposed workers and the control subjects. There was a non-significant increase in mutations at AT sites among mutants from BD exposed workers (52%) compared to control subjects (42%). The frequency of frameshift mutations in butadiene exposed workers (13%) was significantly higher than control subjects (4%). Our preliminary results indicate that butadiene may induce large structural changes in the *hprt* gene in lymphocytes of exposed workers. The diepoxide metabolite of butadiene, a bifunctional alkylating agent, may play a role in butadiene-induced mutagenesis. Butadiene diepoxide was previously shown to cause an increase in deletion mutations in cultured lymphoblasts (Steen AM, et al, Mutagenesis 12, 61-67). (Supported by ES 06015 and ES06676.)

592 OLIGONUCLEOTIDE-INDUCED MUTATION IN CULTURED CELLS: CHARACTERIZATION, MECHANISM, AND IMPLICATION.

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Antisense oligonucleotides are a powerful new class of drugs which inhibit gene expression by interfering with transcription, RNA processing and transport, and translation. Little is known about the potential genotoxic effects of the oligonucleotides. This study demonstrates a link between the interaction of a variety of oligonucleotide analogs with cells and mutations in the cellular genome. Further, reactive oxygen is involved in coupling the oligonucleotide interaction with the cell surface and the mutation frequency. We examined a collection of synthetic oligonucleotides and their structural analogs for binding to cells in culture, production of hydroxyl radicals, and induction of mutations at the HPRT locus of cultured V79 cells. Oligonucleotides composed of phosphodiester, phosphorothioate, and methylphosphonate backbones were found to induce mutations in cell culture. The mutagenic response was not sequence-specific but was dose-dependent. The presence of hydroxyl radical was detected shortly after exposure of cells to these oligonucleotides. Phosphorodiamidate morpholine oligonucleotides, a new structural type containing six-membered morpholine replacing the ribose sugar joined by non-ionic phosphorodiamidate backbone, showed no significant increase in mutation frequency. These observations indicate a novel interaction between oligonucleotides and cultured cells leading to oxidative DNA damage and subsequent mutagenicity.

593 MUTAGENIC POTENTIAL OF ADENINE N^6 AND GUANINE N^2 ADDUCTS OF BUTADIENE MONO- AND DIEPOXIDE.

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In order to determine the biological effects of specific DNA adducts resulting from the interaction of 1,3-butadiene metabolites with DNA, deoxyoligonucleotides have been synthesized with four different adducts both at the N6 position of adenine and the N2 position of guanine. The deoxyoligonucleotides were designed such that the adduct was centrally located within codon 61 and codon12 of the human N-ras gene, respectively. These adducts are those arising from adduction by either the R or S stereoisomer of the monoepoxide (BDO) or the (R, R) or (S, S) isomer of the diepoxide (BDO2) in which the second ring has been hydrolyzed. These adducted oligonucleotides were utilized in *in vivo* and *in vitro* assays designed both to determine their mutagenic potency and to examine specific interactions with *Escherichia coli* polymerases. Each adducted oligonucleotide was ligated into a single-stranded vector M13mp7L2 that was subsequently used to transfect *E. coli*. The resulting mutagenic spectrum for these modified DNAs was stereoisomer specific. In addition, each of three purified *E. coli* polymerases was used to replicate DNAs containing these adducted deoxyoligonucleotides. These assays demonstrate the ability of each individual polymerase to bypass the adduct and divulge any stereoisomer-specific blockage. This is the first report of stereoselectivity related to butadiene-induced mutagenesis. (Supported by NIH P30-ES06676, ES05355, ES07781, P30-ES00267, and T32-ES07253.)

594 RECONSTRUCTED, DIFFERENTIATED AIRWAY EPITHELIAL CULTURES TO DETECT OCCUPATIONAL ASTHMA CAUSING AGENTS.

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Normal human tracheal-bronchial epithelial cells (NHBE) have been cultured using serum free medium to form a three dimensional tissue-like structure that closely resembles the epithelial tissue of the respiratory tract. Histological cross-sections of both the *in vitro* tissue and a normal human bronchiole reveal a four layer, bipartite structure. Transmission electron microscopy revealed numerous cilia on the apical surface of the cultures and dot blot analysis was used to quantify mucin secretion. These cultures were exposed to a saturated atmosphere of some common respiratory asthma inducing agents including toluene diisocyanate (TDI), phthalic anhydride, and formaldehyde. The culture medium beneath the cultures was analyzed for cytokine release using enzyme linked immunosorbent assay (ELISA) kits. Compared with air-exposed controls, the level of prostaglandin E-2 and 15-hydroxy eicosatetraenoic acid (15-HETE) increased by 2-fold or greater after a 45 minute exposure; interleukin (IL)-8 increased by 2-fold following a 4 hour exposure to TDI. No increases above basal levels of IL-6 or tumor necrosis factor (TNF-a) were observed following exposure to any of the materials tested. These data indicate that the newly developed NHBE culture model may be a useful *in vitro* means to detect chemicals that are likely to cause non-immunological occupational asthma.

595 CLARA CELL SECRETED PROTEIN (CCSP) OXIDATION IN THE FIRST WEEK OF LIFE IS INCREASED IN THE TRACHEAL FLUIDS OF INFANTS THAT DEVELOP BRONCHOPULMONARY DYSPLASIA.

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Bronchopulmonary dysplasia (BPD) remains a major cause of morbidity and mortality in prematurely born infants exposed to supplemental oxygen and mechanical ventilation. In the first week of life, infants born prematurely who have evidence of lung inflammation are more predisposed to the development of BPD. This led us to hypothesize that early inflammatory events occurring in the first week of life are secondary to an alteration of the lungs' endogenous anti-inflammatory mediators within the alveolar space. Therefore, this study was designed to test the hypothesis that specific protein oxidation, mediated by, or leading to inflammatory responses would be higher in infants that go on to develop BPD than in infants that do not. Forty-five infants admitted at $\leq$ 29 weeks gestation were studied. Tracheal aspirate samples were collected on days of life 1, 3, and 6 on all intubated infants. The tracheal aspirate fluids were assayed for oxidized proteins by derivatization with 2,4-dinitrophenylhyrazine (DNPH). Oxidized proteins were then identified with N-terminal amino acid sequencing. The infants were evaluated to determine whether they met the criteria for the diagnosis of BPD as defined by a persistent oxygen requirement at 28 days of life, at 36 weeks corrected gestational age and at the time of discharge. A 10 kDa oxidized protein was observed in 31% of the infants that did not develop BPD (n=16), 68% of the infants that did develop BPD (n=19), and 80% of the infants that expired (n=10). This protein was identified as the Clara cell secreted protein (CCSP), which functions as an endogenous anti-inflammatory molecule within the lung. These results suggest that oxidation of CCSP may reduce its function as an anti-inflammatory agent thereby contributing to lung inflammation and injury. Further efforts to determine the precise chemical nature of the oxidation of CCSP are underway.

596 STUDIES OF RESPIRATORY TRACT TOXICITY IN RATS EXPOSED TO MEXICO CITY URBAN AIR.

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The rat has been used as a health sentinel, indicator, or monitor of environmental health hazards; yet this model has not been directly validated against human exposures. Humans in Mexico City show upper respiratory tract lesions and evidence of pulmonary damage related to their environmental exposure. Male and female F344 rats were exposed (24hr/d) to Mexico City urban air (MCA) for up to 7 weeks, while controls were maintained at the same location under filtered air. Prior to these exposures, the nasal passages of normal male rats shipped from the U. S. and housed in Mexico City were examined for mycoplasma infection (none were found); a mobile exposure and monitoring system was assembled and tested, along with supporting histopathology techniques and analysis of rat nasal and lung tissues, using ozone as the exposure atmosphere; and the exposure model (equipment and animals) was transported to Mexico City and validated for a 3-wk period. During the 7-wk study there were 15 1-hr intervals where the average ozone concentration of Mexico City urban air in the exposure chamber exceeded the U.S. EPA standard of 0.125 ppm O3. Prolonged exposure of healthy F344 rats to MCA containing episodically low to moderate concentrations of ozone (and other urban air pollutants) did not induce inflammatory or epithelial lesions in the nasal airways (or in the lung), as measured by qualitative histologic and/or quantitative morphometric techniques. These findings are in agreement with those of previously controlled ozone inhalation studies but are in contrast to previous reports indicating that ozone-polluted MCA causes significant nasal mucosal injury in adults and children living in southwestern Mexico City. Taken together, these findings may suggest that human airways are markedly more susceptible to the toxic effects of MCA than the airways of the F344 rat.

597 MONOCROTALINE PYRROLE (MCTP) ALTERS THE TYPE II PNEUMOCYTE RESPONSE TO INTRANASAL ENDOTOXIN (LPS) ADMINISTRATION IN RATS.

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Monocrotaline pyrrole (MCTP) is a pyrrolizidine alkaloid that causes pulmonary vascular injury with limited type II pneumocyte hypertrophy/megalocytosis *in vivo*. *In vitro*, megalocytosis is seen in cells exposed to MCTP, secondary to the inhibition of normal cell cycle progression and mitosis (Toxicol. Appl. Pharmacol. 1998. 150:37-48). MCTP-induced alterations in cell cycle progression occur in confluent populations, but a stimulus for cell proliferation (ie, subconfluency) enhances the antimitotic and megalocytic effect *in vitro*. We hypothesized that a mitotic stimulus *in vivo* would augment the development of pulmonary cell megalocytosis due to MCTP administration to the rat. Endotoxin (LPS - 100μg per rat), an inducer of transient type II pneumocyte hyperplasia was given intranasally 4 days after MCTP administration (3.5mg/kg, iv). Alterations in lung histomorphology as well as markers of lung injury in bronchoalveolar fluid (BALF) were evaluated at days 6, 7 or 11. Generally, MCTP caused increases in lung weight, BALF protein concentration and BALF lactate dehydrogenase (LDH) activity which (except for increased lung weight on day 6) were unaffected by cotreatment with LPS. LPS given alone induced multifocal type II cell hyperplasia and inflammation, whereas MCTP alone resulted in minimal type II cell hypertrophy and/or megalocytosis. Compared to either agent alone, LPS given after MCTP caused more extensive multifocal inflammation without type II cell hyperplasia, but with more pronounced multifocal hypertrophy/megalocytosis of type II cells. In summary, the addition of a mitotic stimulus (LPS) to the MCTP pulmonary injury model exacerbates type II pneumocyte enlargement in the absence of pneumocyte hyperplasia. (Supported by NIH Grant ES02581.)

598 INTRATRACHEAL INSTILLATION OF ZINC CADMIUM SULFIDE (ZNCDS) IN FISCHER 344 RATS.

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The purpose of this study was to assess the bioavailability and pulmonary toxicity of ZnCdS in rats. Groups of 30 male Fischer 344 rats each were anesthetized and dosed via intratracheal instillation with 5 mg of either ZnCdS, quartz (positive control), or titanium dioxide (TiO_2-negative control) suspended in 0.5 ml saline. A vehicle control group received 0.5 ml saline. Ten animals from each test group were sacrificed at 1 day, 1 week, and 14 weeks after dosing for bronchoalveolar lavage fluid (BALF) analysis and histopathology. The BALF was analyzed for alkaline phosphatase, acid phosphatase, lactate dehydrogenase (LDH), β-glucuronidase (β-glu), total protein, and cell counts. Two separate groups of 24 rats each were dosed as above with either ZnCdS or saline. Eight rats from each group were sacrificed at 1 day, 1 week, and 14 weeks after dosing for determination of cadmium (Cd) and zinc (Zn) concentrations in the lung, liver, kidney and blood. Results indicate that at 1 day after dosing all enzyme activities (except acid phosphatase) and cell counts in BALF from the quartz and ZnCdS groups were significantly higher than TiO_2 and saline groups. At 7 days after dosing, high enzyme activity persisted in the quartz group, while the ZnCdS group showed only LDH and total protein levels significantly higher than the saline group. At 14 weeks after dosing, LDH, total protein, β-glu, and cell counts in the quartz group were significantly higher than all other groups. Histologic examination revealed interstitial inflammation and accumulation of foreign material in the lungs and mediastinal lymph nodes of quartz, TiO2, and ZnCdS treated rats. Metals analyses showed profuse Cd and Zn concentrations in the lung at 1 day, and to a lesser extent, 1week after dosing. In conclusion, ZnCdS appears to cause temporary lung inflammation and is not bioavailable.

599 LUNG MORPHOLOGY AND COLLAGEN DEPOSITION IN AMIODARONE- AND VEHICLE-TREATED HAMSTERS FOLLOWING DIETARY VITAMIN E SUPPLEMENTATION.

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Amiodarone (AM) is a potent antidysrhythmic agent that is limited in clinical use due to the development of numerous adverse effects, including potentially life-threatening AM-induced pulmonary toxicity. Treatment of male golden Syrian hamsters intratracheally with AM (1.83 μmol) or vehicle (0.1 ml distilled H_2O) was performed following 6 weeks of dietary vitamin E supplementation (500 IU d,l-α-tocopherol acetate / kg chow), at which time pulmonary vitamin E content was increased 234% relative to animals on the control diet. At 21 days post-dosing, histological disease index values for the diet and treatment groups were: control diet + vehicle, 5.08 ± 1.34%; vitamin E diet + vehicle, 3.58 ± 0.31%; control diet + AM, 7.25 ± 1.98%; vitamin E diet + AM, 5.58 ± 1.45%. Similarly, pulmonary hydroxyproline contents were: control diet + vehicle, 412 ± 33 μg / right lung; vitamin E diet + vehicle, 339 ± 28; control diet + AM, 521 ± 24; vitamin E diet + AM, 453 ± 73. The increases (p <0.05) in histological disease index and hydroxyproline content data for the control diet + AM group compared to all other groups were indicative of pulmonary fibrosis, while the decreases (p <0.05) of these values in the vitamin E + vehicle group suggest that dietary vitamin E supplementation provides non-specific protection against AM-induced pulmonary toxicity in the hamster. (Supported by Medical Research Council of Canada, grant No. MT-13257.)

600 PULMONARY EFFECTS OF ENDOTOXIN CONTAMINATED METAL REMOVAL FLUID AEROSOLS.

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Endotoxin contamination of metal removal fluid (MRF) aerosols has been implicated in the induction of pulmonary neutrophilia and decrements in airway conductance. In both guinea pigs and rats, acute ozone exposure results in the infiltration of neutrophils into the pulmonary tissues that correlates with increased permeability damage and hyperreactive airways. The present study was performed to determine if the accumulation of neutrophils, caused by endotoxin contaminated MRF aerosols, results in similar permeability damage and airway hyperreactivity. This study exposed male Wistar rats to 10 mg/m^3 MRF aerosols with and without 0.8 μg/m^3 endotoxin. Exposure to MRF aerosols without endotoxin resulted in no significant alterations in bronchial alveolar lavage (BAL)-recovered or lung tissue collagenase digestion-recovered neutrophils. Exposure to MRF aerosols with endotoxin resulted in significant increases in neutrophils, with BAL recoveries increasing from 0.01 (control) to 1.44 million/lung and collagenase digestion recoveries increasing from 6.1 (control) to 32.3 million/lung. These increases returned to control values by 16 hours post exposure. Lung permeability damage, as assessed by BAL recovered albumin, remained unaltered from a control value of 138 μg/ml in all exposure groups. There was also no evidence of hyperreactive airways, since animals exposed to MRF aerosols with and without endotoxin required the same intravenous methacholine dose (32 μg/kg) as control rats to provoke a 200% increase in airway resistance ($PD_{200}R$). These results demonstrate that MRF aerosol exposure, in the absence of endotoxin, did not result in an inflammatory response. In contrast to ozone exposure, the neutrophil accumulation in the BAL and pulmonary tissue brought about by exposure to endotoxin contaminated MRF aerosols did not correlate with permeability damage or hyperreactive airways.

601 EFFECTS OF VITAMIN E ON AMIODARONE-INDUCED CYTOTOXICITY IN ISOLATED HAMSTER LUNG CELLS.

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Life-threatening pulmonary fibrosis limits the clinical use of amiodarone as an antidysrhythmic agent. The ability of vitamin E to protect isolated hamster lung cell types from amiodarone-induced cytotoxicity was investigated *in vitro*. Preparations enriched with alveolar macrophages (95-98%), alveolar type II cells (75-85%) and non-ciliated bronchiolar epithelial (Clara) cells (35-55%) as well as a fraction consisting of unseparated cells were obtained from male golden Syrian hamsters by protease digestion followed by centrifugal elutriation and Percoll® density gradient centrifugation. Vitamin E (300 μM) did not alter viability (measured by trypan blue exclusion) of the cells not treated with 100 μM amiodarone. However, addition of vitamin E to the incubation medium significantly increased cell viability of the amiodarone-treated alveolar macrophage fraction from 67.8 ± 8.5% (mean ± SD) to 79.6 ± 7.9 % at 24 h and from 45.5 ± 13.4 % to 70.6 ± 4.9 % at 36 h (p < 0.01; n=5). Furthermore, vitamin E did not significantly protect any other cell fraction from amiodarone cytotoxicity. Amiodarone content (measured by reverse-phase HPLC) was not altered by vitamin E in any cell fraction, demonstrating that vitamin E does not decrease drug accumulation. These results suggest that vitamin E may counteract initiating events of amiodarone-induced pulmonary toxicity. (Supported by Medical Research Council of Canada Grant No MT-13257.)

602 EFFECTS OF CYTOCHROME P450 (P450) INHIBITORS ON BIO-TRANSFORMATION OF THE TOBACCO SPECIFIC NITROSAMINE 4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE (NNK) IN FRESHLY ISOLATED HUMAN LUNG CELLS.

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P450s and lipoxygenases (LOXs) have both been implicated in human pulmonary NNK metabolism. SKF-525A and 1-aminobenzotriazole (ABT) were used to examine P450 monooxygenase contributions to the biotransformation of NNK in isolated human lung cell fractions from 4 peripheral lung specimens obtained during clinically indicated lobectomy. Cells were incubated with [5-^{3}H]NNK and either SKF-525A (1.0 mM) or ABT (1.0 mM) for 24 hr. SKF-525A significantly inhibited keto reduction of NNK to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) by 72.4 ± 22.9%, 85.1 ± 10.4%, and 67.3 ± 27.1% (_ ± SD, n=4, paired t test) in preparations of isolated unseparated cells, fractions enriched in alveolar type II cells, and fractions enriched in alveolar macrophages respectively. ABT had no significant effect on the formation of NNAL. SKF-525A and ABT had variable effects on formation of other NNK metabolites. Total bio-activation (reflected by the sum of 4 α-hydroxylation endpoint metabolites) was decreased in some patient's cells treated with either SKF-525A or ABT. However, in some cases SKF-525A decreased total α-hydroxylation, whereas ABT either had no effect, or increased metabolite formation. In addition, both SKF-525A and ABT tended to increase detoxification (reflected by pyridine N-oxidation metabolites), particularly in cells producing detectable N-oxide metabolites in the absence of inhibitors. These results suggest a possible role for P450s in human lung cell NNK metabolism. However, NNK metabolism was subject to large variations among cell types and between individuals, and the results do not rule out LOX-mediated NNK biotransformation. In addition, carbonyl reduction of NNK to NNAL, which is reported to be catalyzed by 11β-hydroxysteroid dehydrogenase, is SKF-525A sensitive. (Supported by Medical Research Council of Canada Grant # MT-10382.)

603 CHANGES IN EPITHELIAL INTEGRITY, ALKALINE PHOSPHATASE ACTIVITY AND FIBRONECTIN EXPRESSION IN LUNGS OF RATS EXPOSED TO OZONE.

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The detrimental effects of the air pollutant ozone (O_3) in the lung are dependent on dose, exposure duration, and generally evolve with time post-exposure. This study characterized the time sequence of epithelial injury and fibronectin expression in the lungs of rats exposed to O_3. Bronchoalveolar lavage (BAL) fluid was analyzed for alkaline phosphatase and total protein as markers of epithelial injury and increased permeability, and fibronectin for its role in inflammation and lung injury. The results revealed a time-related increase in total protein in the BAL fluid following a 3 hr exposure of rats to 1 ppm O_3. The protein concentrations peaked at 12 hrs and then declined, but remained higher than the control levels at 24 hrs post-exposure. A similar time-related increase also occurred for BAL fibronectin and alkaline phosphatase activity. However, the return of alkaline phosphatase levels to

baseline prior to a comparable reduction in protein levels suggests repair of injured cells, but a delay in the formation of epithelial junctions that limit the transfer of serum proteins to air spaces. By cytochemistry, alkaline phosphatase activity was detected in association with lung type II epithelial cells and in BAL polymorphonuclear leukocytes (PMNs), but not in macrophages. While a significant increase in cytochemically detectable alkaline phosphatase resulted from the increase in PMN number following O_3 exposure, macrophages constituted the primary cell type responsible for fibronectin mRNA upregulation. The comparability of temporal changes in fibronectin, protein and alkaline phosphatase levels suggest a mechanistic role of fibronectin in lung injury. (Supported by NIEHS grant #ES03521.)

604 ACETAMINOPHEN CYTOTOXICITY IN RAT TYPE II PNEUMOCYTES AND ALVEOLAR MACROPHAGES IN VITRO.

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Acetaminophen (APAP) liver and kidney cytotoxicity is associated with bioactivation by cytochrome P-450 and/or prostaglandin synthetase to a reactive metabolite, which depletes reduced glutathione (GSH), covalently binds to proteins and leads to oxidative stress. Although APAP may also damage the lung, little is known about the mechanism by which this occurs. We, therefore, studied the *in vitro* toxicity of APAP and its effect on intracellular GSH level in rat type II pneumocytes (TII) - either immediately after isolation, or 24h after plating-and alveolar macrophages (AM). Cytotoxicity was evaluated by changes in membrane integrity (LDH assay) as well as by mitochondrial metabolic activity (reduction of MTT), following a 20h incubation with APAP (0.5-20 mM). The reduction of MTT was significantly decreased at ≥ 5 mM APAP in freshly isolated TII and AM, and at ≥ 10 mM in 24h-old TII. APAP caused a significant decrease in LDH retention in the fraction of attached cells in freshly isolated TII, AM and 24h-old TII at ≥ 10 mM, ≥ 15 mM and 20 mM, respectively. This was associated with increase in LDH activity in the fraction of nonattached cells and in the medium. A concentration-dependent decrease of intracellular GSH occurred in freshly isolated TII and AM during a 2h exposure to subtoxic APAP concentrations (0.01-1 mM). In 24h-old TII the GSH concentration was significantly higher than in freshly isolated TII and there were no changes after 2h APAP exposure. Potassium ethyl xanthate (inhibitor of cytochrome P450) and indomethacin (inhibitor of prostaglandin synthetase) decreased significantly APAP-induced GSH depletion in freshly isolated TII and AM. These observations have demonstrated that i) freshly isolated TII and AM are sensitive to APAP-induced toxicity, ii) APAP affects GSH level in these cells, and iii) cytochrome P450 and/or prostaglandin synthetase are possibly involved in APAP-induced cytotoxicity in freshly isolated TII and AM.

605 QUANTIFICATION OF AGGLOMERATION IN THE RAT LUNG FOLLOWING INTRATRACHEAL INSTILLATION EXPOSURE TO SYNTHETIC MINERAL FIBERS.

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Short term biopersistence studies for evaluating the clearance of fibers from the lung have been found to provide an excellent indication of the potential pathogenicity of these fibers. Recently, the European Commission has incorporated both inhalation and intratracheal instillation biopersistence results as one possible criteria for exoneration of fibers from Category 3. However, there has been concern that fiber deposition following intratracheal instillation may not accurately represent fiber distribution in lungs exposed to a fiber-containing aerosols. If fibers do agglomerate, the agglomerates can block the distribution of fibers through the airways and granulomas can form around the fibers which may isolate the fibers inside from the dissolution fluids of the lung. This would produce an artefact which could artificially increase the Intratracheal Instillation biopersistence clearance half-time. In order to quantify agglomeration of fibers in the lung, a novel technique was designed to transform lungs into optically clear specimens allowing the entire airways within intact lung lobes to be directly observed using confocal microscopy and permitting the identification of the fibers therein. Confocal microscopic analysis was performed using laser scanning microscope fitted with a 25 mW argon-ion laser and an upright microscope modified for reflected light imaging. Quantitative measurements of the number (using digital planimetry) and size of fiber agglomerates were made from randomly sampled regions of lung. Aggregate size was determined by measuring the distance (in micrometers) through a fiber agglomeration. The results show strong dependence of fiber agglomeration on fiber diameter and length. When agglomeration is avoided by proper fiber size selection then as shown by our recent studies, both inhalation and intratracheal instillation biopersistence give coherent results.

606 ROLE FOR ICAM-1 AS A SIGNAL TRANSDUCTION MOLECULE IN SILICA EXPOSED MOUSE MACROPHAGES.

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In a mouse model of silica (SI) induced lung injury, SI exposure increases expression of intercellular adhesion molecule-1 (ICAM-1) on lung (alveolar/interstitial) macrophages.To investigate the regulation and role of SI induced ICAM-1 expression on lung macrophages, the mouse macrophage cell line, MH-S (ATCC CRL 2019) was incubated with SI particles (20 ug/ml), PMA (10 ng/ml), TNFα (10 ng/ml) or media for 48 hrs and levels of cell associated ICAM-1 were measured by ELISA. Both TNFα and SI induced a 20% increase in cellular ICAM-1. Immunoperoxidase staining for ICAM-1 revealed that high levels ICAM-1 were detected in approximately 20% of the cells rather than an overall 20% increase in all cells. To study the role of ICAM-1 as a signal transduction molecule, MH-S cells were stimulated with SI, PMA or TNFα (as above) and incubated first with anti-ICAM antibody and then with a goat anti-rat crosslinking antibody. Crosslinking of ICAM-1 resulted in a significant increase in the respiratory burst as detected by the H2DCF oxidation assay. These data suggest that macrophage associated ICAM-1 may play a role in potentiating the phlogistic effects of particle and cytokine stimulation. (Funded by UCRF.)

607 THE ROLE OF CYP2B1 AND CYP2E1 ON M-XYLENE METABOLISM IN RAT PULMONARY AND HEPATIC MICROSOMES, INVITRO.

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Xylenes are volatile organic solvents known to be metabolized by the mixed-function oxidase (MFO) system and have previously been shown to selectively inhibit pulmonary cytochrome P450 (CYP450) activities after i.p. or inhalation exposure. The *m*-tolualdehyde (*m*-ALD) metabolite of *m*-xylene (*m*-XYL) was hypothesized to inhibit pulmonary CYP2B1 activity, at least in part, by CYP2E1-mediated formation and removal of *m*-ALD. CYP2B1 and CYP2E1 substrates, benzyloxyresorufin (BZR) and pyrazole (PYR), respectively, were used to modulate the effects of *m*-ALD formation on CYP2B1 activity, *in vitro*. Formation of *m*-ALD was measured using a 2,4-dinitrophenylhydrazine HPLC method. CYP2B1 was the primary isozyme involved in *m*-ALD formation from *m*-XYL in the lung, while CYP2E1 had a greater role in the liver. Participation of these two isozymes in the formation of *m*-ALD from *m*-XYL was dose dependent in both tissues. Inhibition of CYP2E1 activity, *in vitro*, by 1-heptanol confirmed the relationship between *m*-ALD levels and inhibition of CYP2B1 activity in both lung and liver microsomes. In liver, CYP2E1 played a greater role as supported by decreased CYP2B1 inhibition by 1-heptanol-mediated CYP2E1 inhibition and the subsequent increase in *m*-ALD formation. In lung, 1-heptanol significantly increased the *m*-XYL-induced CYP2B1 inhibition with an associated increase in *m*-ALD formation. In addition, lung and liver microsomes had a time-dependent removal of CYP2B1 inhibition correlated (r=0.97) with removal of exogenous *m*-ALD levels. It can be concluded from these studies that the tissue-selective inhibition of pulmonary CYP2B1 activity is a function of rapid *m*-XYL metabolism by high CYP2B1 activity resulting in *m*-ALD formation which is ineffectively removed by low pulmonary CYP2E1 activity as compared with liver.

608 MONOTERPENES AS SENSORY IRRITANTS.

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The sensory irritancy of the enantiomers of α- and β-pinene (Kasanen et al., Arch. Toxicol. 72; 514, 1998), D-Δ^3-carene and the monoterpene mixture, turpentine, have now been studied using the computerized version of the ASTM E981-84 bioassay. Experimental results revealed that pinenes and carene possess sensory irritation properties; D-pinenes and D-Δ^3-carene being the most potent and L-α-pinene the weakest sensory irritant. RD_{50}

(i.e., the airborne concentration which causes a 50% decrease in respiratory rate) for D-α- and D-β-pinene, and D-Δ^3-carene were almost equal: 1053 ppm, 1279 ppm and 1345 ppm in outbreed OF1 mice, respectively. L-β-pinene was about 4 to 5 times weaker as sensory irritant with RD_{50} of 4663 ppm. For L-α-pinene, the RD_{50} could not be determined because its very weak sensory irritating properties. The RD_{50} of turpentine was 1173 pmm. The turpentine used consisted mainly of D-Δ^3-carene (53.1%), L-β-pinene (14.7%) D-α-pinene (10.6%), L-α-pinene (3.8%) and limonenes (5.6%) indicating that D-monoterpenes might be responsible for the sensory irritation caused by turpentine. For nonreactive chemicals, vapor pressure (P^o) and olive oil-gas partition (L_{Oil}) can be used to estimate their potency as sensory irritant. Thus, for enantiomers with close physicochemical properties, the estimated RD_{50}s will be similar. This can be used to estimate the RD_{50} of a mixture. However, because of the inactivity of L-α-pinene, the 3-dimensional structure must be taken into account. Thus, the target site for nonreactive sensory irritants is most likely a receptor protein capable of chiral recognition and physicochemical descriptors alone may overestimate the potency of some enantiomers in a mixture.

609 GENOTOXICITY AND IMMUNOLOGICAL CHANGES IN ISOCYANATE EXPOSED BROWN NORWAY RATS.

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Methylene di-phenyl diisocyanate (MDI) is an important chemical used to make many polyurethane products such as foams and wood binders. The predominant occupational disease attributed to isocyanates including MDI is asthma, however, the potential for genotoxicity has also been of concern. Diisocyanates are very reactive. MDI can be hydrolyzed to methylene dianiline (MDA), and/or react with primary amines and thiols under physiological conditions. MDA is a carcinogen in animals and is a suspected carcinogen in humans. Brown Norway rats (BNR) were exposed to either 7 (LOW) or 113 mg/m^3 (HIGH) MDI aerosol for 1 hr, 1x/week for 3 weeks with sacrifice 1 week later. A third, acute 135 mg/m^3 (ACUTE) MDI aerosol, 1 hr exposure group was sacrificed within 1 hr of exposure to assess acute eosinophilic inflammation. Bone marrow was recovered from HIGH and LOW groups to assess micronuclei formation in polychromatic erythrocytes (PCE) and peripheral blood for measurement of specific antibodies. Trachea and bronchial alveolar lavage were assessed for inflammatory changes. Dose dependent formation of PCE micronuclei formation was noted in MDI exposed BNR. Preliminary *in vitro* evidence suggest that the genotoxicity may have been mediated through the formation of MDA and/or thioester MDI conjugates. Eosinophilic inflammation measured by histochemical diaaminobenzidine staining of tracheal sections was present only in the acute inflammatory response. T-cells were slightly elevated in the BAL of half of the HIGH MDI exposed BNR, and the cd4/cd8 ratio was normal. MDI-specific IgE was detected in 5/6 and 4/6 of the rats from the LOW and HIGH groups, respectively. MDI-specific IgG was detected in all rats from both groups. These data demonstrates the genotoxic potential along with the allergenic effects of MDI aerosol exposure in the BNR model.

610 RESPONSE OF SHEEP HEMOGLOBIN TO NOSE-ONLY, INHALED NITROGEN DIOXIDE AND CARBON MONOXIDE MIXTURE.

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Nitrogen dioxide (NO_2) and carbon monoxide (CO) are gases produced during fires threatening life and health, because they cause lung injury and impair hemoglobin (Hb) O_2 carrying capacity. If the brain's O_2 supply is reduced, the ability for timely and rational response may be impaired. To study the changes in Hb oxygenation after exposure to NO_2+CO mixture, we used 5 healthy crossbred ewes weighing 70-80 kg, each prepared with a chronic carotid artery loop a month earlier. The sheep inhaled a mixture of (150±10 ppm NO_2+3500±400 ppm CO) for 30 min via the nose using a plastic canine anesthesia mask. Gas concentrations were measured. Arterial (A) and venous (V) blood samples were collected before (Pre), and at 0h, 1h, 4h, and 24h post exposure. Blood was immediately analyzed using a Hemoximeter, and packed cell volume was measured. All sheep survived the exposure, and packed cell volume was not altered. Maximum effect in blood was at 0h post exposure where carboxyHb (COHb) increased 40-fold from <1% to 39.5%, and metHb doubled from 0.8 % to 1.6%. Both returned to control levels by 24h. To follow the kinetics of COHb and metHb formation, we collected blood from two sheep every 5 min during exposure. We found that COHb increased 1% / min, whereas metHb increased at 0.025% / min only. Exposures of rats to similar levels of NO_2 + CO were lethal. This raises a question about extrapolating single species data to humans, and whether small animals alone are suitable for predicting human response or setting environmental and occupational standards.

611 TRICHOTHECENE TOXINS ARE PRESENT IN NON-VIABLE *STACHYBOTRYS CHARTARUM* SPORES.

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Fungi are a significant etiological factor in Sick Building Syndrome. Buildings whose occupants register complaints concerning indoor air quality (IAQ) frequently have high concentrations of Penicillium sp. present in the air and/or Stachybotrys on building surfaces. Stachybotrys sp. are thought to be involved in IAQ problems by virtue of their production of trichothecene toxins. This study determined if spore viability was a prerequisite for the toxigenic properties of S. chartarum, or if the trichothecene toxins were contained in the spores. Toxigenic S. chartarum spores were washed with phosphate buffered saline and incubated with rat alveolar macrophages (RAM) attached to microtiter plates. This treatment caused a dose- and time-dependent loss of RAM viability. Rendering the spores non-viable (NV) with ultraviolet-irradiation did not reduce spore toxicity. Washing S. chartarum spores with absolute methanol extracted the trichothecene toxins and rendered the spores NV. Methanol extraction reduced the toxigenic effect, but, overtime, the RAM were still killed by the S. chartarum spores. These data suggest that at least some of the trichothecene toxins are embedded in the S. chartarum spores and emphasizes the importance of removing S. chartarum spores from the indoor environment regardless of their viability. (These studies were supported in part by QIC Systems.)

612 BIOMARKERS OF CELL PROLIFERATION AND FIBROSIS IN MICE AFTER INHALATION OF ASBESTOS: APPLICATION TO TRANSGENIC MODELS USING LUNG EPITHELIAL CELL-SPECIFIC PROMOTERS FOR MODIFICATION OF MITOGEN-ACTIVATED PROTEINKINASE SIGNALING CASCADES.

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Work in our laboratory has linked activation of certain Mitogen-Activated Protein Kinases (MAPK) to the advent of asbestos-associated apoptosis and proliferation in rodent mesothelial and alveolar type II epithelial cells *in vitro*. These phenotypic endpoints may be linked to the development of pulmonary or pleural fibrosis and/or repair. To address this question, we are developing transgenic mouse models using dominant negative mutant constructs of the Extracellular Signal-Regulated (ERK) and c-Jun-N-terminal (JNK) kinases using lung epithelial cell-specific promoters. The goal of work here was to characterize the development of inflammation, epithelial cell proliferation, and fibrosis in the parental mouse strain, C57Bl/6, after inhalation of NIEHS chrysotile asbestos (~7mg/m^3 air, 6hr/day, 5d/wk). Mice (2 month old, N=5/group/time period) were exposed for periods of time up to 30 days and evaluated for 1) total protein and cell counts in bronchoalveolar lavage fluid (BAL); 2) hydroxyproline content in lung; 3) increases in incorporation of 5-bromo-2'-deoxyuridine (BrdU) in bronchiolar and alveolar type II epithelial cells; 4) histopathology; and 5) procollagen type I message levels in lung. Data show that inflammation occurs in chrysotile-exposed mice as evidenced by increases ($p<.05$) in total protein and increased neutrophils in BALF after 10 and 20 days of exposure. These changes preceded increases in cell proliferation ($p<.05$) at 30 days in bronchiolar and alveolar epithelial cell compartments. Histopathology revealed the development of focal pulmonary fibrosis at 30 days in the absence of increases in lung hydroxyproline or elevations in procollagen type I mRNA levels. Comparative studies using NIEHS crocidolite asbestos are in progress to determine if patterns of inflammation, proliferation and fibrosis are more striking with use of this asbestos type. (Supported by a NIEHS Environmental Pathology Training grant (T32ES07122) and grants from NHLBI and NIEHS.)

613 MODULATION OF OZONE ABSORPTION BY INTERFACIAL PHOSPHOLIPIDS.

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Reactions of ozone (O_3) and nitrogen dioxide (NO_2) with constituents of the lung surface lining layer mediate the net flux of inhaled oxidant into the aqueous phase (reactive absorption). Recent studies have shown that monolayers of dipalmitoyl phosphatidylcholine (DPPC), the major component of alveolar surfactant, modulate NO_2 uptake. When DPPC, deposited on an aqueous surface, was subjected to increased molecular packing (surface compression), NO_2 reactive absorption was significantly reduced. To investigate potential phospholipid (PL) induced effects on O_3 uptake, the rates of O_3 gas phase disappearance (25° C) were evaluated using a specialized exposure-compression apparatus. Determinations of O_3 mass balance across a defined surface area were conducted during graded PL surface compressions. DPPC, dilinoleoyl-PC (DLPC) or palmitoyloleoyl PG (POPG) monolayers were compressed from 121 → 49 Å²/molecule, γ = 72 → 25 dyn/cm respectively. Apparatus constraints prevented further compression. **Results:** 1) At the initial area/molecule level, PL did not alter O_3 (0.6 ± 0.1 ppm) absorption rates. 2) Upon DPPC compression, uptake declined proportional to surface compression (3.5 → 1.2 μg $O_3 \bullet min^{-1} \bullet cm^{-2}$). 3) DPPC-induced reductions occurred regardless of aqueous substrate concentration, composition, or [O_3] (0.3 to 1.0 ppm). 4) Compression of POPG or DLPC monolayers did not significantly decrease uptake. 5) DLPC, extracted from O_3-exposed liposomes, when compressed displayed similar surface tensions and O_3 absorption as previously unexposed. **Conclusions:** Compression of DPPC monolayers to surface tensions which are still greater than those occurring *in vivo* significantly limits O_3 flux into the aqueous phase. Under the exposure conditions employed in these studies, it is unlikely that O_3 reactions with interfacial phospholipids contribute significantly to absorption, nor do such reactions appear to alter PL surface active properties. (HL54696, ESO5749, and HL24075)

614 ADHESION MOLECULES ON BLOOD NEUTROPHILS AND ALVEOLAR MACROPHAGES FROM RATS: MODULATION BY EXPOSURE TO OZONE.

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It is known that exposure of rats to the pulmonary irritant ozone results in an infiltration of polymorphonuclear leukocytes (PMNs) into the lungs. The purpose of this study was to investigate whether the ozone-induced inflammatory process is preceded by a change in the expression of integrins and selectins in peripheral blood PMNs and alveolar macrophages. Female Sprague Dawley rats were exposed to air or ozone (1 ppm, 2 or 4 hrs). Bronchoalveolar lavage (BAL) was carried out and blood was collected via intracardiac puncture at 0 or 18 hrs after the exposure. The percent of neutrophils in the BAL fluid was increased only in animals exposed to ozone for 4 hrs and not for 2 hrs, while the expression of CD18 on alveolar macrophages was lowered at all exposure times. The expression of CD62L on blood PMN was not affected by exposure to ozone, while the expression of CD11b was lowered after 2 hrs, and not after 4 hrs of exposure to ozone. This trend was also observed in experiments in which plasma of ozone-exposed animals was incubated with whole blood obtained from non-exposed animals. In these experiments, the expression of CD11b on PMNs of non-exposed animals was lower after incubation with plasma from 2 hours-ozone-exposed animals, but not after incubation with plasma from 4 hours-ozone-exposed animals. (This work was supported by a grant from the Committee on Prevention and Research in Occupational Health at the Ministry of Labour and Social Welfare.)

615 ACUTE INFLAMMATORY REACTION IN RATS AFTER INTRATRACHEAL INSTILLATION OF MATERIAL COLLECTED FROM A NYLON FLOCKING PLANT.

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The etiologic agent responsible for several cases of interstitial lung disease among workers at a nylon flock plant was unknown. Airborne dust collected at the plant was examined for its inflammatory potential in rat lungs. The endpoints measured were: (1) breathing rates, (2) differential cell counts of bronchoalveolar lavage cells, (3) alveolar macrophage (AM) chemiluminescence, (4) albumin in the acellular bronchoalveolar lavage, and (5) pulmonary histopathology. In the first study, rats received a single dose of the airborne dust sample (10 mg/kg body weight) by intratracheal (IT) instillation. At 1 day post-IT, all inflammatory endpoints were significantly increased ($p<0.05$) versus controls but by 29 days post-IT they did not differ significantly ($p>0.05$) from controls. Histopathology demonstrated mild to moderate, multifocal, suppurative pneumonia usually centered around bronchioles at 1 day post-IT. At 29 days post-IT, pulmonary inflammation was minimal to mild and characterized by alveolar histocytosis usually restricted to the immediate area of retained birefringent fibers. In subsequent experiments, airborne dust was extracted with water and the dust (washed airborne dust) and water extract (soluble fraction) were separated by centrifugation. Nylon dust was prepared in the laboratory by milling uncut nylon strands that had not been treated with finish or dyes which are commonly used in nylon flock plants. Rats were administered a single dose of a dust sample (10 mg/kg body weight) or the soluble fraction (1.3 ml/kg body weight) by IT administration and the same endpoints were measured at 1 day post-IT. The dust samples caused significant increases in all of the inflammatory end points; however the soluble fraction was by far the least active. Histological analysis of the lungs 1 day post-IT confirmed lung inflammation was occurring only after dust instillation and tended to center around bronchioles. The results suggest: (1) nylon flocking generates particles of respirable size which can interact with AM and can be detected in the lung 29 days after exposure, (2) the dusts examined cause an inflammatory response, (3) water extractable agent(s) from airborne dust contribute minimally to the inflammatory response, and (4) the acute inflammatory response to these dusts is substantial when compared to other pathologic occupational dusts previously examined.

616 INTRATRACHEAL INSTILLATION OF WELDING FUMES ALTERS THE PULMONARY CLEARANCE OF *LISTERIA MONOCYTOGENES* IN THE RAT.

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Epidemiological studies have shown an increased incidence of respiratory illness in welders. Stainless steel (SS) welding fumes have been shown to have a greater effect on alveolar macrophage (AM) function than mild steel (MS) fumes. The objective of this study was to evaluate the effects of different welding fumes on the clearance of a bacterial pathogen from the lungs. Fumes were collected during flux-cored manual metal arc (MMA) and gas metal arc (GMA) welding using either SS or MS consumable electrodes. The fume composition was: **1) GMA-SS**: 52.3% Fe, 22.2% Cr, 18.3% Mn, 4.9% Ni, 2.3% Si; **2) GMA-MS**: 89.2% Fe, 8.2% Mn, 2.6% Si; **3) MMA-SS**: 22.3% K, 19.4% Fe, 13.1% Cr, 12.6% Si, 8.2% Ca, 8.0% Mn, 17.4% other. CD/VAF rats were dosed intratracheally with saline (control) or the welding samples at a dose of 1.0 mg/100 g b wt. At 1 and 35 days post-instillation, 5000 *Listeria monocytogenes* were intratracheally instilled into the treated animals. Five days after intratracheal exposure to *L. monocytogenes*, the lungs and spleen were removed, homogenized, and cultured quantitatively on Brain Heart Infusion agar at 37° C. Colony forming units (CFUs) were counted after an overnight incubation. After a 1 day fume treatment, all three welding samples caused a significant decrease ($p<0.05$) in the 5-day clearance of *L. monocytogenes* from the lungs as compared to control. At 35 days post-instillation, the MMA-SS and GMA-SS samples significantly increased ($p<0.05$) *L. monocytogenes* clearance from the lungs as compared to the GMA-MS and control groups. No significant differences were seen among the treatment groups in the number of bacteria cultured from the spleen. We have demonstrated that subchronic exposure to SS fumes may have a greater effect on the pulmonary clearance of *L. monocytogenes* as compared to MS fumes. This is most likely due to differences in fume composition and the greater effect of SS fumes on AM function.

617 APOPTOSIS INDUCTION AFTER SILICA INHALATION IN RATS.

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Occupational exposure to crystalline silica (quartz) has been associated with lung damage and fibrosis. In vitro studies have shown that silica induces apoptosis in human alveolar macrophages and in vivo after intratracheal

instillation in rats. These studies suggested that silica inhalation may stimulate apoptosis and that apoptosis may play a role in the silicotic process. A first step in investigating these questions would be to establish the temporal relationship between silica exposure, apoptosis and lung inflammation and damage. Thus, rats were exposed to filtered air (control) or silica aerosol of 15 mg/m^3 (6 hr/day, 5 days/week) and apoptosis in bronchoalveolar lavage cells (BALC) or lung tissue were determined during a 116 day exposure. The lung inflammatory response to inhaled silica was monitored by determining polymorphonuclear leukocyte and alveolar macrophages (AM) in BALC while lung damage was assessed by measuring acellular bronchoalveolar lavage fluid albumin. In silica-exposed rats, all three parameters were elevated versus controls from 5-41 days of exposure, then dramatically increased thereafter versus controls. Apoptosis in BALC was measured with a commercial ELISA assay kit, and values from silica-exposed rats were normalized to controls. The BALC data indicate that apoptosis was evident after only 5 days of exposure, increased steadily until 20 days of exposure, remained relatively constant through 79 days of exposure and then decreased. Lungs, removed from both control and silica-exposed rats, were fixed with 10% neutral buffered formalin and embedded in paraffin for further examination. Apoptosis was assessed in lung tissue slices using the TdT-mediated dUTP biotin nick end-labeling (TUNEL) assay. The TUNEL assay results were consistent with the observed trends in apoptosis measured in BALC and also indicated that the apoptotic cells were AM. These data indicate that the inhalation of silica stimulates apoptosis as predicted, and the magnitude of the apoptotic response changed during the silica exposure. Finally, the observed acceleration of lung inflammation and damage was associated with a decline in apoptosis, suggesting that apoptosis may play a role in the silicotic process.

618 TEMPORAL RELATIONSHIPS BETWEEN BIOCHEMICAL MEDIATORS OF LUNG DAMAGE AND FIBROSIS AFTER SILICA INHALATION IN RATS.

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Crystalline silica (quartz) is a well established inflammatory and fibrogenic occupational dust. Past studies have established numerous biochemical mediators of these processes, but the temporal relationships between them have not been determined. To investigate these temporal relationships, rats were exposed to filtered air (control) or silica aerosol of 15 mg/m^3 (6 hr/day, 5 days/week) and assays were conducted after 5, 10, 16, 20, 30, 41, 79 and 116 days of exposure. Rat lungs were lavaged to isolate bronchoalveolar lavage cells (BALC) and acellular bronchoalveolar lavage fluid (BALF). Pulmonary inflammation was monitored by measuring BALC polymorphonuclear leukocytes (PMN) and alveolar macrophages (AM) differential cell counts. Compared to control, PMN in BALC isolated from silica-exposed rats were significantly increased after 5 days exposure, remained elevated until 41 days, then increased further. BALC AM also were increased in silica-exposed rats, but only after 41 days exposure. Silica cytotoxicity was monitored by analysis of BALF for lactate dehydrogenase (LDH) and albumin. In silica-exposed rats, both LDH and albumin levels were increased versus control after 5 days exposure, remained relatively constant until day 41, then increased further. AM chemiluminesence, a measure of AM activation and reactive oxygen species production, was higher in silica-exposed rats when compared to control. After 41 days of exposure, lung lipid peroxidation was also higher in silica-exposed rats. BALC secretion of TNF-α and IL-1, when considered on a per cell basis, was higher in silica-exposed versus control rats by 116 days exposure. Lung fibrosis was confirmed by increased hydroxyproline levels in the lungs of silica-exposed but not control rats after 116 days exposure. These data indicate that a progressively severe inflammatory reaction occurs in response to inhaled silica and begins to establish the temporal relationships between the various components of the inflammatory response and the development of pulmonary fibrosis.

619 PULMONARY RESPONSES TO SINGLE VERSUS MULTIPLE INTRATRACHEAL INSTILLATIONS OF SILICA IN RATS.

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The pulmonary toxicity of particles is often studied using a single intratracheal (I.T.) instillation of the material. Criticism has been levied that this procedure is not representative of inhalation exposure. In this study, we compared the pulmonary responses in male F344 rats to a single I.T. instillation of crystalline silica (5 mg/100 gm BWt) given on day 0 with those resulting from 5 consecutive daily I.T. instillations of the dust (1 mg/100 gm BWt per day) with the initial dose given on day 0. Controls received the sterile saline vehicle I.T.. The multiple instillation protocol was used as an experimental surrogate for inhalation exposure. The total amount of silica instilled was the same in the two protocols. Responses were assessed on day 14. The indices of response were cellular differentials recovered by bronchoalveolar lavage (BAL) and the level of albumin in BAL fluid (BALF). With both instillation protocols, the total cells, PMNs, and lymphocytes recovered and albumin levels in BALF were increased significantly in the silica-treated rats versus the respective controls. There were no significant differences between the single and multiple instillation protocols for any of these values for either controls or silica-treated rats. Therefore, at this high dose of silica, where particle overload may be occurring, the single and multiple instillation protocols gave similar pulmonary responses using these endpoints. Responses at other doses of silica and the use of additional endpoints are being investigated.

620 HUMAN BRONCHOEPITHELIAL CELLS CAN DIRECTLY INDUCE LUNG FIBROBLAST GENE EXPRESSION OF EXTRACELLULAR MATRIX PROTEINS AND FIBROGENIC CYTOKINES FOLLOWING ASBESTOS EXPOSURE IN VITRO.

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Direct interactions between human pulmonary epithelial cells and lung fibroblasts, representing two cell types of central regulatory potential in (chronic) lung disease, were studied in a coculture system in vitro as a model for chemical-induced pulmonary lesions such as fibrosis. Membrane cultures of human bronchoepithelial cells (BEAS-2B) were exposed to crocidolite asbestos (2-100μg/cm^2) for 24 h or 96 h and subsequently cocultivated with human lung fibroblast (WISTAR-38) cultures in collagen gels for 48 h or 70 h, respectively. Gene expression of procollagens type I and III, fibronectin (FN) and of the fibrogenic cytokines IL-6 and GM-CSF was determined by RT-PCR. Gene expression of procollagens and FN showed slight but consistent increases less than twofold above control levels after 48 h or 70 h of cocultivation with bronchoepithelial cells pretreated with 50 and 100 μg/cm^2 crocidolite, which was most evident for procollagen type III and FN. Cytokine mRNA levels were already enhanced at the lowest fibre concentration and dose-dependently increased more than twofold above control values after 48 h cocultivation. Constitutive expression of steady-state mRNA levels of both cytokines appeared almost maximally induced in fibroblasts when cocultivated with epithelial cells for 70 h and therefore further enhancement by increasing asbestos concentrations remained less than twofold above controls. 24 h pretreatment of bronchoepithelial cells with nonfibrogenic titanium dioxide could only induce mRNA expression of the cytokines in fibroblasts which occured to a considerably lower extent and only at concentrations above 50 μg/cm^2 TiO_2. Our preliminary results suggest that bronchoepithelial cells can mediate enhanced fibroblast activity in vitro by interacting with fibrogenic agents and thus directly contribute to the development of long-term lung lesions such as fibrosis.

621 CELLULAR INJURY AND REPAIR: REVERSIBILITY OF PULMONARY FIBROTIC LESIONS IN RATS INHALING p-ARAMID RFP.

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Inhalation of asbestos and other fiber-types can induce lung fibrosis. This is considered to be a permanent cellular effect characterized by a progressive tissue thickening response. This study was conducted to assess injury and repair at sites of fiber deposition following high dose inhalation exposures to p-aramid RFP in rats. Rats were exposed to 419 and 772 p-aramid respirable-sized fiber-shaped particulates (RFP) per cubic centimeter (f/cc) for two weeks. Animals were sacrificed and tissue effects analyzed immediately after a 2-week exposure as well as 1, 4, 12, 26, and 52 weeks postexposure. Bronchiole alveolar duct junctions (BADJ) were identified, magnified, and morphometrically analyzed. The volume to surface area ratio (i.e., tissue density or thickness) of BADJs from both exposure groups were compared with sham control rats exposed to filtered air. The results showed that exposures to 419 and 772 p-aramid RFP/cc induced initial increases in tissue density at BADJs for the early postexposure time points. These increases in alveolar tissue volume were most prominent at the 1-4 week postexposure

periods but were not significantly different from controls at 3, 6, and 12 months postexposure. Similarly, morphometric studies measuring alveolar macrophage volume/surface area at BADJs demonstrated a peak at 1 month postexposure but no significant difference thereafter. These results demonstrate that the early fibrotic lesions related to high dose p-aramid exposures are repaired in the absence of continuing exposure, thus indicating reversibility of cellular injury.

622 THE INFLUENCE OF (1→ 3)β -D GLUCAN ON ENDOTOXIN-INDUCED ACUTE PULMONARY INFLAMMATION.

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Organic dust aerosols, known to cause respiratory disease among farmers, are composed of biogenic agents including bacteria, fungi, aeroallergans, excreta, dander, bacterial endotoxins (Etx) and glucans. *Sclerotinia sclerotiorum* (Ss) is an emerging phytopathogenic soybean fungus rich in highly branched, water soluble (1→ 3)β -D glucans to which farmers are exposed. After a first study revealed reduced Etx-induced pulmonary inflammation in mice instilled with Ss extract and Etx, experiments were performed to assess the influence of Ss glucan content in the blunting of Etx-induced inflammation. BALB/cJ mice were intratracheally instilled with 50 μ g of purified scleroglucan (GL) alone, 50 μ g of Ss alone; or 50 μ g of GL or Ss in combination with 0.25 μ g of Etx, or saline (control). Bronchoalveolar lavage (BAL) collected 4 hr postexposure from the Etx group showed an increase in total BAL cells (96% neutrophils) with an increase of MIP-2, TNF-α and IL-6 over controls. GL or Ss exposure alone did not induce changes in BAL cellularity or MIP-2 concentration when compared to controls. However, a reduction of % BAL neutrophils ($p<0.01$) was observed in the GL+Etx group when compared with the Etx group, although total BAL cells remained elevated. The neutrophil response of the Ss+Etx group was similar to the GL+Etx group ($p<0.01$), and total BAL cells were significantly reduced when compared to the Etx group ($p<0.01$). These results suggest that an antagonistic dose-response relationship exists for concomitant GL+Etx exposures and the soluble glucans in *S. sclerotiorum* may be responsible for the reduction of pulmonary inflammation. (Supported by CDC/NIOSH U07/CCU706145 and the Iowa Center for Health Effects of Environmental Contamination.)

623 PULMONARY INFLAMMATORY RESPONSES AFTER EXPOSURE TO OZONE ARE ENHANCED IN ENDOTOXEMIC RATS.

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Inhalation exposure to environmental ozone and systemic exposure to LPS through infection or translocation from the gastrointestinal tract into the circulation are commonplace in people. Acute exposure to ozone causes pulmonary inflammation in rats that is characterized by an early influx of neutrophils (PMNs) into airways. Systemic exposure of rats to small, nontoxic doses of bacterial endotoxin (lipopolysaccharide, LPS) can potentiate deleterious effects of environmental toxicants. To explore effects of a nontoxic dose of systemic LPS on ozone-induced pulmonary inflammation, we evaluated PMN recruitment and inflammatory cytokines in airspaces of Sprague-Dawley rats exposed first to either ozone (1 ppm for six hr) or air and then to either E. Coli LPS (2 mg/kg, i.v. ; $50x10^6$ EU/kg) or its saline vehicle. Three hr after LPS administration, PMNs and cytokines were quantified in bronchoalveolar lavage fluid (BALF). Exposure to ozone alone caused an increase in PMNs and protein recovered in BALF, whereas exposure to LPS alone did not. In rats made endotoxemic after ozone exposure, BALF PMNs were increased compared to rats exposed to ozone alone. Morphometric evaluation of lung tissues showed increased numbers of PMNs in alveolar septa of rats given LPS irrespective of ozone exposure. Examination of inflammatory mediators revealed no or small amounts of tumor necrosis factor-alpha and macrophage chemotactic protein-1 in BALF of rats exposed to LPS or ozone alone, but markedly elevated levels when ozone-exposed rats were made endotoxemic. These results demonstrate that endotoxemia can enhance PMN recruitment into airways of rats exposed to ozone and that this enhancement is associated with markedly elevated release of inflammatory cytokines. (Supported by NIH grant R37ES02581.)

624 ACUTE SMOKE-INDUCED LUNG INJURY IS RELATED TO TUMOR NECROISIS FACTOR-α mRNA GENE AND PROTEIN EXPRESSION IN ALVEOLAR MACROPHAGES.

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Acute inhalation of diesel fuel-polycarbonate plastic (DFPP) smoke causes severe lung injury, leading to acute respiratory distress syndrome (ARDS) and death. It has been reported that the initiation of acute lung injury is associated with activation of pulmonary alveolar macrophages (PAM). To further explore the pathogenesis, PAM of New Zealand rabbits ventilated and exposed to a 60 tidal volume of DFPP smoke *in vivo* were recovered at 1 hour post-smoke. Smoke exposure induced a significant increases in both mRNA and protein levels for PAM tumor necrosis factor-α (TNF-α), when compared to smoke control. Smoke also induced a biphasic response (inhibited at 2 hours, enhanced at 24 hours after cell isolation) in the production of superoxide (O_{2-}) by PAM. However, aerosolized lazaroid U75412E (1.6mg/kg body weight) significantly attenuated smoke-induced expression in PAM TNF-α at the protein level, but not at the mRNA level, and smoke-induced changes in PAM O_{2-}. These changes were paralleled with lung cellular morphological alterations and gas exchange function. This study suggests that highly expressing PAM TNF-α following smoke may be a key contributor to the cascade that establishes an acute injury process and exacerbates oxidant-derived cell injury. Whereas, the lazaroid may ameliorate smoke-induced lung injury by attenuating PAM TNF-α release, in addition to its primary antioxidative mechanism. (Supported by DAMD 17-94-J4001 and NIH ES06694.)

625 PHYSICAL AND CHEMICAL DEGRADATION OF SYNTHETIC VITREOUS FIBERS IN THE LUNG AND IN VITRO.

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Recent rodent inhalation studies of synthetic vitreous fibers (SVFs) and asbestos show a correlation between fiber biopersistence in the lung and fiber toxicity. Inhaled fibers longer than 20 μ m cannot be efficiently cleared by macrophages and therefore can be eliminated only by dissolution or by fragmentation into short segments. In rodent inhalation studies, the fast-clearing, non-toxic fibers demonstrated the following over time in the lung: long fibers cleared more rapidly than short fibers; surfaces became pitted; chemical composition changed; and mean length but not necessarily mean diameter decreased. The slower-clearing, toxic fibers did not show these changes to the same extent or at all. This suggests that incongruent dissolution (leaching) and transverse fragmentation were more pronounced in the non-toxic fibers and enhanced their clearance from the lung. The degradation of 18 fiber glasses was studied in vitro; a correlation between rate of leaching and transverse breakage was found. Additionally, two models of dissolution were demonstrated: (a) constant velocity (CV), the most popular model, which assumes a constant dissolution rate and simple dissolution (all components dissolve at the same rate); (b) diffusion controlled (DC), in which dissolution is incongruent and a hydrated, diffusion-limiting silica gel forms on the fiber surface. 901 fiber glass (MMVF10), which was non-toxic to rodents, fit the DC model. Fibers that fit the DC model underwent rapid transverse fragmentation long before they dissolved completely. Fiberization process (flame vs. air attenuation) strongly influenced in vitro leaching and dissolution rates. Taken together, these studies suggest that biopersistence is an important determinant of fiber toxicity and that, in addition to fiber length and dissolution, leaching and transverse breakage may be important mechanisms of fiber biopersistence.

626 STYRENE METABOLISM BY MOUSE AND RAT ISOLATED LUNG CELLS.

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Styrene has been shown to be pneumotoxic in mice. Rat and mouse pulmonary microsomes are capable of metabolizing styrene to styrene oxide (SO). To determine which cell types in lung are responsible for this metabolism, enriched cell fractions were obtained from mouse and rat lung and comparisons made. The rates of formation of both enantiomers of SO were

determined. The roles of specific cytochrome P450 isozymes were also evaluated using chemical inhibitors. Mouse Clara cells readily metabolized styrene to SO. Diethyldithiocarbamate, a CYP2E1 inhibitor, caused less inhibition of SO formation in Clara cells isolated from mice than in pulmonary microsomes. As in microsomes, 5-phenyl-1-pentyne, a CYP2F2 inhibitor, inhibited the formation of both enantiomers in isolated mouse Clara cells. α -Naphthoflavone, a CYP1A inhibitor, did not inhibit SO formation in isolated lung cells. α -Methyl-benzylaminobenzotriazole, a CYP2B inhibitor, exhibited some inhibition of SO production at 10 μ M and less at 1 μ M in isolated mouse Clara cells. The microsomal and isolated cell studies indicate that CYP2E1 and CYP2F2 are the primary cytochromes P450 involved in styrene metabolism. Styrene metabolizing activity was greater in Clara cells than in type II pneumocytes. It was higher in the mouse Clara cells than in rat Clara cells. R-SO was preferentially formed in mice, and S-SO was preferentially formed in rats. These findings suggest that differences in metabolism may be a reason for the greater susceptibility of the mouse to styrene-induced toxicity. (Supported by NIH grant ES04362 and a gift from the Styrene Information and Research Center.)

627 BIOKINETICS OF AN INHALED ULTRAFINE SILVER AEROSOL IN RATS.

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The possible role of ultrafine particles in the health effects of ambient air pollution makes it important to better understand the disposition of inhaled ultrafine particles deposited in the respiratory tract. Male F344/Crl rats, 12 wk of age, were exposed by inhalation to a radiolabeled ^{110m}Ag with a count median diameter of 25 nm. Rats received an initial body burden (IBB) of approximately 130 Bq at a particle mass of < 1 ng from the 35-min exposure. Immediately thereafter, then at 2, 6, 24, and 72 hr after exposure, groups of four rats were sacrificed to quantify the ^{110m}Ag in selected tissues. The ^{110m}Ag was cleared rapidly, with 53% and 25% of the IBB present at 24 and 72 hr, respectively. Over 98% of the ^{110m}Ag was excreted through the feces. As a percentage of the sacrifice body burden, the activity in lungs was 27%, 7%, and 2% at 6, 24, and 72 hr after exposure, respectively; the activity in the GI tract was 38% and 45% at 24 and 72 hr, respectively; and the activity in liver was 10% and 5% at 24 and 72 hr, respectively. A tissue digestion and ultracentrifugation technique was used to determine whether the ^{110m}Ag activity was present in selected tissues in a particulate or soluble form. Preliminary data suggest that the activity was predominantly particles in the lung and liver, and soluble in the blood. These data describe the biokinetics of an inhaled ultrafine aerosol in the rat and quantify the extent to which ^{110m}Ag is translocated from lung in particulate versus soluble forms. (Research supported by the US DOE Office of Energy Efficiency and Renewable Energy under Cooperative Agreement number DE-FC04-96AL76406.)

628 PHYSICAL CHARACTERISTICS IMPORTANT FOR THE IgE ADJUVANT ACTIVITY OF PARTICLES.

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Diesel exhaust particles (DEP) are reported to increase the allergic immune response to the model allergen ovalbumin. DEP consist of a non-extractable carbon core with a variety of adsorbed chemical substances. DEP have an irregular shape, and form complex aggregates. Because of DEP's chemical composition and tendency to aggregate, it is difficult to determine which physical characteristics are the most important for the adjuvant effect: size, surface area, weight, shape, chemical composition or the number of particles. We performed, therefore, experiments with well characterised, spherical polystyrene particles. These particles are free from adsorbed chemical substances, and they do not so easily aggregate. We used particles with a diameter ranging from 0.06 to 10 μm. We performed four different types of experiments, in which particle size, total weight, total surface area or number of particles, respectively, was kept constant. NIH/Ola mice were given two intraperitoneal injections with polystyrene particles and ovalbumin or ovalbumin alone, over a sixteen days period. The mice were exsanguinated at the end of each experiment, and the serum concentration of IgE anti-ovalbumin was measured. For analysis of the data, graphs were used, one for each experiment. The IgE concentrations and the various physical factors were plotted in each diagram, with one physical factor kept constant. The concentration of IgE anti-ovalbumin increased with decreasing particle size, and also increased with an increasing number of particles. Neither surface area nor weight appeared to correlate with the concentration of IgE anti-ovalbumin. Our findings indicate that particle size and the number of particles are important factors for the IgE adjuvant activity of polystyrene particles, and possibly particles in general.

629 FURTHER CHARACTERIZATION OF EpRE BINDING PROTEINS AND THEIR FUNCTION IN VASCULAR SMOOTH MUSCLE CELLS.

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Ongoing efforts in this laboratory focus on the identification and characterization of proteins that bind and regulate Electrophile Response Elements (EpRE) within *c-Ha-ras* regulatory sequences. The present studies were conducted to further characterize EpRE binding proteins (EBPs) and their regulatory functions in vascular smooth muscle cells (vSMCs). Crosslinking experiments using the consensus *ras* EpRE identified multiple protein complexes that interact specifically with this sequence. A comparison of *c-Ha-ras* protein binding profiles to those in NQO_1 and GSTYa promoters revealed comparable patterns, although the relative abundance of individual complexes appeared to be promoter-specific. To assess the functional relevance of the protein complexes binding to the *c-Ha-ras* EpRE, subsequent experiments were conducted to further evaluate patterns of xenobiotic-inducibility. In agreement with earlier studies showing that activation of EpRE by benzo[a]pyrene (BaP) involves an oxidative mechanism, 3-hydroxy BaP (3-OH BaP) (0.03-3 μM) and BaP 7,8-diol (0.03-3 μM) activated EBP binding in vSMCs. Pretreatment of BaP and H_2O_2-challenged cells with the antioxidant N-acetyl cysteine (NAC) (0.5 mM) reduced oxidant-induced activation of EpRE binding. NAC also reduced constitutive protein binding to this element. Thus, a pathway involving binding of a redox-regulated transcription factor(s) to EpRE may contribute to transcriptional deregulation of *c-Ha-ras* by BaP and related oxidants. (Supported by NIH Grants ES 04849 and ES 09106.)

630 ESTROGEN SIGNALING IN TROUT LIVER: ESTROGEN RECEPTOR AND NON-ESTROGEN RECEPTOR MEDIATED CELLULAR RESPONSE?

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Studies were conducted to determine intracellular hepatic signaling pathways associated with estrogen exposure in rainbow trout. These studies were initiated as a first step in elucidating potential signal transduction pathways associated with exposure to environmental estrogens in fish. Studies include analysis of intracellular pathways that are dependent upon estrogen receptor signaling including vitellogenin and choriogenin biosynthesis, and key pathways thought to act through specific kinase cascades. Exposure of 5mg/kg estradiol to juvenile rainbow trout resulted in a strong and specific "estrogenic" response as measured by the de novo synthesis of vitillogenin and choriogenin in plasma of both male and female trout. Estrogen receptor levels were quantified by both E2 binding and mobility shift assays and demonstrated a significant increase in treated fish. Protein kinaseA (PKA) activity was consistently decreased in estrogen treated males and females, whereas juvenile female trout demonstrated a consistent increase in MAP kinase activity with a concatenate rise in the DNA binding activity of AP-1. Further studies are underway in our laboratories to address whether these cellular responses are estrogen receptor dependent and/or independent signaling events.

631 ANTIESTROGENIC EFFECTS OF 2,3,7,8-TETRACHLORO-DIBENZO-*p*-DIOXIN AND 3,4,4',5-TETRACHLOROBI-PHENYL IN ZR-75-1 HUMAN BREAST CANCER CELLS.

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ZR-75-1 human breast cancer cells respond to treatment with 17β-estradiol (E_2) with an increased rate of pre-confluent cell growth and increased levels of mRNA encoding cathepsin D and estrogen receptor α (ER-α). In this

study, antiestrogenic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 3,4,4',5-tetrachlorobiphenyl (PCB 81) were investigated in ZR-75-1 cells. Effects of TCDD and PCB 81 on E_2-induced cell proliferation, estrogen metabolism, and the levels of RNAs encoding cathepsin D, ER-α, and the E_2-metabolizing cytochromes P450, CYP1A1 and CYP1B1, were evaluated. RNA levels were determined by using the competitive reverse-transcription polymerase chain reaction. The increase rate of pre-confluent cell proliferation elicited by 1 nM E_2 (a 1.4-fold increase over control) was completely blocked by co-treatment with 10 nM TCDD or 200 nM PCB 81. Similarly, elevations in the levels of cathepsin D and ER-α RNAs elicited by 1 nM E_2, 3- and 3.9-fold increases over control, respectively, were completely blocked by co-treatment with 10 nM TCDD or 200 nM PCB 81. Both TCDD (10 nM) and PCB 81 (200 nM) caused marked elevations in the levels of CYP1A1 RNA, eliciting 70- and 50-fold elevations over control, respectively. Levels of CYP1B1 mRNA were elevated 7-fold by exposure to 10 nM TCDD and 6-fold by exposure to 200 nM PCB 81. Rates of CYP1A1 and CYP1B1-catalyzed E_2 metabolism at the C-2, C-4, C-6α and C-15α were highly elevated by exposure to 10 nM TCDD or 200 nM PCB 81; E_2 2-hydroxylation was elevated 48-fold by exposure to 10 nM TCDD and 44-fold by exposure to 200 nM PCB 81. These results indicate that both TCDD and PCB 81 are potent antiestrogens in ZR-75-1 cells *in vitro*, and implicate a marked stimulation in the rate of estrogen metabolism in the antiestrogenic mechanisms of the two xenobiotics. (Supported by ES 04913.)

632 SENSITIVE MOLECULAR ASSESSMENT OF XENO-ESTROGEN ACTION IN THE UTERUS AND IN ENDOMETRIAL CELL LINES.

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Receptor dependent function of steroid hormones is known to be tissue specific. Therefore, potential advers effects of xenoestrogens have to be assessed in a tissue specific manner. The aim of the presented study was to assess xenoestrogen action in RUCA-I rat endometrial adenocarcinoma cells in vitro and in the uterus of DA/Han rats in vivo by analyzing the expression of estrogen-responsive marker genes. In vivo ovariectomized, syngeneic DA/Han rats were were treated for 3 days p.o. with bisphenol A (BPA; 200 mg/kg/d), p-tert.-octylphenol (OP; 200 mg/kg/d), o,p-DDT (500 mg/kg/d), daidzein (Dai; 500 mg/kg/d) and ethinylestradiol (100 μg/kg/d). In vitro RUCA-I cells were cultured on matrigel in a serum free defined medium and treated with the above substances in a concentration dependent manner. Estradiol and the antiestrogen ICI 182780 were used as controls. Gene expression was analyzed by either in vivo labelling and consecutive SDS-PAGE, Northern blotting or semiquantitative PCR. Regulation of expression of complement C3 in RUCA-I cells showed that Dai, OP and BPA were weakly estrogenic, whereas o,p-DDT had almost no effect. In vivo, ethinylestradiol and o,p-DDT down regulated clusterin expression dramatically and up regulated complement C3 expression. Treatment with Dai and OP down regulated clusterin expression significantly, indicative of a weak estrogenicity. BPA was ineffective in vivo. In conclusion, all compounds tested showed estrogenic effects in RUCA-I cells or rat uteri or both. Comparing the relative potencies of xenoestrogens in vivo and in vitro three different pictures arise: the relative potencies of Dai and OP were comparable in vitro and in vivo, BPA proofed to be weakly estrogenic in vitro and almost ineffective in vivo, conversely o,p-DDT exhibited a strong estrogenicity in vivo with an almost undetectable activity in vitro.

633 STRESS PROTEINS IN MOUSE UTERUS INCREASE UPON TREATMENT WITH ESTRADIOL, METHYL-TESTOSTERONE, MEDROXYPROGESTERONE AND FLUTAMIDE.

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We have previously shown that treatment of mice with estrogens changes uterine morphology and increases the levels of chaperon proteins in the uterus. These effects were seen with β -estradiol, tamoxifen (a mixed estrogen agonist/antagonist), diethylstilbestrol (a non-steroidal estrogen), and bisphenol A (a weak estrogen). Neither of these responses were observed following treatment with the glucocorticoid, dexamethasone. The changes in chaperon proteins correlated with uterine swelling and histological changes consistent with a typical estrogenic response. Here we examined the effect of three other compounds: methyltestosterone (MET at 10 ug/mouse/day), a weak androgen and anti-glucocorticoid; medroxyprogesterone (MED at 2 ug/mouse/day), a progestin; and flutamide (FLUT at 150 ug/mouse/day), a non-steroidal anti-androgen. B6C3F1 ovariectomized mice were treated with test compound, corn oil or estradiol for 4 days. Uteri were then removed and weighed. Uterine histology was examined, and stress protein levels were determined by Western blot analysis. Blots were probed with commercially available antibodies to the stress proteins hsp72, hsp73, hsp90α and grp94, among others. MED, MET, and FLUT produced increases in stress proteins similar to those seen with estradiol treatment, although they did not elicit uterotrophic effects. Thus, the stress protein response is not induced by uterine tissue alteration.

634 THE ANTIESTROGEN ICI 182,780 REVERSES THE EFFECTS OF β-ESTRADIOL ON UTERINE MORPHOLOGY AND STRESS PROTEIN LEVELS.

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We previously reported that β-estradiol (E2) tamoxifen, diethylstilbestrol, and bisphenol-A (a weak estrogen) increased uterine weights, altered histological morphology, and increased the levels of several stress proteins (hsps) in the uteri of mice. The present study evaluated the ability of the "pure" anti-estrogen ICI 182, 780 (ICI) to reverse the effects of E2 on uterine hypertrophy and hsp induction. Ovariectomized B6C3F1 mice were injected subcutaneously daily for 4 days with either corn oil (CO), 40 ng of E2, or a low (2 μg) or high (20 μg) dose of ICI alone or in combination with E2. Administration of ICI alone did not result in changes in uterine weights compared to control levels (CO). Co-administration of the high, but not the low, dose of ICI and E2 reversed the uterotrophic and body weight gain effects of E2 to near CO levels ($P<0.001$). The increases in the uterus of the levels of hsp90α, hsp72, hsp73 and of the glucose regulated protein, grp94 caused by E2 were decreased by either ICI treatment, whereas ICI alone had no effect on stress protein levels. Thus, stress protein induction by E2 could be prevented by co-administration of the anti-estrogen ICI.

635 IDENTIFICATION OF ELEMENT IN THE *bcl*-2 GENE PROMOTER REQUIRED FOR ESTROGEN RESPONSIVENESS.

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The *bcl*-2 protooncogene is expressed in diverse tissues and cells, and plays an important role as an inhibitor of apoptosis. Treatment of estrogen receptor (ER)-positive MCF-7 human breast cancer cells with 1 nM 17β -estradiol (E2) resulted in a 2.8-fold increase in *bcl*-2 mRNA levels. Results of initial studies demonstrated that E2-responsiveness of *bcl*-2 was associated with a 360 bp region (-1647 to -1289) of the *bcl*-2 gene promoter. Further deletion analysis showed that a construct containing the -1602 to -1532 region of the promoter (pBcl-2a) was also E2-responsive in transient transfection assays, and a series of constructs, pBcl-2b (-1602/-1577), pBcl-2c (-1577/-1532), and pBcl-2d (-1552/-1532), were prepared to identify functional enhancer elements. In transient transfection studies with pBcl-2a, pBcl-2b, pBcl-2c and pBcl-2d in MCF-7 cells, treatment with E2 resulted in a 2.2- to 3.2-fold induction of reporter gene activity suggesting that functional elements were present in both upstream and downstream regions of the 70 bp sequence. The upstream 25 bp sequence (-1602 to -1577) contained two GC-rich elements that bound Sp1 protein in gel mobility shift assays, and cotreatment with ER enhanced retarded band formation. Mutational analysis confirmed that both Sp1 binding sites are required for hormone-inducibility. Current studies are focused on analysis of the E2-responsive downstream 21 bp sequence (-1552 to -1532), and these results will be discussed. (Welch Foundation and NIH CA76636)

636 A GC-RICH SITE IN THE THYMIDYLATE SYNTHASE GENE PROMOTER IS REQUIRED FOR ESTROGEN-RESPONSIVENESS IN MCF-7 HUMAN BREAST CANCER CELLS.

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It has previously been reported that 17β -estradiol (E2) induces DNA synthesis and thymidylate synthase (TS) activity in MCF-7 cells. This study investigated the molecular mechanism of this response using a series of constructs containing the following TS gene promoter sequencess: -441 to +28 (pTS1), -229 to +28 (pTS2),-140 to +28 (pTS3) and -229 to -140 (pTS4). Ten nM E2 induced a 2.2-fold increase in chloramphenicol acetyl transferase (CAT) activity in MCF-7 cells transiently transfected with pTS2, whereas minimal induction was observed for pTS1 or pTS3. In cells transfected with pTS4, E2 induced a 2.8-fold increase in CAT activity, thus indicating that the -229 to -140 region of the TS gene promoter contained elements required for E2-mediated transactivation. This region of the promoter contains two GC-rich sites at -299 to -220 and -150 to -140, and pTS4m1 and pTS4m2 are mutated at each of these two sites, respectively. Results of transient transfection studies demonstrated that only pTS4m1 was E2-responsive indicating that the downstream GC-rich site was required for hormone inducibility. In gel mobility shift assays, recombinant Sp1 protein formed a retarded band with the -150 to -140 region of the TS promoter, and co-incubation with estrogen receptor protein enhanced Sp1-DNA binding. The results indicate that TS is another E2-inducible gene in MCF-7 cells that is regulated via ER/Sp1 interactions at a specific GC-rich site. (Welch Foundation and NIH CA76636)

637 ANTIESTROGENIC ACTIVITY OF DIINDOLYLMETHANE ANALOGS IN HUMAN BREAST CANCER CELLS: STRUCTURE ACTIVITY RELATIONSHIPS.

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The anticarcinogenic effects of cruciferous vegetables are due, in part, to indole-3-carbinol and its acid-catalyzed metabolites including diindolylmethane (DIM). This study characterizes the *in vitro* activity of DIM and 18 structurally-related analogs in T47D and MCF-7 human breast cancer cells. Data from cell proliferation assays in T47D cells treated with DIM and 18 halo- and alkyl-substituted analogs show that none of these compounds alone (0.1-10 μ M) induced cell proliferation, whereas 1 nM 17β -estradiol (E2) induced up to an 18-fold increase in cell number. In cotreatment studies (E2 + 0.1-10 μ M DIMs), 5 μ M concentration of all analogs inhibited 50-90% of E2-induced cell proliferation, whereas 5,5N -dichloro- and 5,5N -dimethyl-DIM inhibited 51% and 53% of E2-induced cell growth, respectively, at 1 μ M concentration. Similar results were obtained in MCF-7 cells. IC_{50} values for competitive binding to the rat hepatic aryl hydrocarbon receptor (AhR) varied from 6.9×10^{-10} to 2.4×10^{-6} M, and the most active congeners were DIM, 5,5N -dibromo- and 5,5N -dichloro-DIM. DIM and the substituted analogs (10-100 μ M) exhibited minimal induction (<2-fold) of ethoxyresorufin *O*-deethylase (EROD) activity, whereas TCDD (10 nM) exhibited a maximal 45-fold induction response in T47D cells. The response profile for DIM and its structural analogs, namely low AhR-mediated induction of EROD activity and high antiestrogenic activity, indicates that substituted DIMs represent a new class of AhR-based antiestrogens for treatment of breast cancer. (Supported by the State of Texas Advanced Technology Program.)

638 ESTROGEN RECEPTOR (ER) Sp1 PROTEIN INTERACTIONS WITH ER COACTIVATORS.

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Research in this laboratory has demonstrated that the estrogen receptor (ER) and Sp1 proteins physically interact and mediate 17β -estradiol (E2)-dependent transcriptional activation through binding GC-rich elements. Coactivator proteins, such as ERAP-140, SRC-1 and A1B1, bind ER and enhance hormone-dependent transactivation via interaction with promoter sequences containing estrogen-responsive elements (EREs). Interactions of coactivators with ER/Sp1 and Sp1 proteins were investigated by coimmunoprecipitation and gel mobility shift assays. Incubation of [^{35}S]-labeled ERAP 140 and SRC-1 with ER, Sp1 or ER/Sp1 (combined) proteins, followed by addition of Sp1 or ER antibodies, showed that both coactivators coimmunoprecipitated with Sp1/ER and ER proteins. Gel mobility shift assays showed that the intensity of the ER/Sp1-DNA retarded band was decreased by both coactivators in a concentration-dependent manner suggesting that coactivators compete with Sp1 protein for the ER resulting in decreased Sp1-DNA binding. Using [^{32}P]-labeled GC-rich oligonucleotides and combinations of Sp1 (alone), Sp1+ER and Sp1+ER+coactivators, supershifted binary or ternary protein-DNA complexes were not observed in gel mobility shift assays. Current studies are focused on effects of coactivators on ER/Sp1-mediated transcriptional activation using GC-rich promoters.

639 MOLECULAR MECHANISM OF ESTROGEN-INDUCED IGFBP-4 GENE EXPRESSION.

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IGFBP-4 is an inhibitory insulin-like growth factor (IGF) binding protein expressed in a variety of tumor cell lines. Treatment of MCF-7, T47D and ZR-75 breast cancer cells and ECC-1 endometrial cancer cells for 6 hr with 10 nM 17β -estradiol (E2) resulted in induction of IGFBP-4 mRNA levels, and this response was decreased in cells cotreated with the antiestrogens 4N -hydroxytamoxifen (OHT) and ICI 182,780. Western blot analysis with IGFBP-4 antibodies showed that E2 caused a 6-fold increase in immunoreactive glycosylated protein in MCF-7 cells. The 5N -promoter region (-1214 to +18) of IGFBP-4 gene was cloned into a vector upstream from the firefly luciferase reporter gene and E2 induced a 10-fold increase in luciferase activity in MCF-7 cells transiently transfected with this construct. Deletion analysis of this region of the IGFBP-4 gene promoter identified two GC-rich sequences at -559 to -553 and -72 to -64 that were important for E2-induced transactivation. Gel mobility shift assays using ^{32}P-labeled IGFBP-4 promoter sequences -569 to -540 and -83 to -54 showed that Sp1 protein bound these oligonucleotides to form a retarded band, and intensity of the band was competitively decreased after co-incubation with unlabeled IGFBP-4 derived and "consensus" Sp1 oligonucleotides. Mutation of the GC-rich sites within these sequences resulted in loss of retarded band formation. Wild-type human estrogen receptor (hER) did not bind directly to the IGFBP-4 oligonucleotides; however, hER enhanced Sp1-DNA binding in a concentration-dependent manner. Results of this study demonstrate that at least two GC-rich sequences at -559/-553 and -72/-64 are required for induction of IGFBP-4 gene expression by E2. (Welch Foundation and NIH CA76636)

640 HALODIINDOLYLMETHANES: NATURAL PRODUCT DERIVATIVES WITH ANTIESTROGENIC AND ANTITUMORIGENIC ACTIVITIES.

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Indole-3-carbinol (I3C) conjugates in cruciferous vegetables dimerize under acidic conditions to form diindolylmethane (DIM). Both I3C and DIM bind with the aryl hydrocarbon receptor (AhR) and exhibit antitumorigenic and antiestrogenic responses. Halogenated DIM analogs were synthesized and examined as antiestrogens in *in vitro* and *in vivo* assays. All compounds used in this study competitively bound to the AhR (IC_{50} values ranged from 2.6×10^{-9} to 2.0×10^{-7} M). In the female B6C3F1 mouse uterine model, 100 mg/kg of 5,5N -dichlorodiindolylmethane (5,5N -Cl_2DIM) and 6,6N -dichlorodiindolylmethane (6,6N -Cl_2DIM) significantly inhibited 17β -estradiol (E2)-induced uterine peroxidase activity (41 - 67%) and progesterone receptor levels (62 - 25%), and only minimally affected uterine wet weight. DihaloDIMs also inhibited growth of 7,12-dimethylbenzanthracene-induced mammary tumors in female Sprague-Dawley rats, and their order of potency was 5,5N -dibromo . 6,6N -dichloro > 5,5N -difluoro > 5,5N -dichloroDIM at doses of 5 or 1 mg/kg every other day. DihaloDIMs also significantly inhibited E2-induced proliferation of MCF-7 and T47D breast cancer cells at concentrations < 5 μ M, thus exhibiting a broad spectrum of antiestrogenic responses. (Supported by the State of Texas Advanced Technology Program.)

641 CROSSTALK BETWEEN THE Ah RECEPTOR AND ESTROGEN RECEPTOR SIGNALING PATHWAYS IN REGULATION OF TRANSFORMING GROWTH FACTOR α.

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17β -Estradiol (E2) induces transforming growth factor-α (TGFα) gene expression in human breast cancer cell lines, and TGFα acts as an autocrine

growth factor. Previous studies in this laboratory have demonstrated that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) inhibits E2 and TGFα-induced cell proliferation, and the molecular biology of this interaction has been further investigated using the pTGFα-1145/5 construct which contains the -1145 to -5 sequence from the TGFα gene promoter linked to a luciferase reporter gene. Ten nM E2 induced luciferase activity 4- to 5-fold in MCF-7 human breast cancer cells transiently cotransfected with TGFα-1145/5 (5 μ g) and a human estrogen receptor (hER) expression plasmid (0.5 μ g). Cotreatment of transfected cells with TCDD inhibited E2 induced levels. Two downstream (-200 to -252) imperfect palindromic estrogen responsive elements (EREs) have been characterized, and the possible role of other upstream promoter sequences was investigated using the TGFα-1145/377 construct containing a -1145 to -377 TGFα gene promoter insert. In MCF-7 cells cotransfected with 5 μ g of TGFα-1145/377 and 0.5 μ g hER, 10 nM E2 induced luciferase activity 4.5-fold. A perfect ERE half-site in proximity to three Sp1 sites within the -1145 to -377 region of the TGFα gene promoter are required for E2-responsiveness, and the induction response can be inhibited by TCDD. Two potential inhibitory core pentanucleotide dioxin response elements (iDREs) within the -1145 to -377 region of the promoter have been identified at -485 and -465, and current studies are focused on the function of the iDREs in mediating the antiestrogenic response in breast cancer cells. (NIH ES04176)

642 VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) GENE EXPRESSION IS DOWNREGULATED BY 17β -ESTRADIOL (E2) IN HEC1-A HUMAN ENDOMETRIAL CARCINOMA CELL LINE.

M Stoner and S Safe. *Department of Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX, USA.*

Vascular endothelial growth factor (VEGF) is a potent angiogenic factor expressed by many cancer cell lines and is implicated in the neovascularization necessary for tumor growth and metastasis. The molecular mechanisms of 17β -estradiol (E2)-induced VEGF gene expression have been investigated in HEC1-A human endometrial cancer cells. After treatment with 10 nM 17β -estradiol (E2), total RNA was extracted, and semiquantitative RT-PCR showed that E2 decreased VEGF mRNA levels by approximately 50% compared to controls. Transient cotransfection of a human VEGF gene promoter luciferase reporter construct [VEGF*ApaI*-Luc (-131/+379)] and human estrogen receptor α (hERα) expression plasmid into HEC1-A cells produced a dose-dependent 40% decrease in luciferase activity in cells treated with E2 (0.1 nM to 1 μ M) compared to controls. Furthermore, 3N and 5N deletion analysis of VEGF*ApaI*-Luc produced VEGF*NheI*-Luc (-131/+57) and VEGF *SmaI*-Luc (-85/+379); and in transient transfection assays in HEC1-A cells, E2 caused a 40% decrease in luciferase activity. Decreased luciferase activity in HEC1-A cells transiently transfected with VEGF*ApaI*-Luc and treated with 10 nM E2 was specifically reversed after cotreatment with 1 μ M ICI 182,780, a pure antiestrogen, but was not affected by tamoxifen, another antiestrogen. Since the VEGF gene promoter contains no consensus estrogen response elements (ERE), we are currently investigating ERα action through one or more transcription factors that bind Sp1, AP1 or AP2 sequences found between positions -85 to -27. (Welch Foundation and NIH CA76636)

643 AhR-MEDIATED ANTIESTROGENICITY AND ANTITUMORIGENICITY OF DIINDOLYLMETHANE AND ANALOGS.

K Ramamoorthy, A McDougal, M Gupta and S Safe. *Department of Veterinary Physiology & Pharmacology, Texas A&M University, College Station, TX, USA.*

Diindolylmethane (DIM) is the dimerization product of indole-3-carbinol (I3C), an antitumorigenic compound commonly found in cruciferous vegetables. DIM has previously been shown to inhibit 7,12-dimethylbenzanthracene (DMBA)-induced mammary tumor growth and 17β -estradiol (E2)-induced cell proliferation of breast cancer cells. Research in this laboratory has shown that the antitumorigenic and antiestrogenic responses are mediated via the aryl hydrocarbon receptor (AhR). DIM and several alkyl-substituted analogs were synthesized and examined for their antiestrogenic activity *in vivo* and *in vitro*. In the DMBA rat mammary tumor studies, 2,2N -dimethylDIM (2,2N -Me_2DIM) and 1,1N,2,2N -tetramethylDIM (1,1N ,2,2N -Me_4DIM) were significantly antitumorigenic at a dose of 1 mg/kg every other day for 3 weeks, and 1,1N -dimethylDIM (1,1N -Me_2DIM) was significantly antitumorigenic at a dose of 5 mg/kg. At these same doses, no induction of hepatic CYP1A1-dependent activities were observed. The female B6C3F1 mouse uterine model was also used for *in vivo* studies, and effects of E2 (0.02 μ g/day) and E2 + substituted DIMs (100 mg/kg) on progesterone receptor levels, uterine peroxidase activity and uterine wet weight were determined. 1,1N -Me_2DIM and 2,2N -Me_2DIM were both significantly antiestrogenic, and methyl-substituted DIMs also inhibited E2-induced proliferation of T47D breast cancer cells at concentrations # 5 μ M. DIM and several substituted analogs exhibit antiestrogenic and antitumorigenic activity in both *in vivo* and *in vitro* models and represent a group of relatively nontoxic AhR-based antiestrogens. (Supported by the State of Texas Advanced Technology Program.)

644 ANTI-INFLAMMATORY EFFECTS OF TRITERPENOIDS ARE MEDIATED THROUGH PEROXISOME PROLIFERATOR ACTIVATED RECEPTORS (PPARS).

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Triterpenoids are dietary factors that have been demonstrated to have a protective role in regulating cancer formation and progression. Studies have shown that the naturally occurring triterpenoids, oleanolic acid (OA), and the synthetic derivatives TP82 and TP151, mediate these chemopreventive effects by functioning as anti-inflammatory agents. However, the molecular mechanism by which these compounds regulate these anti-inflammatory responses have not been delineated. In this study, we show that these oleananes differentially bind and activate the nuclear receptors peroxisome proliferator receptor α and γ (PPARα and PPARγ), both of which have been shown to modulate inflammatory responses. Transactivation experiments demonstrate that OA and TP151 activate PPARα and PPARγ, respectively, while TP82 activates both PPAR isoforms. Moreover, these compounds directly bind PPARγ and promote adipocyte differentiation. Evaluation of the anti-inflammatory response shows that these terpenes inhibit inducible nitric oxide synthase (iNOS) promoter reporter activity via disruption of NFκB signaling and this response is dependent upon PPARγ. The dose-dependent effects observed for the most potent triterpenoid (TP151) are identical to those reported for the natural PPARγ ligand, 15-deoxy-$\Delta^{12,14}$-PGJ2 (15d-PGJ2). These data identify a novel class of PPARα and PPARγ ligands and provide one of the molecular components for the anti-inflammatory and chemopreventive action of these compounds.

645 HEPATOCYTE PLASMA MEMBRANE ATP-DEPENDENT TRANSPORT PROTEIN EXPRESSION FOLLOWING INDUCTION OF HEPATO CELLULAR PROLIFERATION IN VIVO.

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Peroxisome proliferators are a class of rodent hepatocarcinogens that act as nongenotoxic tumor promoters. While the mitogenic and carcinogenic activity of peroxisome proliferators require the nuclear peroxisome proliferator-activated receptor (PPARα), the mechanisms of PPARα regulation are poorly understood. Previous studies suggest mitogenic growth factors that act through the extracellular signal-regulated kinase (ERK) pathway, including insulin and EGF, modulate PPARα activation and the mitogenic activity of peroxisome proliferators. Furthermore, PPAR proteins have been shown to be phosphorylated after treatment with insulin or EGF. We are investigating whether there is crosstalk between the pathways activated by peroxisome proliferators and these mitogenic growth factors. Our results demonstrate that exposure of primary mouse hepatocytes to peroxisome proliferators, including trichloroacetate, Wy-14,643, and clofibrate, results in a dose-dependent phosphorylation and activation of ERK. Peroxisome proliferator-induced ERK phosphorylation was blocked when cells were pretreated with the MEK (ERK kinase) inhibitor, PD098059, or the phosphatidylinositol 3-kinase (PI3K) inhibitors, LY294002 and apigenin, suggesting that both MEK and a PI3K protein are involved in this response. Under serum-free conditions, Wy-14,643 induced a modest (2-fold) increase in the incorporation of bromodeoxyuridine (BrdU) into primary hepatocytes. However, concentrations of PD98059 which block Wy-14,643-induced ERK phosphorylation also block the stimulation of BrdU incorporation. Our results, therefore, indicate that activation of the ERK pathway through a PI3K-dependent mechanism may play a significant role in promoting the mitogenic activity of peroxisome proliferators.

646 STEREOSPECIFIC ACTION OF NICOTINE ON NASAL NICOTINIC ACETYLCHOLINE RECEPTORS.

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The responses of the olfactory and the trigeminal system in the nose of humans and rats to nicotine vapor were investigated. An olfactometer was used to stimulate the nasal mucosa with R- and S-nicotine vapor applied randomly at different concentrations. a) In anaesthetized Wistar rats extracellular trigeminal single cell units were recorded from the cranial ipsilateral Gasserian ganglion. An aqueous solution of hexamethonium (41.4 mmol/l) applied to the nasal mucosa inhibited responses to both R- and S-nicotine, indica-ting the involvement of nicotinic acetylcholine receptors. Neuronal activity induced by S-nicotine was significantly greater than that induced by R-nicotine (Anova, $\alpha = 0.05$), indicating stereospecificity of the nicotinic receptor. b) For humans, individual thresholds for odorous, burning and stinging sensations were determined. Enantiomeric differences were seen only for the burning threshold (factor 3) and the stinging threshold (factor 2). Trigeminal activation in the nose was quantified by recording negative mucosa potentials (NMPs) and by estimating the intensity of burning and stinging sensations. NMP amplitudes were two times higher and intensity estimates 2 to 6 times higher for S-nicotine ($p < 0.001$). These observations indicate that in humans and rats the nasal olfactory and the nasal trigeminal system are both involved in the sensory detection of nicotine: receptors in the olfactory epithelium respond equally to R- and S-nicotine. Their threshold concentration is 2 µg/l. Trigeminal receptors are able to discriminate between R- and S-nicotine at concentrations above 35 µg/l and involve a stereospecific nicotinic acetylcholine receptor. (Sponsored by Philip Morris USA.)

647 UV LIGHT INDUCED MODULATION OF RPA PHOSPHORYLATION BY EXPRESSION OF ATM PROTEIN.

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Ataxia telangiectasia (AT) is a rare human autosomal recessive disease that is characterized by sensitivity to radiation and genomic instability. The gene mutated in AT, *ATM* encodes a 350-kDa protein that has a C-terminal region homologous to the catalytic subunit of phosphatidylinositol 3-kinases (PI 3-kinases) which have either lipid or protein kinase activity. An important response in a number of cell types exposed to ultraviolet radiation (UV) includes hyperphosphorylation of RPA, a single-stranded DNA-binding protein involved in DNA replication and repair. To examine the relationship between ATM and RPA phosphorylation following UV treatment two experimental approaches were used. First, while normal cells (CH3ABR) hyperphosphorylated RPA following UVC exposure, a normal cell line expressing an antisense ATM mRNA showed reduced ATM protein levels and failed to hyperphosphorylate RPA. Second, an AT cell line (AT1ABR) that lacks the ATM protein showed decreased hyperphosphorylation of RPA after UVC exposure. However, when recombinant ATM protein was expressed in AT1ABR cells, hyperphosphorylation of RPA p34 was stimulated after UVC exposure. These results suggest that RPA phosphorylation can be either directly or indirectly regulated by the expression of ATM protein and may provide a role for regulation of DNA replication and contribute to genomic stability after toxicant exposure.

648 DOSE ESTIMATING EXPOSURE MODEL (DEEM) ARCHITECTURE TO ESTIMATE RELEVANT TOXICOLOGICAL DOSE.

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Physiologically based pharmacokinetic (PBPK) models are often used to estimate toxicologically relevant dose. Such mechanistic models, based on actual physiologic, biochemical, and thermodynamic parameters, are very useful for high- to-low dose, route-to-route, and species-to-species extrapolations. Typically, models have been developed to address health impacts of a specific chemical or a focused. In addition to the extensive physiologic, biochemical, and thermodynamic data necessary, meticulous and exhausting computer coding is required. Even after all this, considerable testing and code verification is necessary before the system can be applied to a risk assessment. The research presented attempts to develop an architecture that simplifies the formulation of case specific models. While totally generic PBPK models are not feasible, nor even desirable, this type of standardized flexible framework can greatly enhance the ease of use by risk assessors and toxicologists. The DEEM is structured so that any number of metabolic and binding schemes can be simulated. The user merely specifies which metabolites circulate and which remain in the organ where they are first formed. Alternatively, the model framework can be used to simulate exposure to several compounds simultaneously. With DEEM the user may also specify metabolic inhibition, induction, and enzyme resynthesis. Thus, by simply inputting the proper physiologic, biochemical, thermodynamic, and exposure parameters the user can quickly formulate his or her own case specific model without the need to write and compile any new code. We have used this framework to model possible toxicologically relevant doses for chloroform, trichloroethylene, methy tertiary butyl ether (MTBE), and hexachlorobenzene. Model simulation results are presented in this paper. NOTICE: The U.S. Environmental Protection Agency (EPA), through its Office of Research and Development, funded this research and approved this abstract as a basis for an oral presentation. The actual presentation has not been peer reviewed by the EPA.

649 DEVELOPMENT OF A DYNAMIC SYSTEMS MODEL OF PESTICIDE EXPOSURE, DOSE, AND BIOMARKERS.

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Multi-media and multi-pathway exposure modeling techniques are needed to properly assess aggregate exposure to infants and children as required under the Food Quality Protection Act (FQPA). To help address this need, a modeling system is being developed by linking a multi-pathway residential pesticide exposure model to a physiologically based pharmacokinetic (PBPK) human dose model using the MATLAB® Simulink® icon-based dynamic system modeling software. The exposure model is based partially on selected scenarios from the *Standard Operating Procedures (SOPs) for Residential Exposure Assessment* as used by EPA's Office of Pesticide Programs for aggregate exposure assessments under FQPA. The linked exposure-dose model system was used to reconstruct exposure and dose estimates for selected exposure scenarios from urinary metabolite data. The dose reconstructions were compared with estimations of dose based on assumptions and chemical specific dislodgeable residue and passive dosimetry measurements for the SOP scenarios. The comparisons suggest that the SOPs tend to overestimate actual "typical" exposures. Notice The U.S. EPA Office of Research and Development funded this research. The abstract was reviewed and approved. The oral presentation has not been reviewed. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

650 QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIP (QSAR) FOR PREDICTING THE POTENTIAL DERMAL DOSE AND DISPOSITION OF ORGANOPHOSPHORUS INSECTICIDES.

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Reconstruction of dermal dose and estimation of the disposition of dermal doses through the use of Physiologically Based Pharmacokinetic (PBPK) modeling have been restricted to single organophosphorus insecticide chemicals. With the enactment of the Food Quality Protection Act (FQPA), exposure modeling techniques are needed to assess the aggregate exposure of infants and children to a broad range of organophosphorus insecticides with differing chemical structures. To this end, a database was compiled containing a number of physicochemical parameter values (Log P, Hammett σ, F, R, Taft E_s, molecular weight, Van der Waals volume, and molar refractivity) and percutaneous absorption, pharmacokinetic, and metabolic parameter estimates, for a series of 15 aromatic organophosphate pesticides. The molecular parameters were obtained from published literature or estimated

using molecular modeling software (HyperChem® ChemPlus™). Pharmacokinetic, metabolic, and percutaneous absorption parameter estimates were obtained from the published literature. Results indicate that these quantitative structure-activity relationship (QSAR) techniques can provide reasonable estimates of pharmacokinetic, metabolic, and skin absorption parameter values for individual OP insecticides and mixtures for use in PBPK modeling. (The U.S. EPA Office of Research and Development funded this research. The abstract was reviewed and approved. The oral presentation has not been reviewed. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.)

651 A PRIORI DETERMINATION OF THE LUMPING OF TISSUE COMPARTMENTS.

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Biologically-based kinetic (BBK) models that simulate the kinetic behavior of volatile organic chemicals divide the body into four compartments, one which corresponds to an actual tissue (liver) and three that represent lumped tissues: the richly perfused tissues, comprised of those tissues most profusely supplied with blood vessels (heart, brain, spinal chord, hepatoportal system, kidney, GI tract and endocrine glands); the slowly perfused tissues comprised of those tissues that are supplied by relatively fewer blood vessels (muscle and skin), and the fat compartment that includes fat depots in the body and the bone marrow. This approach has become standard in BBK modeling but the conditions under which this assumption is valid has not been investigated. The objective of this paper is to develop mechanistically-based guidelines that will allow lumping of several tissues into a single tissue compartment to be based on sound kinetic principles. Of all the parameters necessary to simulate the kinetics of chemicals in a BBK model, only those of tissue volumes, tissue perfusion rates, tissue:blood partition coefficients and metabolic clearance influence the equilibration time for tissues referred to as tissue time constants (TCs, (hr)). The TC is the time required for 63% of the steady-state tissue compartment concentration to be reached and tissues with similar TCs can be lumped together to form a single compartment with no loss in the predictive power of the model. To test this hypothesis, a BBK model consisting of eight tissue compartments (liver, brain, kidney, richly perfused tissues, fat, bone marrow, skin, and muscle) was constructed. Subsequently, the brain and kidney compartments were sequentially lumped into the richly perfused compartment, the bone marrow compartment was incorporated into the fat compartment and lastly the skin and muscle compartments were lumped together to form the slowly perfused tissues compartment. This lumping resulted in a seven, six, five and four compartment models, respectively, which were used to simulate the kinetic behavior of several generic chemicals in rats and humans. The results of the present study indicate that appropriate lumping of tissues depends both on the chemical and the species being modeled.

652 PREDICTION OF THE PHARMACOKINETICS OF DRUGS WHICH ARE HIGHLY METABOLIZED IN LIVER.

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Physiologically-based pharmacokinetic (PBPK) modeling is an effective tool for predicting the plasma concentration-time profile of drugs in mammals. At the present time, simulation of the pharmacokinetics (PK) of drugs can be attempted by providing data on tissue:blood partition coefficients ($P_{t:b}$) and constants for the metabolism (hepatic extraction ratio (E)) and the oral absorption (K_a) as the input in PBPK models. The numerical value of $P_{t:b}$ can be predicted using a mechanistic equation which takes into account the retention of drugs by the lipids, proteins and water. However, data on constants need to be determined experimentally for each new drug. It is known that for highly metabolized drugs (HMDs) in liver, the E value approaches the unity. Whereas, the value of K_a can be estimated by fitting one set of data on plasma concentration-time profile measured following an oral absorption with the simulations obtained using a classical PK model. Then, for HMDs for which an extensive metabolism is suspected principally in liver, their PK after oral or i.v. administration can potentially be predicted using a PBPK model. The objective of the present study was to develop an approach for predicting the PK in rats of HMDs extensively metabolized in liver. The approach consisted of using a conventional PBPK model framework (11 compartments) in which (i) the $P_{t:b}$ values were calculated using a validated mechanistic equation from data on drug solubility in lipids and water and assuming based on literature observations that the magnitude of the protein binding in tissues and blood is identical, (ii) E was set equal to an average value of 0.975 considering that the exact numerical value of E for HMDs ranged probably between 0.95 and 1 (where 1 is hypothetical), and (iii) the K_a value was estimated by fitting using a classical PK approach. The plasma concentration-time profiles in rats of tetrabenazine (after an oral absorption of 1 mg/kg) and of biperiden (after an i.v. injection of 3.2 mg/kg) simulated using the above PBPK model, were comparable to those obtained under *in-vivo* conditions. The PBPK model validated in this study could be a useful mechanistic tool for predicting the PK of HMDs with a minimum of experimental studies.

653 ESTIMATION OF TISSUE:BLOOD PARTITION COEFFICIENTS OF DRUGS BY TAKING INTO ACCOUNT THEIR SOLUBILITY AND PROTEIN BINDING.

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The tissue:blood partition coefficients ($P_{t:b}$) is a composite constant that represents two properties of a drug in tissues and blood, namely, solubility and protein binding. If these two properties can be quantified, estimations of $P_{t:b}$ of drugs could be accomplished. The objective of the present study was to develop a mechanistic approach for estimating the $P_{t:b}$ of drugs in mammals by taking into account their solubility and binding in tissues and blood. The approach consisted of developing an equation that integrates data on drug solubility in neutral lipids, phospholipids, and water with data on protein binding. Based on this equation, $P_{t:b}$ of drugs were calculated from the ratio of the sum of the unbound and bound forms between the tissue and blood. The unbound form in tissues (and blood) was assumed to correspond to the drug solubilities in lipids and water, which were calculated using a recently published methodology. Whereas the magnitude of the protein binding (i.e., relative to the bound form) compared to the drug solubilities in lipids and water was quantified from data on the unbound fraction observed in each tissue and blood. The data on the unbound fraction were obtained from experimental studies published in the literature. The estimated rabbit, rat and human $P_{t:b}$ (brain, fat, kidney, heart, intestine, liver, lung, muscle, skin, spleen, milk) were compared with experimentally-determined data reported in literature for eight drugs (ethoxybenzamide, acetaminophen, prednisolone, barbital, phenobarbital, pentobarbital, thiopental, paracetamol). The estimated $P_{t:b}$ values were close to the experimental ones with an average ratio of 1.01 (S.D. = 0.32, r = 0.89, n = 48). Additionally, experimental data on the unbound fraction in tissues were compared with the ones in blood for the above drugs. The unbound fraction data for tissue differed by an average factor of 1.25 (S.D. = 0.50, n = 48) to the ones observed in blood. The use of the mechanistic equation validated in this study for estimating $P_{t:b}$ should allow to improve the prediction compared to the current standard approach, where the solubility in tissues and blood is assumed to be the same for all the drugs.

654 CRITICAL DETERMINANTS AND IMPACT ON PHARMA-COKINETICS (PK) OF BLOOD PROTEIN BINDING OF VOLATILE ORGANIC CHEMICALS (VOCs).

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The blood:air partition coefficient ($P_{b:a}$) is an important input parameter for predicting the PK of VOCs. The rat $P_{b:a}$ of some VOCs predicted on the basis of lipid and water solubility alone are lower than the experimental $P_{b:a}$ values. This discrepancy is hypothesized to be caused by binding to blood proteins. The objectives of the present study were to: (i) develop and validate an algorithm based on the identification of critical determinants of the blood protein binding of VOCs, and (ii) develop an approach to evaluate the impact of protein binding on the $P_{b:a}$ and PK of VOCs using physiologically-based PK (PBPK) models. Analysis of the various critical determinants of protein binding suggested that only chemicals without an oxygen atom in their structure, having a log oil:water partition coefficient>1, and a geometric volume <300 Å^3 would display binding. An algorithm based on the above observations was developed and applied to identify VOCs for which blood protein binding should be negligible [ether (ETH), methyl ethyl ketone (MEK), and isooctane (ISO)] and those for which protein binding is likely to be important [ethylbenzene (EBZ), bromoform (BRM), chloroform (CHM), and chlorobenzene (CBZ)]. Vial equilibration studies showed that PCs were similar for rat blood and oil/water samples (mean±SE, n=7-8) for ETH (9.24±0.75, 9.53 ±1.16), MEK (139±6, 159.3±8), and ISO (1.92±0.4, 2.88±0.5), whereas the PCs displayed a marked difference for EBZ (50.8±1.3, 7.13±1.6), BRM (161±5, 28.9±3.4), CHM (16.9±1.1, 3.4±0.75),

and CBZ (61.8±2.8, 8.3±2.35). These results confirm that the additional consideration of binding to blood proteins is essential to adequately predict rat $P_{b:a}$ and PK of EBZ, BRM, CHM, and CBZ. Following the estimation and inclusion of the association constant for protein binding into PBPK models of VOCs, the apparent $P_{b:a}$ (=[free+bound blood concentration]/ambient concentration) as a function of exposure concentration was generated. The apparent $P_{b:a}$ of VOCs was dose-dependent. The blood protein binding of VOCs and its dose-dependency elucidated in this study are fundamental to the conduct of scientifically sound extrapolations of the high dose to low dose behavior of VOCs using PBPK models.

655 A QUANTITATIVE STRUCTURE-TOXICOKINETIC RELATIONSHIP (QST_KR) MODEL FOR ALIPHATIC HYDROCARBONS.

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The blood and tissue concentrations of chemicals resulting from an inhalation exposure may be predicted using information on their molecular structure. For example, if the contributions of each molecular fragment of a chemical to its blood concentration (Cb) can be quantified, then Cb for any related chemical can be predicted by summing the contributions of all molecular fragments in its structure. Such a Free-Wilson additive QST_KR model has not been developed previously. The objective of this work was to develop a QST_KR model for inhaled aliphatic hydrocarbons in rat. The literature data on steady-state venous blood concentrations (Cb_{ss}) of n-hexane, n-heptane, n-octane, n-nonane, n-decane, 2-methyl heptane, 2-methyl octane, 2-methyl nonane, 1-octene, 1-nonene and 1-decene in rats exposed to 100 ppm of these chemicals were analyzed using a commercially available software (QSAR-PC®). The resulting QST_KR model was of the form: $Cb_{ss} = A + \Sigma f_S * C_S$, = contribution of $-CH_2-CH_2-CH_2-CH_2-CH_2-$ to Cb_{ss} (+6.8009), f_S = frequency of occurrence of each substituent S on either end of the above basic aliphatic chain (positions 1 and 5), and C_S = contribution of each S to Cb_{ss}. The numerical values of S at position 1 were -3.253, +7.4410, and -2.0190 for H, CH_2=, and $(CH_3)_2$, respectively. For carbon position 5, the values were -1.195, -1.6905, +0.7095, +1.2552 and +2.8223 for CH_3, C_2H_5, C_3H_7, C_4H_9 and C_5H_{11}, respectively. This Free Wilson-type QST_KR model explained 98.8% of the variability in Cb_{ss} among the aliphatic hydrocarbons investigated in the present study. This approach permits to assess the contribution of each S to the toxicokinetics of related compounds. As such, these results can be used to design or choose chemicals with desired toxicokinetic properties, on the basis of specific number, nature and location of S in their molecular structure. This work represents the first attempt to characterize and delineate the quantitative contributions of specific molecular fragments to the toxicocokinetic behavior of volatile organic chemicals.

656 AN INTEGRATED PHYSIOLOGICAL PHARMACOKINETIC MODEL FOR HIGHLY LIPOPHILIC ORGANOHALOGEN SUBSTANCES.

C Emond and K Krishnan. *TOXHUM, Université de Montréal, Montréal, Québec, Canada.*

Physiologically-based pharmacokinetic (PBPK) models have been developed and validated for several organohalogen substances in rodents and humans. These models typically consist of four to six tissue compartments interconnected by systemic circulation. Further these models contain chemical-specific descriptions of certain processes (i.e., receptor binding, biliary excretion, blood protein binding, tissue diffusion coefficients, intestinal reabsorption). A PBPK framework to integrate the models for highly lipophilic organohalogens has not been previously developed. The objective of this work was to construct an integrated PBPK model for simulating the kinetics of highly lipophilic organohalogens. This PBPK model included blood, liver, skin, richly perfused tissues, slowly perfuse tissues, and adipose tissue compartments. This model consisted of input parameters that were kept constant (i.e., tissue:blood partition coefficients, tissue volumes, and blood flow rates) and parameters for which numerical values changed from chemical to chemical (i.e., binding association constants, metabolic rates, biliary clearance, tissue diffusion coefficients and gut reabsorption rate). The tissue:blood partition coefficients were calculated by dividing the fraction of neutral lipids in tissues with the fraction of neutral lipids in whole blood. Blood flow rates and volumes of tissues were obtained from the literature. The numerical values of other chemical-specific parameters were obtained from previously published PBPK modeling work. The resulting integrated model has been used to simulate the pharmacokinetics of several organohalogen substances including 2,2',4,4',5,5'-hexabromobiphenyl, 2,2',4,4',5,5'-hexachlorobiphenyl, and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). This integrated model provides simulations that are comparable to the individual chemical models that have previously been published. The simulations of this model are being used to derive the toxicokinetic equivalency factors for organohalogen substances, using TCDD as the reference chemical.

657 PHYSIOLOGICAL MODELING TO CHARACTERIZE ADULT-CHILDREN DIFFERENCES IN PHARMACOKINETICS (PK).

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Adult-children differences in PK of chemicals may result from age-related changes in physiological, physicochemical and biochemical parameters. A review of the literature suggests that there is no systematic, significant age-related change in tissue lipid and water composition (i.e., the determinants of tissue:blood and blood:air partition coefficients (Pba)). Whereas the hepatic extraction ratio (E) may vary between children and adults for some chemicals, the E value is likely to be 1 for highly metabolized chemicals (HMCs) in all cases. Several physiological parameters, on the other hand, change as a function of age. The objectives of the present study were to develop a physiologically-based PK (PBPK) model that accounts for age-related changes in physiological parameters and to simulate toxicokinetics of HMCs in children and adults. Literature data on physiological parameters (i.e., body weight, cardiac output, ventilation rate and organ volumes) were collected for children of several age groups (newborn – 18 years). Using these data, algorithms relating each physiological parameter to age (or body weight) have been developed. These newly developed algorithms were then introduced in a validated adult human PBPK model for HMCs to create a framework that considers child development. Simulations of inhalation exposure to a given ambient concentration of HMCs (e.g., chloroform) indicated that the initial and steady-state venous blood and liver concentrations of parent chemical are comparable between children of different age groups (except newborns) and adults. This observation can be explained by the fact that the steady-state concentrations are solely determined by factors that do not change with age (i.e., E, which in the case of HMCs is likely to be equal to 1 for all age groups, the fraction of cardiac output flowing through liver, Pba, and the ratio of ventilation rate to the cardiac output). Overall, this study suggests that no adult-children differences in the parent chemical concentrations of HMCs are likely to be observed during inhalation exposures. The PBPK modeling framework developed in the present study can be used to compare PK of chemicals (with varying E values) in adults and children for other exposure routes and dose surrogates.

658 EXPERIMENTAL EVALUATION AND PHYSIOLOGICAL MODELING OF THE PHARMACOKINETICS OF 3, 5-XYLIDINE IN RATS.

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Human exposure to xylidines results from their formation as degradation products of certain pesticides and pharmaceutical products, and from their use in the production of azo dyes. Of the six isomers of xylidine, the 3,5-isomer is the most potent inducer of methemoglobinemia. There is no information available in the literature regarding the pharmacokinetics (PK) of 3,5-xyldine (XYL) in rats or humans. The objectives of this study were: (i) to characterize the PK of XYL in the rat and (ii) to construct a physiologically-based PK (PBPK) model for XYL using animal-replacement methods. The PK of XYL was characterized by analyzing the tissue and blood concentrations at 0.12 – 24 hr following a single iv dose of 0.24 to 0.60 mmol/kg or an oral dose of 1.2 to 4.8 mmol/kg. The concentrations of XYL in the various tissues followed the general order: adipose tissue > kidney > brain > liver and muscle. The PBPK model for XYL consisted of five compartments namely stomach, liver, fat, richly-perfused tissues (RPT) and slowly-perfused tissues (SPT), interconnected by blood. All physiological parameters were obtained from the literature. The tissue: blood partition coefficients (PC) were estimated from the log n-octanol:water PC (2.17) of XYL, its pKa (4.79), pH of tissues, and the proportion of lipids and water in the blood and each tissue. For the biochemical parameters, hepatic extraction ratio was set at 0.1 or 0.9. The PBPK model then generated a range of possible XYL concentrations in the blood and tissues of the rat for each iv dose provided as input. Subsequently, an oral absorption constant of 0.1 hr^{-1} was used to simulate the range of plausible concentration profiles of XYL following oral

dosing. The experimental PK data collected following oral and iv administrations, in general, fell within the range of predictions obtained with the PBPK model. The predictions of the PBPK model can be linked with a pharmacodynamic model for methemoglobinemia, for assessing the risks of human exposure to XYL.

659 PHYSIOLOGICALLY-BASED MODELING OF THE PHARMACOKINETICS OF C8 – C10 1-ALKENES AND ISO-ALKANES IN THE RAT.

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Several C8 – C10 1-alkanes and iso-alkanes occur as environmental contaminants. The kinetics of these substances have previously been studied in rats. Physiologically-based pharmacokinetic (PBPK) models for these chemicals have not been developed. The objective of this study was to develop and validate PBPK models for several 1-alkenes (1-octene, 1-nonene, and 1-decene) and iso-alkanes (2-methyl heptane, 2-methyl octane, and 2-methyl nonane) in the rat. The model structure consisted of four compartments (liver, adipose tissues, slowly perfused tissues and richly perfused tissues) interconnected by systemic circulation. The rat physiological parameters were obtained from the literature. The tissue:blood and blood:air partition coefficients (PCs) were predicted from n-octanol:water and water:air PCs estimated from molecular structure information and tissue/blood composition data. The hepatic extraction ratio was initially set at 0 and then at 1. The PBPK model was used to simulate upper and lower limits of venous blood and tissue concentrations of 1-alkenes and iso-alkanes (corresponding to situations of E = 0 and E = 1, respectively) for any given exposure scenario. The simulated envelopes were compared with previously published experimental data obtained in rats exposed to 100 ppm of these chemicals for 12 hr. The simulated range of values contained most of the experimental data available for all three 1-alkenes and iso-alkanes. The PBPK model developed and validated in the present study should be useful for predicting the kinetics of 1-alkenes and iso-alkanes for other exposure concentrations, scenarios and species.

660 COMPARISON OF SOFTWARE ENVIRONMENTS FOR PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL SIMULATION.

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Physiologically Based Pharmacokinetic (PBPK) simulation modeling involves solving a series of linear differential equations, which are usually of first order. This task is most often carried out by numerical solution of the differential equations using a digital computer. Continued development of computer software automating algebraic calculations and the operations of calculus has resulted in several alternative software environments capable of simulation modeling. For many individuals, the Advanced Continuous Simulation Language (ACSL), produced by MGA Software (Concord, MA), is the simulation environment with which they are familiar. Alternatives to ACSL include Mathematica by Wolfram Software (Cambridge, MA), which is an example of a generalized mathematical solution engine, and MathCad by MathSoft, INC (Cambridge, MA), an example of the "notebook" user interface to an underlying mathematical computation engine. Each of these software environments has its own operating characteristics and it is of interest to compare their use in the development and solution of a PBPK simulation model. This presentation will give examples of equation development, equation solution and graphing of results using each of these three programs.

661 USING PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING TO MINIMIZE ANIMAL TESTING AND ASSOCIATED COSTS UNDER USEPA'S HAZARDOUS AIR POLLUTANTS TEST RULE.

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In June of 1996, the USEPA proposed a test rule under the Toxic Substances Control Act (TSCA) which would require manufacturers and processors of 21 hazardous air pollutants (HAPs) to test these compounds for specific health effects identified as data gaps. As an alternative to conducting these tests, the USEPA solicited proposals regarding the use of pharmacokinetic studies that would permit the extrapolation of toxicity information from other routes of exposure (i.e., oral) to fill gaps in the database of existing inhalation studies. Case studies are presented for two HAPs, 1,2-dichloroethane and 1,1,2-trichloroethane. Physiologically based pharmacokinetic (PBPK) modeling was used to reduce toxicity testing costs and animal usage in three ways. First, where toxicity studies are available for the oral route, route-to-route extrapolation of the results was performed to fill data gaps identified by USEPA. Second, where data gaps could not be filled by existing studies, toxicity testing will be conducted by the oral route (at considerable savings over testing by the inhalation route), after which PBPK modeling will be used to extrapolate the results to the inhalation route. Lastly, the PBPK model will be used to identify appropriate test doses for the toxicity studies, thereby eliminating the need to conduct dose range-finding studies. Additional metabolic studies were designed to refine the existing PBPK model so that it may be applied to the toxic endpoints of interest under the test rule. By taking advantage of existing oral toxicity studies and using the best available science, the need to further animal testing was greatly reduced. Additionally, by reducing toxicity testing costs by nearly one million dollars for each chemical, limited research funds may be better focussed on filling true data gaps.

662 EFFECT OF BROMODICHLOROMETHANE (BDCM) ON VENTILATION IN THE MALE F-344 RAT DURING GAS UPTAKE INHALATION EXPOSURE.

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BDCM is a volatile disinfection by-product of water chlorination within the trihalomethane group which has been found to be carcinogenic in rodents. Gas uptake is a closed inhalation exposure system used to measure rodent metabolism of volatiles non-invasively. The actual estimation of metabolism requires knowledge about the rodent's physiological state during the exposure. The question as to whether BDCM affects ventilation during gas uptake exposure has arisen due to previous inhalation experiments. In this study, 90-day old male F-344 rats were exposed to BDCM at initial concentrations of 200, 800, 1200 and 3200 ppm. The closed inhalation system functioned as a whole body plethysmograph via use of a differential pressure transducer to provide signals from which ventilation frequency, tidal volume, and ventilation rate were derived. Our data shows a marked decrease in ventilation (as minute volume) in the exposed animals compared to controls for each exposure concentration. The percent differences between the time before exposure and the time subsequent to the start of the exposure were also used to determine potential differences. When taking the average of the percent differences for all the data after start of exposure, the difference between controls and exposed was at least two-fold. The impact of the observed decrease in ventilation on the ability to estimate metabolic parameters is currently being evaluated via pharmacokinetic modeling. In summary, preliminary results indicate that BDCM markedly decreases ventilation during closed inhalation exposures used for the measurement of its metabolism. (This abstract does not necessarily reflect EPA policy.)

663 IN VITRO TO *IN VIVO* EXTRAPOLATION FOR TRICHLOROETHYLENE METABOLISM IN HUMANS.

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The use of *in vitro* systems for metabolic studies has distinct advantages and disadvantages. While human hepatocytes and microsomes generate data for comparisons among species and *in vitro* systems, these are generally performed on milligram recovered microsomal protein or million isolated hepatocytes. Recently, *in vitro* data have been used in quantitative predictions of *in vivo* metabolic capacity. Because many data on the distribution and metabolism of trichloroethylene (TRI) in humans exist, we have examined the metabolism of TRI as a case study in the development of a method to extrapolate metabolic rates from *in vitro* to *in vivo*. Kinetic studies undertaken with isolated human hepatocytes have demonstrated that TRI is well-metabolized in culture with a Km of 266 $\pm$ 202 ppm (mean $\pm$ S.D.) in headspace and a Vmax of 16.1 $\pm$ 12.9 nmol/hr/million viable hepatocytes. To provide a basis on which to extrapolate these hepatocyte-mediated and previously published microsome-mediated metabolic rates to the intact liver, we determined that human liver contains approximately 116 x 10^6 hepatocytes and 20.8 mg microsomal protein per gram, based on DNA recovery and glucose-6-phosphatase activity, respectively. Thus, comparison of apparent Vmax values between these *in vitro* systems can be based on 179 ug microsomal protein/10^6 isolated hepatocytes. The microsomal apparent Vmax value of 1589 pmol/min/mg microsomal protein extrapolates to 17.07 nmol/hr/10^6 hepato-

cytes and to 6.8 mg/hr/kg body mass, while the hepatocyte apparent Vmax extrapolates to 6.9 mg/hr/kg body mass. Our extrapolated Vmax value agrees well with other recently published model-predicted Vmax values for TRI in humans, but slightly underpredicted metabolism when incorporated into an existing TRI physiologically-based pharmacokinetic (PBPK) model. The quantitation, extrapolation and inclusion of extrahepatic and CYP-independent TRI metabolism may increase the predictive value of this approach.

664 APPLICATION OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR ETHANOL TO QUANTIFY THE BIOAVAILABILITY IN MALE RATS.

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A portion of ingested ethanol does not reach the systemic circulation. Metabolism by gastric and hepatic alcohol dehydrogenase (ADH) has been implicated as a possible mechanism for this decrease in bioavailability. However, the extent to which each pathway contributes is not known with certainty. Variations in gastric metabolism are also thought to contribute to interindividual differences in sensitivity to the adverse effects of ethanol. The purpose of this study was to use a PBPK model for ethanol in the male rat to estimate the relative contribution of gastric and hepatic metabolism to the bioavailability of ingested ethanol and to evaluate factors that affect this process. The PBPK model was also used to re-evaluate conclusions drawn from previous studies. The current model accounted for competition between incoming ethanol from the gastrointestinal tract and recirculating ethanol, whereas previous methods only quantified the effect of ethanol entering the liver from the GI tract on the overall rate of metabolism by the liver. The model predicted that the decrease in bioavailability of ethanol was due to metabolism by both gastric and hepatic ADH. Metabolism by hepatic ADH was responsible for a greater loss in bioavailability than gastric ADH, particularly at doses below 1.5 g/kg. As the dose increased above 2 g/kg gastric and hepatic metabolism contributed almost equally to the decrease in bioavailability. The contribution of hepatic ADH was most sensitive to variations in the hepatic ADH V_{max}. Similarly, the contribution of the gastric mucosa was most sensitive to its relative capacity for metabolism. Bioavailability was also sensitive to the absorption rate and was dose-dependent. Dose dependence of bioavailability is due to the saturable nature of the gastrointestinal and hepatic metabolism of ethanol. In summary, this study shows that, when ethanol bioavailability is examined using a PBPK model, both gastric and hepatic metabolism affect systemic exposure to ethanol.

665 INVESTIGATION OF THE ENHANCED SUSCEPTIBILITY OF THE AGED RAT TO CARBON TETRACHLORIDE (CCl4) BY DEVELOPMENT OF A PHYSIOLOGICALLY–BASED PHARMACOKINETIC (PBPK) MODEL WITH AGE–SPECIFIC INPUT PARAMETERS.

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Previous studies in our laboratory have shown that aged male F-344 rats are much more susceptible to CCl4–induced acute hepatotoxicity than their younger counterparts. The objectives of the present investigation were to develop a PBPK model for CCl4 in the F–344 rat with age–specific input parameters, and to use the resulting model to investigate the mechanism(s) underlying the increased susceptibility of the aged animals. Closed chamber experiments were conducted with individual aged (22 – 23 months old) male F–344 rats (n=2–4/concentration CCl4). Closed chamber data previously collected in our laboratory from young (65 – 72 days old) male F–344 rats (Evans et al., 1994) were reanalyzed with recently available age– and strain–specific physiological parameters. Age–specific values for cardiac output and blood flow were taken from Delp et al. (1998); fat volume, liver volume and partition coefficients for the old rats were determined in our laboratory. Values for the young rats were taken from the literature. Using averaged data for each inhaled CCl4 concentration, the numeric estimates of Vmaxc and Km, optimized with Simusolv©, were 0.32 mg/hr/kg and 1.16 mg/l, respectively, in the young rats and 0.85 mg/hr/kg and 1.47 mg/l, respectively, in the old rats. Assessment of hepatotoxicity in the young and aged rats that were used for estimation of metabolic rate constants revealed substantially greater toxicity in the aged rats. In conclusion, the increased metabolism in the aged F–344 rats is consistent with their increased susceptibility to CCl4. (Abstract may not reflect EPA policy. DJS was supported by a U.S. EPA STAR fellowship.)

666 COMPARATIVE TOXICOKINETICS OF CARBON TETRACHLORIDE IN RATS, MICE, AND HAMSTERS.

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The toxicity and toxicokinetics of CCl_4 have been studied under a variety of exposure scenarios. However, no study has comprehensively assessed the uptake, metabolism and tissue distribution of CCl_4 in species other than the rat. Therefore, species differences in the *in vivo* metabolism of CCl_4 were evaluated using groups of 3 F344 rats, 14 B6C3F1 mice or 5 Syrian hamsters exposed for 6 hr to 40-1200 ppm CCl_4 in a closed, recirculating gas-uptake system. For each species, an optimal fit of the family of uptake curves was obtained by adjusting the affinity (K_m in mg/l) and capacity (V_{max} in mg/hr/kg BW) parameters for metabolism using a physiologically based pharmacokinetic (PBPK) model. The results show that across species, the mouse appeared to have a higher capacity (V_{max} 1.15) and lower affinity (K_m 0.34) than the rat, while the hamster had both a higher capacity (V_{max} 1.44) and higher affinity (K_m 0.15) than the rat. This suggests that the hamster metabolizes more CCl_4, and at a faster rate, than either rats or mice. To further evaluate species differences, toxicokinetic studies were conducted using groups of 24 animals exposed by nose-only inhalation to ^{14}C-labeled CCl_4 for 4 hours. Groups of 4 animals/species were sacrificed, and tissues collected for analysis, immediately post exposure, and at 2, 6, 24, and 48 hrs post exposure. Exhaled breath, urine, and feces were collected from animals held for the 48-hr sacrifice time point. These studies show that tissue distribution and clearance rates for CCl_4 differ among species. For example, the relative extent of exhalation of $^{14}CO_2$ was hamster >/= mouse > rat, which is consistent with the gas uptake studies. This information is being used to improve the current PBPK model for CCl_4. (Supported by U.S. Department of Energy under Cooperative Agreement No. DE-FC04-96AL76406.)

667 PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING OF HUMAN DERMAL ABSORPTION OF CHLOROFORM FROM BATHWATER.

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The kinetics of chloroform in the exhaled breath of human volunteers exposed dermally via bathwater were analyzed using a physiologically based pharmacokinetic (PBPK) model. A significant decrease in the amount of chloroform exhaled (and thus bioavailability) was observed as exposure temperatures were decreased from 40 to 35 to 30° C. The blood flow to the skin and skin permeability coefficient were both varied to reflect the temperature-dependent changes in physiology and exhalation kinetics. At 40° C, there were no differences in exhalation kinetics of chloroform between males and females. Therefore, similar skin permeability coefficients were determined (~0.06 cm/hr) at a skin blood flow rate of 18% of the cardiac output. At 30 and 35° C, however, male volunteers absorbed and exhaled more chloroform than comparably exposed females resulting in lower skin permeability coefficients calculated for females. At these lower temperatures, the blood flow to the skin was also reduced reflecting the vasoconstriction and shunting of capillary blood from the skin surface to conserve body heat. Simulations of the dermal uptake of chloroform from bathwater were compared to other potential routes of exposure. Assuming an average human will take a 30 min bath at 40° C and consume 2 L of drinking water/70kg body weight/day at the MCL for total trihalomethanes, the relative contribution of dermal uptake of chloroform to the total body burden is predicted to be approximately 18%. At the lower temperatures, the predicted relative contribution of the skin to total body burdens decreases to 1-6%. (Sponsored by the USEPA 68-D4-0023; this research has not been subjected to Agency review and does not necessarily reflect the views of the Agency, and no official endorsement should be inferred.)

668 PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING OF ETHYLENE GLYCOL ETHERS AND ACETATES IN PREGNANT RATS.

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Ethylene glycol monomethyl ether (EGME), ethylene glycol monoethyl

ether (EGEE) and ethylene glycol monoethyl ether acetate (EGEEA) have been found to cause teratogenic effects in rodents and non-human primates. For each of these chemicals, the acetic acid metabolites (methoxy acetic acid and ethoxy acetic acid, respectively) are the likely putative teratogens. PBPK models of EGME/MAA and EGEEA/EGEE/EAA disposition in pregnant rats and humans were developed based on the framework from an existing PBPK model for EGME in mice. The model was parameterized for rats using data from studies in which EGME was administered intravenously and orally. Pharmacokinetic studies were conducted in which the exposure conditions of critical toxicity studies in rats were reproduced. Specifically, pregnant rats were exposed to either 10 or 50 ppm EGME or 50 or 100 ppm EGEEA for 6 hours/day for 5 days beginning on gestation day 11. Maternal blood was collected at hours 1,3 and 5 during the exposure on day 5 and for up to 48 or 96 hours post-exposure for EGEEA and EGME exposed animals, respectively. Fetuses were collected at 6 and 14 hours post-exposure and urine was collected for 18 hours post-exposure by placing the animals in individual metabolism cages. The PBPK model accurately predicted observations from the inhalation studies. Internal dose measures (area under the MAA/EAA maternal plasma curve) for the NOELs and LOELs for both EGME and EGEEA were calculated using the PBPK model. The rat PBPK model was then scaled to humans using published information on the metabolism of these glycol ethers in human tissues and the known physiology of pregnancy in humans. The PBPK model was used to determine the workplace exposure concentration at which pregnant women would achieve the same internal dose measure as those in rats exposed at the NOELs in critical toxicology studies.

669 AN APPLICATION OF SENSITIVITY ANALYSIS WHEN USING A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR CHLOROFORM IN RATS.

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A non-invasive technique used for the determination of metabolism for volatile organic chemicals is gas uptake exposure. The question arises as to whether this technique is best for the determination of metabolic parameters. Sensitivity analysis, a numerical tool, was used to obtain an improved experimental design to estimate metabolism (Vmax and Km) for gas uptake inhalation exposures. We used a previously published five compartment model for chloroform, a disinfection by-product of water chlorination. The resulting simulations of liver concentration over time for four different (100, 500, 1000 and 3000 ppm) initial concentrations were used. The calculated sensitivity coefficients (SC) were related to changes in liver concentration (CL) when metabolic rate (Vmax) was changed by ± 10%. The SCs reflected the change in liver concentration given a change in Vmax over exposure time. The largest changes in absolute value for CL correspond to where the largest absolute value in SCs occur. At 100 ppm, the largest change in CL and SCs were observed within the first hour. For 500 ppm, the largest change in CL and SCs were observed within 4 hours. The largest departure from zero indicated the largest potential change in Vmax that is measurable, and these departures suggest the best sampling times. In contrast, 1000 and 3000 ppm had no peaks or valleys, but kept increasing with exposure length, implying no optimal sampling time. Sensitivity analysis suggested an optimal concentration or exposure length useful in the determination of metabolic parameters. In summary, the technique of sensitivity analysis can be helpful in planning improved measurements for future experiments. (This abstract does not necessarily reflect EPA policy.)

670 EFFECT OF AGE ON THE DISPOSITION OF TRICHLORO-ETHYLENE (TCE) USING PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING: COMPARISON BETWEEN ALLOMETRIC EXTRAPOLATION AND PHYSIOLOGICAL DATA.

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TCE is an environmental contaminant in both air and water which was chosen as a model volatile organic compound. PBPK modeling has traditionally used allometric scaling when comparing across different species. The question arises as to whether this same type of scaling can be used to account for the effect of aging within a given species. In this study, we compared the predicted tissue concentrations obtained using a PBPK model for TCE using allometric scaling. The comparison used aged rats scaled from adult physiological values with those obtained using measured flows in aged (24 month) F344 rats. The PBPK model consisted of the following compartments: blood, fat, liver, brain, muscle, kidney, and rest of the body. A one hour constant level exposure was simulated for concentrations ranging from 0.1 to 4000 ppm TCE. In general, the results of this modeling effort indicate that the most obvious differences between the measured and scaled cases were seen at the lower concentrations. The simulated concentrations using experimental data for fat blood flow were consistently larger than those using extrapolated values. For the liver, the simulations using measured flows had concentrations that were lower than those predicted when using the extrapolation. On the other hand, blood predictions using measured values were higher than the extrapolated for the lower concentrations and then the reverse was observed at the higher concentrations. In summary, preliminary PBPK modeling for TCE indicates that allometric scaling may not accurately account for changes due to aging. (This abstract does not necessarily reflect EPA policy.)

671 DEVELOPING A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL TO DESCRIBE METHYLENE CHLORIDE KINETICS AT THE SUBCELLULAR LEVEL.

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Physiologically based pharmacokinetic (PBPK) models are becoming increasingly popular in their application to human health risk assessment. By and large, these models have been used to predict internal doses at the organ level (i.e., peak concentration in the liver). To date, a PBPK model that describes chemical kinetics at the subcellular level has not been developed. A PBPK model was originally developed for methylene chloride in 1987. This model serves as the primary basis for human health risk assessments prepared by USEPA and OSHA. Over the past ten years, a great deal of information has been collected regarding the toxicity, kinetics, and mechanism of action for methylene chloride. For example, recent evidence indicates that differences in subcellular localization of glutathione S-transferase theta [1-1], the key enzyme involved in metabolic activation of methylene chloride, may explain differences across species in their relative susceptibility to methylene chloride-induced carcinogenesis. A panel of six experts on the kinetics, toxicology, and risk assessment concluded that the existing model for methylene chloride no longer reflects the current state of knowledge, and needs to be updated. For this reason, the existing PBPK model was refined to describe the subcellular localization of metabolic activity for glutathione transferases. In addition to extensive cross-species information on cytochrome P-450 2E1 activity, the refined PBPK model will rely on all of the published and unpublished data available for the subcellular localization of glutathione-S-transferase theta activity in the lung and liver of rodents and humans.

672 PHARMACOKINETIC MODELING OF OCTAMETHYLYCYCLOTETRASILOXANE (D_4) IN RATS: SINGLE AND REPEAT INHALATION EXPOSURE.

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Male and female Fischer 344 rats were exposed to 7, 70 or 700 ppm octamethylcyclotetrasiloxane (D_4) for a single 6-hr exposure or for 15 consecutive 6-hr exposures. Blood, liver, lung, fat and exhaled breath D_4 concentrations, and urinary metabolite excretion data from these rats formed the basis for physiologically based pharmacokinetic (PBPK) modeling. The initial model had blood, fat, liver, richly perfused and slowly perfused tissues that were all well-mixed aggregates. Partition coefficients and metabolic rate constants were estimated from the time course data. This model provided reasonable simulation of uptake, metabolism of D_4, and blood/tissue D_4 concentrations during exposure but underestimated by orders of magnitude blood/tissue D_4 in the post-exposure period and failed to account for the gradual decline in the rate of exhalation after exposure. Addition of deep-lipid compartments within tissues, multiple fat compartments and a transported-lipid compartment to blood provided a better simulation of post exposure blood/tissue concentrations as well as the rate of exhalation. The combination of high lipophilicity and low blood:air partitioning makes D_4 a unique material requiring special consideration for dealing with transport

between these stores and more hydrophobic regions in the body. (Supported in part by the Silicones Environmental Health and Safety Council.)

673 DEVELOPMENT OF A PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PB-PK) MODEL FOR ISOPROPANOL AND ACETONE.

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Due to the widespread use of isopropanol, the EPA promulgated a Final Test Rule in 1989 requiring that isopropanol be tested for pharmacokinetics, subchronic toxicity, and acute and subchronic neurotoxicity. The goal of the testing was to provide the experimental basis for a more quantitative human health risk assessment for isopropanol exposure. A PBPK model was developed for isopropanol and its major metabolite, acetone. Isopropanol kinetics is described as flow-limited, with metabolism occurring in the liver, lung, and skin compartments. Acetone formed in the different metabolizing organs becomes available for clearance into the venous blood supply or can be further metabolized to carbon dioxide, which is then exhaled. The PBPK model was able to simulate isopropanol and acetone kinetics in the rat after dosing by five different routes of exposure (intravenous bolus, intraperitoneal injection, oral, inhalation, and dermal). With this validated PBPK model describing the kinetics in the rat, it would then be possible to extend this pharmacokinetic description to predict and evaluate kinetics of both isopropanol and acetone in humans. (This work was supported by both the Isopropanol and Acetone Panels of the Chemical Manufacturers Association.)

674 DEVELOPMENT OF A PBPK MODEL FOR THE ANALYSIS OF H_2S KINETICS FOR DIFFERENT ROUTES OF EXPOSURE.

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Hydrogen sulfide (H_2S) inhalation is the chief chemical exposure hazard associated with the production of natural gas in gas fields containing high sulfur contamination. Potential exposure to H_2S also occurs at sites of decaying organic matter or inorganic sulfide under reducing conditions. The great hazard from H_2S exposure results from the combined effects of deadening the olfactory system, preventing warning of exposure, and inhibiting cytochrome oxidase in the respiratory centers of the central nervous system, producing apnea. The dose-response curve for H_2S lethality is very sharp, with concentration and not duration as the primary determinant of toxicity. Because of the occupational hazard of H_2S lethality, most animal studies addressing H_2S effects and pharmacokinetics have been conducted at lethal exposure levels or doses, lasting less than five minutes in duration. In order to make it possible to better understand the dose-response relationship for H_2S, a simple preliminary physiologically based pharmacokinetic (PBPK) model of H_2S pharmacokinetics was developed. The model assumes that upon inhalation H_2S is taken up in the blood stream according to the blood/air partition coefficient. Blood/air and tissue/blood partition coefficients were based on data from short-term inhalation studies reported in the literature. Metabolism of H_2S/HS- to sulfate was modeled as occurring exclusively in the liver. The kinetics of sulfate and its excretion was described with a simple one compartment pharmacokinetic model. Metabolism of H_2S to sulfate species was determined by fitting the model to H_2S kinetic data, and the appearance of sulfate in the urine. Overall metabolism of H_2S was adequately described with a capacity (Vmaxc) of 1.0 mg/hr (scaled by body weight to the three-quarters power), and an affinity (Km) of 1.0 mg/L. The completion and utilization of an interspecies PBPK model should reduce current uncertainties in the evaluation of the potential human health effects of H_2S.

675 PHYSIOLOGICALLY BASED TOXICOKINETIC MODELING OF INHALED ETHYL *t*-BUTYL ETHER IN HUMANS.

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Physiologically-based toxicokinetic (PBTK) models are useful tools to relate external exposure to internal doses and biological exposure markers. A PBTK model was developed for the gasoline additive ethyl *t*-butyl ether (ETBE). The compartments used to describe the kinetics of ETBE correspond to lungs (including arterial blood), liver (first order metabolism-based on experimental results), fat, rapidly perfused tissues, resting muscles, and working muscles (to account for work load). The same set of compartments and, in addition, a urinary excretion compartment were used for the metabolite *t*-butyl alcohol (TBA). Organ volumes and blood flows were calculated from individual body build. Estimates of individual metabolic parameters were obtained by fitting human experimental data, i.e. individual blood, urine and exhaled air data (ETBE and TBA) from 8 volunteers exposed to 5, 25, and 50 ppm ETBE (2 hr, 50 W, Nihlén *et al. Toxicol. Sci., in press*) with WinNonlin. Biomarker levels at end of shift (reflect the exposure during the same day) and next morning prior to shift (indicate several days of exposure) were predicted with Simnon. Workload (0 to 100 W) increases all biomarker levels by 2-fold at end of shift, and by 3-fold next morning. Only slightly increased biomarker levels are seen at end of shift during 5 consecutive work days. Predicted interindividual variability in biomarker levels in 8 subjects illustrate that variability is higher next morning than at end of shift, and higher for TBA than for ETBE. Monte Carlo simulations suggest that ETBE in blood and exhaled air at end of shift is highly sensitive to exposure fluctuations, whereas ETBE next morning and TBA in urine and blood are less sensitive to fluctuations. These simulations suggest TBA as a suitable biomarker for ETBE.

676 PHYSIOLOGICALLY BASED MODELING OF HUMAN EXPOSURE TO DICHLOROMETHANE VAPOR USING MARKOV CHAIN MONTE CARLO SIMULATIONS.

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The kinetics of volatile substances can be assessed with the aid of physiologically based toxicokinetic (PBTK) models. However, such modeling is often performed without assessing the reliability of the parameters or their variability in the population. This can be addressed with Markov chain Monte Carlo (MCMC) simulations. By using existing data on the physiological parameters, including their distributions, improved estimates of the parameters are produced, again as distributions rather than point estimates. Åstrand *et al.* (1975) exposed 14 male volunteers to dichloromethane (DCM) vapor at 250 and 500 ppm during moderate to heavy exercise. Engström and Bjurström (1977) exposed 12 male volunteers to 750 ppm during moderate exercise. Extensive physiological (including pulmonary and alevolar ventilations as well as oxygen consumption) and kinetic (DCM in venous and arterial blood, adipose tissue and exhaled air, carboxyhemoglobin (HbCO) in venous blood) data were collected in both studies. Thus, the DCM data sets were deemed suitable for testing this approach. The kinetic data were fit to a PBTK model for DCM, including HbCO, based on Andersen *et al.* (1991). The results show that already by using prior information on the parameters, these experimental studies are described reasonably well by our model. Furthermore, by using the information supplied by the experimental data it is possible to improve the reliability of the distributions of the physiological parameters. (The study was financially supported by the Swedish Council for Work Life Research.)

677 DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR METHYLEUGENOL IN RATS AND MICE AFTER SINGLE INTRAVENOUS AND ORAL DOSES.

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Methyleugenol is a naturally occurring flavoring agent found in nutmeg, mace, and clove oil. It is used also extensively in perfumes. Methyleugenol possesses structural similarity to the carcinogens safrole, isosafrole, estragole and has been shown to form DNA and protein adducts when administered orally to rats. Methyleugenol is rapidly absorbed and metabolized in the liver, with P450-dependent hydroxylation followed by formation of sulfates and glucuronides.We have developed a PBPK model to describe the kinetics of methyleugenol administered to rats and mice by single intravenous injection or by single oral gavage. Absorption from the GI tract is assumed to be rapid but not instantaneous, distribution is described as flow-limited, and metabolism of methyleugenol in the liver is described as saturable. Comparison of the optimized model to data from animals administered methyleugenol multiple times by oral gavage suggests auto-induction of hepatic metabolism.

678 IN VITRO AND IN VIVO KINETIC ANALYSIS OF LIPOPHILIC COMPOUNDS:TETRACHLOROBENZYL-TOLUENES (UGILEC 141).

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Tetrachlorobenzyltoluenes (TCBTs) are present in the technical mixture of Ugilec 141, which was used in Europe as a replacement for polychlorinated biphenyls (PCBs) in the 1980s. The kinetics of some TCBT isomers in the rat were studied using in vitro and in vivo techniques. A preliminary PB-PK model, consisting of 5 compartments (blood, liver, fat, slowly perfused and richly perfused tissue) was established. Partition coefficients were estimated on forehand based on lipophilicity of the compounds and composition of the tissues. In vitro-derived metabolic rate constants for the liver were scaled to the in vivo situation. Metabolic rate constants were also determined from model optimization to in vivo-derived data. Thus, it is demonstrated that the in vitro-derived rate constants differ 3- to 4-fold from the optimized values. The estimated partition coefficients were mostly in accordance with the actual values observed in vivo. In addition, it was observed that diffusion of these compounds from adipose and muscle tissue to the blood compartment influences the elimination from the body. The present study demonstrates that in vitro-derived metabolic parameters can be used for independent parameterisation of PBPK models for lipophilic compounds.

679 SIMULATION OF GLYCYRRHIZIC AND GLYCYRRHETIC ACID KINETICS AFTER INTRAVENOUS AND ORAL ADMINISTRATION TO RATS AND HUMANS WITH A PBPK MODEL.

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Glycyrrhizic acid (GD), isolated from the dried root of *Glycyrrhiza glabra*, is used in a variety of products (e.g. as a sweetener in liquorice and chewing tobacco and for its anti-inflammatory properties in clinical therapeutics). Due to 11-β-hydroxydehydrogenase inhibition, GD and its aglycon glycyrrhetic acid (GA) can cause symptoms such as hypertension and edema. The kinetic behavior of GD and GA was studied with a rat PBPK model, for both intravenous and oral administration. Body distribution and elimination were modeled physiologically using literature data from *in vitro, in situ* and *in vivo* studies. This model explains 65% and 88% of the variation in the experimental plasma concentrations of GD and GA after iv doses of 5-50 mg/kg. Variation in the GD bile appearance was explained for 99%. To simulate kinetics of GD and GA after oral dosage, the model was extended with a gastrointestinal tract submodel. This physiologically describes GD and GA transfer to and absorption from stomach, and intestines. Intestinal conversion of GD in GA by commensal bacteria was incorporated in this model. Simulations show that this extended PBPK model is able to simulate the oral absorption of GD and GA in rats. Extrapolation of the PBPK model to humans was also successful. The a priori predicted maximum GA plasma concentration (Cmax) after 130 mg GA in oral suspension to human volunteers (equivalent to 150 g liquorice sweets) was 1.29 mg/l, whereas an average Cmax value of 1.50 mg/l was reported in 16 volunteers.

680 ESTIMATION OF SMOKE UPTAKE FROM CIGARETTE PRODUCTS USING A VALIDATED PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) NICOTINE MODEL.

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A validated physiologically-based pharmacokinetic (PBPK) model has been applied to estimate nicotine absorption as a surrogate for smoke uptake from smoking a single cigarette (Chang et al., 1995 and Chang et al., 1998). In this study, 20 smokers smoked two types of cigarette products in separate sessions. During each session, subjects smoked two cigarettes of usual brands or a test product that primarily heats tobacco, every 30 minutes for one hour. Blood samples were taken before smoking and 5, 7.5, 10, and 30 minutes after smoking each individual cigarette. Serum nicotine was quantified by radioimmunoassay (RIA). Nicotine uptake was estimated by model simulation to best fit data with input of subjects' parameters. There were no statistical differences in nicotine uptake between smoking test product compared to usual brands, nor between the 1st (1.11 mg ± 0.64 mg) and the 2nd (0.85 mg ± 0.31 mg) test cigarette smoked. However, the mean nicotine uptake from usual brands was statistically different between smoking the 1st (1.35 mg ± 0.55 mg) and the 2nd (1.04 mg ± 0.38 mg) cigarettes. Our results indicate that the large interindividual variability in smoke uptake causes great difficulty in empirically defining smoke uptake for any given cigarette product.

681 A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR PENTACHLOROBENZENE: APPLICATION TO A MEDIUM-TERM LIVER FOCI BIOASSAY.

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A physiologically-based pharmacokinetic (PBPK) model for pentachlorobenzene (PeCB) was modified to incorporate the temporal changes following a partial hepatectomy and describe the disposition within the dosing schedule of a medium-term liver focus bioassay. The study consisted of a 6-week course of daily gavage administration of PeCB in a corn oil vehicle (100 μmoles/kg/day, 7 days/week). One week after the start of gavage dosing, a two-thirds partial hepatectomy was performed followed by time course measurements of PeCB in blood, liver, kidney and fat. Concentrations of PeCB in the fat have a peak concentration at approximately 300 hours after the first gavage treatment (i.e., 132 hours post-surgery). The increase is due to a mobilization of fat and is consistent with previous reports of post-surgery lipogenic enzyme induction. Blood and liver concentrations also rise dramatically following surgery as parent chemical is released from the fat during mobilization. In the kidney, the time-course tissue concentration of PeCB displays a complex behavior that is dependent on both a temporary decrease in hepatic alpha2u protein synthesis and the mobilization of fat. The construction of the PBPK model allows for the physiological changes following surgery by adjusting model parameters representing fat stores, alpha2u synthesis, and metabolism. When compared with the experimental data, the PBPK model was able to accurately describe the changes in tissue concentrations after surgery and provide a means for evaluating the dose-response relationship between the growth of preneoplastic lesions and target tissue concentrations with the medium-term bioassay. This work was supported by AFOSR (f49620-94-1-0304) and NIEHS Superfund Basic Research Program (P42 ES05949).

682 ELEVATED PROLACTIN (PRL) PRIOR TO PUBERTY AND THE DEVELOPMENT OF PROSTATITIS IN ADULT WISTAR RATS.

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Previously, we reported that alterations in rat maternal serum PRL (sPRL) lead to an increased incidence of lateral prostate inflammation (LPI) in the offspring. Based on these results, we hypothesize that if the prepubertal male is made hyperprolactinemic from exposure to estrogen (E) or dopamine (DA) receptor blockers, there would be lasting effects on the prostate in adulthood. To test this hypothesis, pups were exposed daily to a DA receptor blocker, pimozide (PIM, 20 mg/kg, sc), estradiol benzoate (EB, 8 mm silastic capsule) or EB + the DA receptor agonist, bromocriptine (BR, .417 mg/kg, sc, 2 X day) or the environmental estrogen, bisphenol A (BPA) (50mg/kg, sc) from postnatal day (PND) 22 to 32. On PND28, half the males were killed by decapitation 1 h after dosing. sPRL levels were increased in the PIM, EB, and BPA groups, but sPRL was comparable in the vehicle and EB + BR males. At 120 days of age, the incidence of LPI (as determined by a myeloperoxidase assay for neutrophil conentration) in the PIM, EB and BPA groups was greater than the comparable controls. In contrast, EB + BR did not induce LPI. Thus, prepubertal hyperprolactinemia, whether it is induced by estrogen receptor agonists or DA receptor blockers, is associated with an increase in LPI in the adult. (This abstract does not reflect EPA policy.)

683 IN UTERO ANTI-ANDROGEN TREATMENT REDUCES POST-NATAL ENERGY METABOLISM IN RAT SERTOLI CELLS.

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Human epidemiological studies have suggested a general decrease of quantity and/or quality of spermatozoa in developed countries, and studies on wildlife have shown abnormalities in anatomy and sexual behavior of some

male animals (alligators, bird...). These alterations have thought to be related to the lesions produced by endocrine modulators during the intra-uterine life. In order to test this hypothesis, pregnant Sprague Dawley rats were treated with an anti-androgen, flutamide (0, 2, 10, 50 mg/kg/day; from day 6 up to delivery). At post-natal day 15, male offspring were sacrificed, testes were collected and the primary cultures of Sertoli cells were prepared following enzymatic digestion. Culture media were collected for lactate assay by an enzymatic method and total RNA were extracted. Messenger RNA expression was analyzed using semi-quantitative RT-PCR. Treatment of animals by flutamide during the fetal period resulted in a decrease of lactate production by Sertoli cells (approximately 60% at all doses). In an attempt to explain this decrease and to determine the level of flutamide action, we studied the expression of genes implicated in the production and transport of lactate. The expression of lactate dehydrogenase A (LDHA) messenger (isoform involved in the conversion of pyruvate into lactate) was decreased (about 48% of inhibition) at 10 mg/kg/day. In addition, the level of lactate transporter, MonoCarboxylate Transporter (MCT) 1 and 2, mRNA was decreased in Sertoli (45% and 70%, respectively) at 10 mg/kg/day. In conclusion, the energy metabolism (production of lactate) in Sertoli cells appears to be altered in animals exposed *in utero* to flutamide. As lactate represents the preferential energy substrate for germ cells, it is possible that flutamide, by a decrease of lactate production in Sertoli cells could affect the maturation of germ cells.

684 IN UTERO EXPOSURE TO FLUTAMIDE: EFFECTS ON DEVELOPMENT OF THE MALE REPRODUCTIVE SYSTEM OF THE RAT.

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The present study examined the development and function of the reproductive system of the male rat after in utero flutamide exposure during the routine dosing period recommended by EPA for developmental toxicity studies. Pregnant Sprague Dawley rats were administered flutamide in methylcellulose daily from gestation day 6 up to delivery at 0, 2, 10 and 50 mg/kg/day. In order to examine effects on the development of the reproductive system, male offspring were examined and sacrificed at specific periods between weanling and adult age (day 3, 15, 28-35 and 90). In addition, sperm analyses were performed on postnatal day 90. Maternal weight gain, parturition, pup weights and survival were unaffected by flutamide exposure at 2 and 10 mg/kg/day. In utero exposure of male offspring at 10 and 50 mg/kg/day resulted in feminization of the external genitalia and strongly reduced development of the reproductive tract. On postnatal day 3 prostate and epididymis were macroscopically affected. However, hypospadia, vaginal pouches, persistence of nipples, absence of testicular descent, reduced testis, epididymis, vas deferent, seminal vesicle and prostate weights were observed at 10 and 50 mg/kg/day from postnatal day 15. In addition, a dose-related reduction of sperm count, motility, velocity parameters of caudal epididymis sperm was noted at these doses. No treatment-related changes in sperm parameters were noted at 2 mg/kg/day. At this dose, the only significant effects were hypospadia and persistence of nipples on postnatal day 15 and 90. These findings demonstrated that flutamide administered from gestation day 6 up to delivery irreversibly altered the male reproductive system and function. In the context of hormonal disruption, the present study has identified some potentially sensitive endpoints and critical periods which could be used in discussion for risk assessment.

685 GESTATIONAL/LACTATIONAL EXPOSURES TO AROCLOR 1254 INDUCE SEVERAL P450-DEPENDENT ACTIVITIES, INCLUDING METHOXYCHLOR DEMETHYLATION, IN RAT HEPATIC MICROSOMES.

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Polychlorinated biphenyls (PCB's), such as Aroclor 1254, are potent inducers of cytochrome P450 (CYP) in adults. Experiments were conducted to determine whether exposure of rats to Aroclor 1254 during gestation and lactation induces hepatic microsomal P450's, with special interest in the proestrogenic insecticide methoxychlor (MXC) whose didemethylated metabolite dihydroxyphenyl trichloroethane (DHPTE) binds to estrogen receptors. Pregnant female rats were administered 4 mg/kg-day of Aroclor 1254 in safflower oil p.o. from gestation day 6 through weaning, and offspring were sampled on postnatal days (PND) 15, 23, 31, and 70. PCB treatment substantially induced CYP 1A1, 2B1 and 3A2, as determined by Western blots, on PND 15 and 23 (while PCB exposure was still occurring), with moderate induction still present on PND 31 (after PCB exposure had ceased for 9 days), and no induction apparent at PND 70. No induction was observed in CYP 2C11 or 4A1, but sex-dependent development of CYP 2C11 was observed in males. Ethoxyresorufin and pentoxyresorufin *O*-dealkylases were induced 100- and 25-fold, respectively, on PND 15, 15-fold on PND 23 and 31, and not induced on PND 70. Desulfuration activities with parathion and chlorpyrifos were induced 10- and 27-fold, respectively, on PND 15, and the induction declined with age, but a 2-3-fold induction was still present at PND 70. A-Esterases were slightly induced. An age-related increase in the ability of microsomes to produce the monodemethylated MXC metabolite and DHPTE was observed, and the production of DHPTE was induced in the PCB-treated animals at PND 15, 23 and 31. Exposure to PCB's during development may have the potential to alter the organism's response to MXC-induced reproductive toxicity by modifying the amount of estrogenic metabolite produced. (Supported by EPA R825296.)

686 ANTIANDROGENIC EFFECTS OF 2,2-BIS(4-CHLOROPHENYL)-1,1-DICHLOROETHYLENE IN NEONATAL AND FETAL RATS.

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The insecticide dichlorodiphenyltrichloroethane (DDT) was used extensively in the US during the middle part of this century. Although banned in 1970, DDT and its major metabolite 2,2-bis(4-chlorophenyl)-1,1-dichloroethylene (p,p'-DDE) continue to be persistent environmental contaminants. p,p'-DDE acts as an antiandrogen in both *in vitro* and *in vivo* systems. Male rats exposed to high doses of p,p'-DDE *in utero* display feminized external morphology in the form of reduced anogenitial distance and retained thoracic nipples. *In vitro* studies indicate p,p'-DDE antagonizes androgen binding to the androgen receptor and alters transcription of androgen-regulated expression vectors in cell culture. The purpose of this study is to further characterized the antiandrogenic impact of p,p'-DDE upon the developing male. Time-pregnant Long Evans rats were gavaged with either 50,100, or 200 mg/kg p,p'-DDE during gestation days 14-18. Endpoints investigated include the evaluation of post-natal day 21 neonatal organ weight as an indicator of antiandrogenic activity and the quantitation of androgen receptor mRNA expression in gestation day 19 fetal tissues using RT-PCR. Neonatal livers in experimental progeny exposed to 50 or 100 mg/kg p,p'-DDE were increased in size, indicative of microsomal enzyme activation due to continual exposure to p,p'-DDE via lactation. Prostate tissue from exposed male pups displayed a dose-dependent reduction in weight, indicative of potential antiandrogenic action by p,p'-DDE. Conversely, ovaries from exposed females tended to be larger in the exposed groups, suggesting the ovaries may be a potential target for p,p'-DDE. Preliminary results from fetal tissues exposed *in utero* to 200 mg/kg/day imply an alteration of androgen receptor mRNA expression. These results further demonstrate the ability of p,p'-DDE to antagonize androgen action in fetal and neonatal rat tissues. (Funded by a National Research Council-USEPA/NHEERL Research Associateship. This abstract does not reflect USEPA policy.)

687 PRENATAL EXPOSURE TO ANTIANDROGENS ALTERS THE RESPONSIVENESS OF PROSTATE TO *p,p'*-DDE IN MALE ADULT RATS AND INDUCES PROSTATIC INFLAMMATORY RESPONSES.

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1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE) is a persistent environmental contaminant and a demonstrated antiandrogen in the rat. This study was conducted to investigate the response of adult male rats to a DDE exposure following their prenatal treatment with DDE or the nonsteroidal antiandrogen flutamide. Pregnant Long-Evans rats were dosed by gavage with 0, 10, or 100 mg *p,p'*-DDE/kg/day or 40 mg/kg/day flutamide from gestation day 14 to 18. At the age of 80 days, the male offspring in each treatment group were divided into two subgroups (n = 4 to 9). One group received DDE by gavage at 70 mg/kg/day for 4 days, while the other received the vehicle (corn oil). Prenatal antiandrogen treatment was found to cause a reduction (18, 31, and 53%, for 10 and 100 mg DDE and flutamide groups, respectively) in the ventral prostate (VP) weight on postnatal day 84. The postnatal DDE treatment reduced the VP weight in only the 0 and 10 mg

DDE groups (31 and 19%, respectively) compared to their corresponding second vehicle controls, while the 100 mg DDE and flutamide groups were not affected. Adult serum testosterone and prostate tissue mRNA level of androgen receptor in either the postnatal DDE or control group correlated poorly with the VP weights. In contrary, high correlations were found in the postnatal DDE treatment group between VP weights and the mRNA levels of prostatein C3 or testosterone-repressed prostatic message 2 (r2 = 0.82 and 0.99, respectively). In addition, histological evaluation of prostates from three prenatally exposed rats with grossly abnormal prostate appearance revealed a chronic suppurative inflammatory response. These results indicate that prenatal antiandrogen treatment alters ventral prostate responsiveness to subsequent DDE exposure. (Supported in part by the Chlorine Chemistry Council.)

688 AH RECEPTOR AND ARNT PROTEIN CONCENTRATIONS IN RAT PROSTATE: EFFECTS OF AGE AND 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN.

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The developing male rat reproductive tract is a target of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity, thought to be mediated through the aryl hydrocarbon receptor (AHR) and its dimerization partner, the AHR nuclear translocator (ARNT) protein. Thus, studies focused on the effects of age and TCDD exposure on AHR and ARNT protein concentrations in Total Tissue Lysates derived from reproductive organs of male rats using quantitative Western blotting techniques. AHR protein levels in the ventral prostate (VP) and dorsolateral prostate (DLP) of developing rats decreased with age, declining approximately 70% between postnatal day (PND) 1 and 21. ARNT protein levels decreased with age in the DLP, but not VP. High levels of AHR and ARNT protein were also detected on GD 16, 18, and 20 in fetal urogenital sinus (UGS). *In utero* and lactational TCDD exposure (1.0 ug/kg dam, GD15) did not alter ARNT protein levels, but reduced prostatic AHR protein levels on PND 7 and delayed the developmental decrease in AHR protein in VP and DLP. Acute exposure of rat pups to TCDD (5 ug/kg, 24 hr), down regulated prostatic AHR protein on PND 7, but not PND 1. Thus, AHR down regulation and TCDD-mediated toxicity in male rat reproductive tract do not correlate, but the expression of AHR protein appears to correlate with normal prostate differentiation. (Supported by ES08980 and ES01332.)

689 PROFILES OF EFFECTS OF ANTIANDROGENIC PESTICIDES AND TOXIC SUBSTANCES ON SEX DIFFERENTIATION.

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Antiandrogenic chemicals alter sex differentiation by a variety of mechanisms and, as a consequence, they induce different profiles of effects. Here, we describe the effects of 10 known or suspected antiandrogens from high dose studies which used over 1500 male pups from 250 litters with more than 40 different treatments conditions. Chemicals included vinclozolin (V, 3-200 mg/kg/d), procymidone (P, 25-200), iprodione (I, 100), chlozolinate (C, 100), linuron (L, 100), p,p' DDE (100), ketaconazole (K, 12-100), dibutyl (DBP, 500) and diethylhexyl (DEHP, 750) phthalate, and the Leydig cell toxicant ethane dimethane sulfonate (EDS, 50). Anogenital distance (AGD), nipple/aerola development and reproductive organ weights were measured. Six of the 10 chemicals altered male sexual differentiation (V, P, DBP, DEHP, L, and DDE). C and I will be retested at a higher dosage level. To date, dose response (DR) studies have been conducted with V, P, K, L (no AGD) and DBP (no AGD). The AR-ligands, V, P, and DDE all produced a "flutamide"-like profile. DBP and DEHP, which do not appear to be AR ligands, produced a different profile which included a surprising incidence of epididymal and testicular lesions. L, an AR ligand, induced a low incidence of hypospadias along with an unanticipated degree of malformed epididymides, suggesting that L may display additional mechanisms of endocrine toxicity. A model based in these AGD and body weight data will be presented that separates specific from nonspecific effects on AGD. We also found that an increase in aerolas/nipples at d 13 was always accompanied by permanent alterations or malformations. Examination of DR curves revealed that 1) threshold and 2) nonthreshold responses were common, but only one endpoint displayed a U-shaped DR. In summary, AGD and aerola/nipple incidence are sensitive to antiandrogens regardless of the mechanism of action, but we currently are unable to predict epididymal or testicular malformations from these early effects. (Disclaimer: These abstracts do not reflect USEPA policy.)

690 DOSE-RESPONSE FOR ALTERED MALE REPRODUCTIVE DEVELOPMENT AND FUNCTION INDUCED BY DI(*n*-BUTYL) PHTHALATE.

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Di(*n*-butyl) phthalate (DBP) is a commercially important plasticizer and ubiquitous environmental contaminant. Previous limited dose-response studies with DBP that reported alterations in male reproductive development and function failed to establish a NOAEL. An extensive dose-response study was conducted in which pregnant CD rats were given DBP by gavage at 0, 0.5, 5, 50, 100 mg/kg/day (n = 19—22) or 500 mg/kg/day (n = 11) from gestation day 12 to 21. In male offspring, anogenital distance was decreased at 500 mg DBP/kg/day. Retained areolas or nipples were present in 31 and 90% of male pups at 100 and 500 mg/kg/day, respectively. Preputial separation was not delayed by DBP treatment in males with normal external genitalia, but cleft penis (hypospadias) was observed in 5/58 rats (4/11 litters) at 500 mg/kg/day. Agenesis of the epididymis (21/58 rats in 9/11 litters), vas deferens (15/58 animals in 9/11 litters), and ventral prostate (1/58 animals) occurred at 500 mg/kg/day. In mature F1 males, the weights of the testis, epididymis, dorsolateral and ventral prostates, seminal vesicles, and levator ani/bulbocavernosus muscle were decreased at 500 mg/kg/day. Widespread seminiferous tubule degeneration was seen in 2/67 rats (in 2/10 litters) and 13/23 rats (in 3/5 litters) at 100 and 500 mg/kg/day, respectively. Focal interstitial cell hyperplasia (8/23 rats in 3/5 litters) and adenoma (1/23 rats in 1/5 litters) was observed at 500 mg/kg/day. For this 10-day fetal exposure to DBP, the NOAEL and LOAEL were 50 and 100 mg/kg/day, respectively. This is currently the lowest NOAEL for the toxicity of DBP.

691 DEVELOPMENTAL AND REPRODUCTIVE EFFECTS OF LOW-DOSE, STEADY-STATE MATERNAL 2,3,7,8 TETRACHLORODIBENZO-P-DIOXIN (TCDD) ADMINISTRATION.

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When a single dose of TCDD is administered during pregnancy, developmental alterations are seen if the fetal concentration is at 13 ppt or higher. In the current study, 0 or 10 ng TCDD/kg/d was administered to 90 d old (16/group) LE Hooded female rats for 90 d prior to mating, and during mating and gestation. This dosing regime has been reported to result in about 10 ppt TCDD in the fetus. Estrous cycles were monitored for 28 d prior to mating from 150-180 days of age. TCDD-treatment attenuated the normal age-related disruptions of estrous cyclicity and fertility (fertility = 50 % for control versus 87.5 % in the TCDD group, $p < 0.03$). The number of pups, pup weight and sex ratio were not affected by TCDD-treatment. However, infantile viability was reduced ($p < 0.0005$) from 71 % in controls to 32 % in the TCDD group. We did not anticipate an effect of this magnitude or this level of mortality in controls. As hypothesized for the F1, the age of eye opening was accelerated from 34% in controls to 70 % in the TCDD group at 15 d (p 0.005), body weight at weaning and the ages at puberty in males and females were unaffected. In addition, 23.2 % of F1 TCDD females displayed a persistent vaginal thread versus 0 % in the control group ($p < 0.04$ one tailed-t-test). However, in TCDD F1 males (only ten survived), anticipated reductions in ejaculated and epididymal numbers, and cauda epididymal weight, but these measures were not affected by TCDD. In summary, while some TCDD-like effects were seen in exposed offspring (accelerated eye opening and vaginal threads), reproductive effects were not seen in the treated male offspring. The increased incidences of normal estrous cyclicity and fertility in TCDD-treated females may result from the antiestrogencity of TCDD as endogenous estrogen in continuously cycling rats has adverse effects on hypothalamic-pituitary neuroendocrine function. (These abstracts do not reflect USEPA policy.)

692 REPRODUCTION TOXICITY OF DI-2-ETHYLHEXYL PHTHALATE (DEHP).

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DEHP was investigated in a reproduction toxicity range-finding study using Wistar rats. The study was sponsored by an industry group and was con-

ducted under GLP. Dietary dose levels of 0; 1,000; 3,000 and 9,000 ppm (about 100; 300 and 900 mg/kg body weight/day) were administered to 10 males and 10 females (F0 generation) and F1 pups. Selected F1 pups were raised and mated (F1 generation) to generate F2 pups, which were sacrificed at day two after birth. DEHP had no effect on fertility up to 9,000 ppm (F0 and F1 generation). Increased liver weights were seen in F0 parental animals at 3,000 and 9,000 ppm and in F1 male parental animals at 9,000 ppm. At 9,000 ppm reduced food consumption, impaired body weight gain (both generations) and reduced weights of testes and epididymies (F1 generation only) were also noted. There was a reduced number of pups (F1 and F2 pups) and increased pup mortality (F1 pups) at 9,000 ppm. At the age of 15-18 days post partum nipple anlagen were noted in 29 out of 35 high dosed male F1 pups. This phenomenon was transient since only one out of six surviving F1 males showed nipple persistence at necropsy. Detailed external examination of F1 rats after weaning revealed no structural abnormalities, including the external genital organs. However, the examined male F2 pups had a reduced anogenital distance and anogenital index at 9,000 ppm, while females were not affected. Thus, although the high dose of DEHP induced some signs of postnatal developmental toxicity in male progeny, no effect on fertility and no abnormalities of external genital organs were found.

693 CHANGES IN THE REPRODUCTIVE ORGANS OF THE MALE RATS EXPOSED MATERNALLY TO 2,3,7,8-TETRA-CHLORODIBENZO-P-DIOXIN (TCDD).

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Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) on endocrine functions during developmental stage has gained much attention in terms of health risk assessment of this highly toxic compound. Some researchers reported that the transplacental exposure to TCDD during developmental stage of fetuses caused reproductive disorders, including decrease of sperm numbers, reduced size of genital organs, and also feminized behavior. We here examined relatively low dose TCDD effects on male reproductive system. Pregnant Holtzman rats (JCR) were given a single oral dose of 0, 12.5, 50, 200 or 800 ng TCDD/kg body weight on gestation Day 15, and male offspring were sacrificed on Day 2, 49 or 63 after birth. There was no TCDD effects in litter size, birth weight and sex ratios of pups for all groups. Anogenital distance of Day 2, 49 and 63 male rats showed a significant decrease in the highest dose of TCDD. Prostate was slightly reduced in size. However, there were no changes on testis and epididymal weights by TCDD administration even at the 800 ng/kg dose on Day 49 and 63. In addition, daily sperm production and sperm reserve on Day 63 were not changed by TCDD. GC/MS analysis with the abdominal fat tissue clearly showed the increased amounts of TCDD were still retained in these offspring. These results suggested that relatively low dose of TCDD could affect the external genital organs, but the internal genital organs including testis seems to be less sensitive.

694 INHIBITION OF EMBRYONIC CATALASE BY ARSENICALS AS A POTENTIAL MECHANISM OF DYSMORPHOGENESIS.

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The adverse developmental effects of arsenicals are well established. Exogenous antioxidants (e.g. glutathione peroxidase) ameliorate the dysmorphogenic effects of arsenite and to a lesser degree arsenate in whole embryo culture. To determine the origin of reactive oxygen species (ROS) we measured embryonic catalase activity at varying times after exposure to arsenicals in CD–1 mouse conceptuses in culture. M arsenite (that induces 100% malformations) produced a progressive decrease in embryonic catalase activity. After 6H of exposure there is a trend towards a reduction in activity (13% reduction) and a 33% reduction after 12H of exposure. By 24H catalase activity was reduced 75% by 5μ M arsenite. Catalase activity was reduced 45% by 4 μ M and 21% by 3 μ M arsenite after 24H. 20μ M arsenate produced a 25% reduction and 10 μ arsenate produced no effect on catalase activity after 24H. Therefore, one mechanism for ROS accumulation produced by arsenicals appears to be an inhibition in endogenous antioxidant defenses. In order to determine if inhibition of catalase would produce malformations, 3–6 somite stage conceptuses were exposed to 3–amino–1,2,4–triazole (3–AT), an irreversible catalase inhibitor. 3–AT produced dysmorphogenesis in embryos after a 26H culture period. The heart and pharyngeal arches were most sensitive to 3–AT and neural tube closure defects are not a major effect of 3–AT exposure. Therefore, inhibition of catalase does not reproduce the full spectrum of arsenical–induced dysmorphogenesis, but does induce the facial and heart effects. These studies suggest that the dysmorphogenic effects of arsenicals are not produced by a single biochemical effect. (This abstract does not necessarily represent US EPA Policy and was partly funded by EPA/UNC Toxicology Research Agreement CT902908 T901915.)

695 GENETIC CONTROL OF THE DIFFERENTIAL SENSITIVITIES OF THE SWV AND C57BL/6 MOUSE STRAINS TO CADMIUM-INDUCED TERATOGENICITY.

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As with other toxicological endpoints, sensitivity to teratogen-induced birth defects varies greatly among inbred strains of mice, demonstrating the importance of genetic background in determining susceptibility. However, the identity of the genetic factors underlying these strain differences is unknown. In a comprehensive strain comparison, C57BL/6N mice were found to be very sensitive to the induction of forelimb ectrodactyly by treatment with 4 mg/kg cadmium chloride on day 9.0 of gestation, with 73% of fetuses displaying the defect, while SWV mice were entirely resistant. A whole-genome linkage analysis has been initiated to identify the genetic factors controlling this strain-difference. This procedure involves the genotyping of backcross animals at densely spaced genomic markers to compare segregation of the limb defect with the segregation of genomic regions. 54.0 % of the 1009 microsatellite markers that have been tested were found to be polymorphic between SWV and C57BL/6, and of these, 177 were selected to assemble a genome-spanning array at an average spacing of 8.2 centimorgans. A panel of 206 fetuses has been generated from a backcross utilizing male F1 hybrids and female C57BL/6N mice treated on day 9.0 of gestation with 4 mg/kg cadmium chloride. The fetuses have been phenotyped for the presence of forelimb ectrodactyly and are currently being genotyped at each of the markers in the genome spanning array. To date, 116 markers have been genotyped in the complete panel of backcross fetuses. While preliminary analysis has not revealed any loci with significant LOD scores, several loci with scores suggesting linkage have been identified, possibly indicating that sensitivity to cadmium-induced forelimb ectrodactyly is polygenic in nature.

696 EARLY EMBRYONIC LOSS INDUCED BY DIPHENYLTIN DICHLORIDE (DPTCl) IN RATS.

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The present study was conducted to evaluate the adverse effects of DPTCl on initiation and maintenance of pregnancy after administration during early pregnancy in rats. Following successful mating, female rats were given DPTCl by gastric intubation at 0, 4.3, 8.6, 17.2, 25.8 or 34.3 mg/kg on days 0-3 of pregnancy or days 4-7 of pregnancy (sperm day = day 0 of pregnancy). Female rats were sacrificed on day 20 of pregnancy and pregnancy outcome was determined. In females successfully mated, DPTCl caused complete failure in implantation, which was evidenced by total absence of any implantation site, and the pregnancy rate was significantly decreased after administration of DPTCl on days 0-3 at 25.8 mg/kg and on days 4-7 at 34.4 mg/kg. The incidence of preimplantation embryonic loss per litter was significantly increased after administration of DPTCl on days 0-3 at 17.2 (45.4%) and 25.8 mg/kg (62.1%) and on days 4-7 at 25.8 (28.4%) and 34.3 mg/kg (67.4%). In females having implantations, the numbers of implantations and live fetuses per litter and the incidence of pre- and postimplantation embryonic loss per litter in the groups given DPTCl on days 0-3 were comparable to those in the control group. The incidence of postimplantation embryonic loss per litter was significantly increased after administration of DPTCl on days 4-7 at 34.4 mg/kg. It can be concluded that DPTCl during early pregnancy caused embryonic loss and that DPTCl has greater effects on reproduction when administered earlier than later stages of blastogenesis.

697 ACQUISITION OF A MULTIPLE DRH EXTINCTION SCHEDULE OF REINFORCEMENT IN RATS EXPOSED DURING DEVELOPMENT TO METHYLMERCURY.

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Female rats were exposed to 0, 0.5 or 6.4 ppm Hg as methyl mercury in drinking water beginning at least 4 weeks before mating and ending on postnatal day 16. Female offspring (12 control, 10 low, and 12 high) represented six litters in each group. Concentrations of mercury in the brain for the low and higher-dosed groups were 0.5, and 9.5 ppm respectively at birth and 0.04 and 0.53 ppm at weaning. At 4 mos., female offspring were trained to lever press under Multiple Differential-Reinforcement of-High-Rate Extinction (MULT DRH-N:T EXT) schedules of reinforcement. Under the extinction schedule, lever-pressing had no scheduled consequences. Under the DRH-N:T schedule, a food pellet was delivered whenever N responses occur within T seconds. Two acquisition protocols were examined. First, the DRH parameter imposed in three successive sessions was 3:1, 5:2, and 9:4, values selected so that the same average inter-response time (i.e., rate) was required by the schedules. When the rats did not acquire the schedules in single sessions, as Bornhausen, Musch, & Greim (1980) reported, we implemented each of the three schedules individually until the rats acquired stable performance under each. Acquisition of the schedule was analyzed and marginal effects were apparent. This failed to replicate a similar experiment by Bornhausen et al. (1980) showing effects of mercury on acquisition of a Multiple DRH EXT schedule of reinforcement. (Supported by ES06466 from NIH.)

698 IN VIVO DETERMINATION OF CELL CYCLING RATES IN EMBRYONIC RAT NEURAL CELLS: DEVELOPMENT OF A BIOLOGICALLY-BASED TOXICODYNAMIC MODEL FOR METHYL MERCURY RISK ASSESSMENT.

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We are developing a linked toxicokinetic-toxicodynamic model of the effects of methyl mercury (MeHg) in the fetal rat. Key components of the toxicodynamic model are the cell cycling rates for fetal neuroepithelial cells. While these rates have previously been determined *in vitro*, *in vivo* data are preferred for use in quantitative modeling. We are using osmotic pumps to deliver 5-bromo-2'-deoxyuridine (BrdU) to the dam at a constant rate of 0.55 mg/hr. BrdU, a thymidine analog, is incorporated into fetal DNA during the S phase of the cell cycle. Fetal midbrain tissues are harvested and evaluated with a FITC-conjugated anti-BrdU Ab and flow cytometry. Preliminary results indicate that the fraction of cells incorporating BrdU (and therefore engaged in replication) is 60±15% (mean ± SE) between gestational days 12 and 13. Cell cycle information for untreated cells has been incorporated into our toxicodynamic model and the model demonstrates good correlation ($r = 0.9$) with midbrain cell number data we have collected between gestational days 12 and 16. Our future plans are to use this system to investigate the effect of MeHg on cell cycle rates. Comparison of cell cycle data obtained *in vivo* with that we have previously obtained *in vitro* will provide a valuable indicator of the utility of *in vitro* data for quantitative risk assessment.

699 DEVELOPMENTAL METHYLMERCURY ADMINISTRATION DECREASES SYNAPTOSOMAL SIALYLTRANSFERASE ACTIVITY AND ALTERS SYNAPTIC EXPRESSION OF SIALOGLYCOCONJUGATES.

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Defective neural structuring and neurological disturbances are recognized consequences of developmental methylmercury (MeHg) exposure, but the underlying molecular mechanisms are poorly understood. MeHg-induced perturbation of neural structure and function may involve alterations in the expression and/or regulation of molecules integral to synaptic structure, such as sialoglycosylated carbohydrates. Sialic acid residues attached to these molecules influence many biological functions, including synaptic adhesion, receptor function, and neurotransmission. To investigate the effects of MeHg on synaptic sialoglycoconjugates, rat pups were dosed with 7.0 mg/kg MeHgCl (s.c.) on alternate days from postnatal days 3-13 and killed on days 15, 30, or 60. Sialyltransferase activity was assayed in purified synaptosomes from rat hippocampus and cerebellum. Expression of sialoglycoproteins was determined by western blotting of synaptosomes and probing with the lectins *Maackia amurensis II*, *Sambucus nigra*, and Wheat Germ Agglutinin. Experiments revealed a significant decrease in synaptosomal sialyltransferase activity in synaptosomes of MeHg-treated rats at PND15 and PND30. In PND15 hippocampus, a 15% reduction was observed while a 30% reduction in sialyltransferase activity was seen on PND30. Lectin immunoblotting indicated decreased expression of various sialoglycoprotein species in the cerebellum and hippocampus of MeHg-treated animals. Altered expression of synaptic glycoconjugates in development may contribute to the behavioral and morphological disturbances observed following MeHg poisoning. (Supported by ES-04976, ES-05022 and a US EPA Graduate Fellowship [PMD].)

700 ACTIVATION OF HUMAN PLACENTAL PHOSPHOLIPASE A_2 BY NICOTINE AND COTININE: A RISK FACTOR FOR SMOKING WOMEN DURING PREGNANCY.

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Tobacco smoking by pregnant women increases frequency of spontaneous abortions and preterm births. Human labor is associated with enhanced intrauterine phospholipid metabolism and production of prostaglandin E_2 (PGE_2), which induces labor and initiates uterine contractions and maintains homeostasis of placental blood flow. Therefore, we studied (a) activation of placental phospholipase A_2 (PLA_2) by nicotine and cotinine using 1-palmitoyl-2-[1-^{14}C]arachidonylphosphatidylethanolamine (PE, 2.2 nmol) as substrate, precursor for formation of PGE_2, and (b) the influence of nicotine and cotinine on the effects of PGE_2 on placental vasculature in perfused human placental cotyledon. These studies gave the following results: (1) Both cotinine (EC_{50} 470-400 fM) and nicotine (EC_{50} 18-32 pM) activated PLA_2 in human placental tissues in low concentrations. (2) Increasing concentrations of PGE_2 (10-150 ng/ml) increased umbilical perfusion pressure by 170 ± 10% (N = 6) of control (100%). Cotinine (2 m g/ml) enhanced this effect at all concentrations of PGE_2. Nicotine (2 m g/ml) prevented the effect of PGE_2. These observations indicated that cotinine was more potent to activate PLA_2 and formation of PGE_2, and potentiate the vasoconstrictive effects of PGE_2 on the fetal placental circulation. Nicotine releases placental acetylcholine, a vasodilator of placental arteries. Acetylcholine stimulates muscarinic receptors of endothelial cells resulting in the release of endothelium-derived relaxing factor (EDRF), and possibly nitric oxide. Therefore, nicotine prevents the vasoconstrictive effects of PGE_2 through the release of EDRF. Cotinine is inactive at nicotinic and muscarinic receptors. Therefore, accumulation of cotinine, the major metabolite of nicotine, in fetal circulation may contribute to production of PGE_2 and induction of preterm labor and spontaneous abortions. (Supported by the Smokeless Tobacco Research Council, Inc.)

701 ACTIVITY OF CHOLINESTERASE ENZYMES FOLLOWING A SINGLE DERMAL DOSE OF CHLORPYRIFOS ALONE, OR IN COMBINATION WITH METHYL PARATHION IN SPRAGUE-DAWLEY RATS.

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A single subclinical dermal dose of 30 mg/kg (15% of LD_{50}) of chlorpyrifos (*O,O*-diethyl*O*-3,5,6-trichloro-2-pyridyl phosphorothioate) caused maximum inhibition of maternal and fetal brain acetylcholinesterase (AChE) after 24 hr of dosing, corresponding to activity of 48% and 67% of control, respectively. When combined with a single dermal dose of 10 mg/kg (15% of LD_{50}) of methyl parathion (*O,O*-dimethyl*O*-4-nitrophenyl phosphorothioate), peak inhibition of maternal and fetal brain AChE occurred at 24 hr, corresponding to 35% and 73% of control, respectively. Recovery of maternal brain AChE activity was slower following combined exposure compared to chlorpyrifos alone, corresponding to activity of 60% and 76% of control, respectively. Similarly, while fetal brain AChE activity returned to normal96 hrs after chlorpyrifos treatment, it was 69% of control following combined exposure. Activity of maternal plasma butyrylcholinesterase (BChE) was inhibited to 41% and 40% 24 hr after application of chlorpyrifos, or mixture of chlorpyrifos and methyl parathion, respectively. No significant inhibition has been detected in fetal plasma BChE following application of chlorpyrifos alone, or in mixture with methyl parathion. Peak inhibition of maternal liver BChE took place 24 hr after application of chlorpyrifos alone, or in combination with methyl parathion, corresponding to activity of 80% and 61% of control, respectively. Activity of BChE recovered to normal at 96 hr,

following application of chlorpyrifos, or combination of chlorpyrifos and methyl parathion. Mixture of chlorpyrifos and methyl parathion caused more inhibition in placental AChE and BChE enzymes compared to chlorpyrifos alone. Maximum inhibition of placental AChE occurred 12 hr, and 24 hr after application of chlorpyrifos, or mixture of chlorpyrifos and methyl parathion, corresponding to activity of 75% and 56% of control, respectively. No significant inhibition of placental BChE was detected following application of chlorpyrifos, or mixture of chlorpyrifos and methyl parathion. Combined dermal exposure of chlorpyrifos and methyl parathion resulted in persistent inhibition and slower recovery in maternal and fetal ChE enzyme activity.

702 INHIBITION AND RECOVERY OF MATERNAL AND FETAL CHOLINESTERASE ENZYMES FOLLOWING A SINGLE DERMAL DOSE OF DIAZINON ALONE, OR IN COMBINATION WITH METHYL PARATHION IN SPRAGUE-DAWLEY RATS.

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Pregnant Sprague-Dawley rats (14-18 days of gestation) were treated with a single subclinical dermal dose of 65 mg/kg (15% of LD_{50}) of diazinon (*O,O*-diethyl*O*-2-isopropyl-6-methylpyrimidinyl phosphorothioate), or combination of diazinon with a single subclinical dose of 10 mg/kg (15% of LD_{50}) of methyl parathion (*O,O*-dimethyl*O*-4-nitrophenyl phosphorothiate). Following an application of diazinon, maximum inhibition of maternal and fetal brain acetylcholinesterase (AChE) occurred after 24 hr of dosing, corresponding to activity of 50% and 80% of control, respectively. When diazinon was combined with methyl parathion, peak inhibition of maternal and fetal brain AChE took place at 24 hr, corresponding to activity of 46% and 37% of control, respectively. Recovery of maternal and fetal brain AChE activity following application of diazinon alone was to 80% and 90% of control in the mother and in the fetus, respectively. Recovery of maternal and fetal brain AChE activity was slower following combined exposure compared to diazinon alone, corresponding to activity of 75% and 72% of control in the mother and in the fetus, respectively. Peak inhibition of maternal and fetal plasma butyrylcholinesterase (BChE) following diazinon application occurred at 24 hr, corresponding to activity of 75% of control. Exposure to combination of diazinon and methyl parathion caused maximum inhibition of maternal and fetal plasma BChE after 12 hr and 72 hr of treatment, corresponding to activity of 12% and 70%, respectively. Fetal plasma BChE activity recovered to 100% of control following application of diazinon, and combination of diazinon and methyl parathion. Activity of maternal plasma BChE remained inhibited after 96 hr, corresponding to 32% of control. Diazinon alone, or combination of diazinon and methyl parathion did not cause significant inhibition of maternal liver BChE or placental AChE and BChE enzymes. The faster recovery of fetal ChE enzymes is attributed to the rapid *de novo* synthesis of these enzymes in the fetus compared to the mother. Combination of diazinon and methyl parathion resulted persistent inhibition and slower recovery of maternal and fetal acetlcholinesterase enzymes activity.

703 AN EVALUATION OF THE EFFECTS OF MGI 114 ON ORGANOGENESIS IN RATS AND RABBITS.

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MGI 114 (6-hydroxymethylacylfulvene) is a semi-synthetic derivative of the mushroom toxin Illudin S with potent antitumor activity and is currently in Phase I-II human clinical trials. The drug was administered by daily intravenous injections to pregnant rats and rabbits to evaluate its teratologic potential. Based on the results of dose range-finding studies, doses of 0, 0.04, 0.1 and 0.4 mg/kg/d (gestation days 6-17) were used in rats and 0, 0.05, 0.15, and 0.3 mg/kg/d (gestation days 7-20) in rabbits. Body weights, food consumption and clinical observations were recorded. Laparohysterectomy's were performed (d20 in rats and d29 in rabbits). Uteri were weighed, implantation sites evaluated, the number of corpora lutea counted, and fetus viability examined. Each fetus was examined morphologically (external, visceral, and skeletal). In rats, maternal toxicity consisting of reduced mean body weights, food consumption, and gravid uterine weights, was observed at 0.4 mg/kg/d. Developmental toxicity (reduced fetal body weights, increased postimplantation losses, microphthalmia, and skeletal variations) was also observed at 0.4 mg/kg/d. In rabbits, maternal toxicity consisting of two abortions at 0.3 mg/kg/d and reduced mean body weights and food consumption was observed at doses ≥ 0.15 mg/kg/d. Developmental toxicity in rabbits, at doses ≥ 0.05 mg/kg/d, included malformations (lobular agenesis of the lung, paragonadal vascular proliferation, costal cartilage anomalies, fused sternabrae, and 14th full ribs) and skeletal variations. The NOAEL for developmental toxicity was considered to be 0.1 mg/kg/d in the rat study and <0.05 mg/kg/d in the rabbit study.

704 EFFECTS OF DIETHYLENE GLYCOL MONOMETHYL ETHER AND ETHYLENE GLYCOL MONOMETHYL ETHER ON *IN VITRO* CHONDROGENESIS.

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Chick embryo limb bud mesenchymal cells, when grown in tissue culture under conditions conducive for chondrogenesis, differentiate into chondrocytes. Ethylene glycol monomethyl ether (EG) and diethylene glycol monomethyl ether (DEG) were added directly to cell cultures in log concentrations (EG=0.00097 - 97mg/ml; DEG=0.00101 - 101mg/ml) to determine their effects on chondrogenesis and cell proliferation. After five days, cultures were fixed and chondrogenesis analyzed by staining with alcian green for proteoglycans and measuring absorbance at 600 nm wavelength. Subsequently, cell proliferation was analyzed by staining with crystal violet and measuring absorbance at 570 nm wavelength. Significant difference from control cultures was determined using a Dunnett's T-test ($p<0.05$). At the highest concentration tested, proteoglycan content was significantly different from controls for both chemicals (1.4% of control for DEG and 1.6% of control for EG), but the decrease did not appear to be concentration-dependent. For cell proliferation, all treatments were significantly different from control and showed a concentration-dependent pattern. Cell proliferation ranged (from the lowest to the highest concentration) from 73.7% to 1.8% of control for DEG and from 83.6% to 2.4% of control for EG. In conclusion, as chemical concentration increased for DEG and EG, a concentration dependent decrease in cell proliferation occurred. For proteoglycans, the decrease is likely a result of general cell toxicity because it does not appear to be concentration dependent. (Supported in part by US Air Force Office of Scientific Research and Hatch Funds to MAS.)

705 CHARACTERIZATION OF OCHRATOXIN A (OTA) UPTAKE AND DEVELOPMENTAL TOXICITY USING THE FETAX SYSTEM.

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The mycotoxin ochratoxin A has been observed to be teratogenic in rat, mouse, hamster and chick models with craniofacial abnormalities being the most commonly noted malformations. Possibly due to its almost ubiquitous nature in foodstuffs, all human blood samples tested to date have proved positive for the toxin. Its half-life in humans has been demonstrated to be well in excess of that in rodents and OTA has been associated with the occurrence of Balkan Endemic Nephropathy (BEN) as well as the formation of urothelial tract tumors in humans. However, the role of OTA in the current incidence of human teratogenic events remains to be elucidated. The aim of this study was to characterize the teratogenic effects of OTA in the FETAX assay, a system, which has previously been shown to be sensitive to human teratogens. Embryos incubated with 2?Ci tritiated OTA showed a time-dependent increase in uptake of radioactivity over 24, 48 and 72 hours, with a two-fold increase in uptake subsequent to the opening of the oral cavity ie feeding reflex (96h). Following standard range-finding experiments, stage 8-11 *X. laevis* embryos were selected and exposed to nM concentrations of the toxin for 96h essentially in accordance with ASTM standard protocol with medium and toxin renewal at 48h only. Embryos were fixed and mortality, malformation and growth inhibition, were assessed. An LC_{50} of 200nM was noted and OTA-exposed embryos displayed a specific and reproducible craniofacial narrowing (EC_{50} 60nM) which was inconsistent with generalized growth retardation known for other teratogens or embryotoxicants. No increased incidence of other common malformations, such as incomplete gut coiling or axial defects were observed in the presence of the toxin. At higher concentrations (200-400nM), a decrease in average embryo length by up to 20% was noted. The type of malformations and growth retardation observed suggests a specifically localised incipient cytotoxicity or inhibited cell proliferation mediated by OTA.

706 RETINOIC ACID-INDUCED HINDLIMB MALFORMATIONS IN WOOD FROGS (*RANA SYLVATICA*).

S J Degitz, P A Kosian, E A Makynen and G T Ankley. *U.S. EPA, MED, Duluth, MN.*

Recently, high frequencies of hindlimb malformations have been reported in amphibians across North America. The phenotype of the malformations is similar to those produced by retinoids in mammalian, avian and amphibian species. The objective of this study was to determine if retinoids induced hindlimb malformations in the wood frog (*Rana sylvatica*). Stage 28 tadpoles were exposed to 0, 250, 500, 750, 1000 and 1250 ng/ml of all-trans retinoic acid (RA) for 24 h. Water and tissue samples were collected at 0, 4, 12 and 24 h and analyzed via HPLC to characterize aqueous degradation and tissue residues in exposed tadpoles. Tadpoles were held until forelimbs emerged, and prepared for skeletal analysis. RA concentrations in water dropped rapidly to 14% of target concentrations within 4 h, and were near detection limits within 12 h. Tissue RA concentrations were greatest at 4 h (10-50 μg/g) and declined rapidly by 24 h (0.1-7 μg/g). A number of unidentified metabolites were observed and are being further investigated. We found a 48% incidence of hindlimb defects at the lowest concentration, and this increased to 68% at the 1250 ng/ml exposure. The malformations ranged from minor effects on the digits to severe disruption of the femur, tibia/fibula, tibiale/fibulare and digits. This is in contrast to experiments conducted with the northern leopard frog (*R. pipiens)* in which the same concentration did not induce hindlimb malformations. Differences in the developmental rate between these two species may explain their seemingly differential sensitivity to pulse doses of RA.

707 LIFE-STAGE SPECIFIC EFFECTS OF ALL-TRANS RETINOIC ACID ON GREEN FROG (*RANA CLAMITANS)* EMBRYOS AND TADPOLES.

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It has been suggested that the large number of malformed frogs recently observed in North America may be occurring as a result of disruptions in developmental pathways regulated by retinoic acid. Therefore, a series of experiments were undertaken to examine life-stage specific effects of all-trans retinoic acid (RA) on the North American anuran, green frog (*Rana clamitans)* embryos and tadpoles. Stage 8 embryos were exposed to 0, 3, 6.25, 12.5, 25 and 50 ng/ml RA for 24 h. Embryos were then moved to clean water and at stage 22, tadpoles from all treatments were sub-sampled and scored for dysmorphogenesis. In addition, stage 28 tadpoles were exposed to RA concentrations of 0, 250, 500, 750, 1000 and 1250 ng/ml. These tadpoles were held until forelimbs emerged and prepared for skeletal analysis. In the embryo exposures, RA concentrations of 12.5 ng/ml and above induced lethality, with all animals dying at the 50 ng/ml treatment. Surviving animals from the 12.5 ng/ml and higher treatments exhibited an increase in dysmorphogenesis of the tail, notochord, eye, forebrain and cement gland. Moreover, dose-related delays and growth reductions were observed in treated embryos when compared to the controls. These results were in contrast to the stage 28 tadpole exposure where RA concentrations of 1250 ng/ml did not produce lethality. Overall, our experiments show that RA is teratogenic to *R. clamitans* embryos, with animals approximately 100 times more sensitive to RA-induced lethality than stage 28 tadpoles.

708 DYSMORPHOGENESIS AND LETHALITY OF MINK FROGS (RANA SEPTENTRIONALIS) EXPOSED TO ALL-TRANS RETINOIC ACID AT DIFFERENT LIFE STAGES.

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An extremely large number of malformed frogs have been found in Minnesota, as well as in other states, and Canadian Provinces. The documented malformations have striking similarities to those produced by retinoids in developing mammalian, avian and amphibians species. The spectrum of malformation is quite broad indicating insult over a broad developmental period. The objective of this study was to examine the stage specific sensitivity of mink frogs, (*Rana septentrionalis*), to retinoid-induced dysmorphogenesis and lethality. Embryos were exposed to all-trans retinoic acid (RA) at stage 8, 26, or 28. Stage 8 embryos were exposed to 0, 6.25, 12.5, 25 and 50 ng/ml RA for 24 h. Stage 26 and 28 tadpoles were exposed to 0, 250, 500, 750, 1000 and 1250 ng/ml RA for 24 h, held until forelimbs emerged and preserved for skeletal examination. Concentrations of 6.25 ng/ml and above produced increased lethality, and dose-related increase in dysmorphogenesis of the eye and forebrain in the stage 8 exposed embryos. In addition to these effects, concentrations of 12.5, 25 and 50 ng/ml produced an increase in tail, notochord, and cement gland dysmorphogenesis as well as developmental delay. This was in sharp contrast to later stage exposures in which RA concentrations as high as 1250 ng/ml did not produce lethality. Not only do these experiments show that RA is teratogenic, but that the embryo is at least 200 times more sensitive to RA-induced lethality than stage 26 and 28 tadpoles.

709 EFFECTS OF CONTINUOUS OR INTERMITTENT INHALATION AND ORAL EXPOSURE OF 4-BROMO-2-NITROPHENOL ON THE WISTAR RAT'S DEVELOPMENT.

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4-Bromo-2-Nitrophenol (BNP) is a structure analog (isomer) of the 3-Bromo-4-Nitrophenol which is used as lampricide. Wistar rats were exposed to BNP by whole body inhalation at concentrations of 0, 0.01, 0.14 or 1.0 mg/m^3 for 24 hr/day (24-hr group); and for 4 hr/day at concentrations of 0.14, 1.0, 2.8 mg/m^3 (4-hr group); or by gavage at doses 0, 0.3, 1.0, 6 mg/kg/day (oral group). All animals were treated 7 days/week throughout gestation. General toxic effects on the dam's liver and kidneys were found only at the maximum concentrations/doses in all three experimental groups. Significant depression effects on the maternal CNS was registered in the oral and 24-hr groups but not in the 4-hr group. This depression of the CNS was also registered at the highest of the tested concentrations/doses. BNP treatment by all types of exposure increased embryolethality, retarded weight, length and skeletal ossification of the fetuses, and affected postnatal development of the offspring. Significant suppression in the open field was shown for horizontal movement and orientation reactions of the offspring in the 24-hr group; horizontal movement, orientation reactions and emotionality for the 4-hr group and for the orientation reaction and emotionality in the oral group. These changes were dose/concentration-dependent and statistically significant at the higher levels of exposure. Therefore, depression influence of BNP on the CNS of mothers and their offspring was shown. Analyzing the LOAELS (Lowest Observed Adverse Effect Levels) for the reproductive effects of BNP suggests that developmental toxicity is enhanced approximately 10 fold by both inhalation routes in comparison to an oral route.

710 FISH EMBRYOTOXICITY AND TERATOGENICITY OF PHARMACEUTICALS, DETERGENTS AND PESTICIDES REGULARLY DETECTED IN SEWAGE TREATMENT PLANT EFFLUENTS AND SURFACE WATERS.

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Recent chemical analysis of sewage treatment plant effluents (STPE) and surface waters in southern Germany revealed surprisingly high concentrations of pharmaceuticals, especially NSAID's, pesticides and detergents. In view of this laboratory experiments were conducted using the previously established zebrafish embryo assay DRETA (*Danio Rerio* Embryotoxicity and Teratogenicity Assay, Dietrich et al., Toxicol. Sci., 42(1-S), 1279, 1998) to determine the potential detrimental effects of a limited but representative selection of environmentally present compounds to zebrafish embryos. The compounds tested were: Diclofenac, Phenylbutazone, Acetyl salicylic acid, Ibuprofen, Indomethacin, Naproxen, *bis*-Phenol A, Nonylphenol, and Carbendazim. Caffeine, Ethanol and Valproic acid served for further validation of the DRETA and as positive controls. All compounds tested demonstrated embryotoxicity and induced malformations in the zebrafish embryos within the 96 hours of exposure. However, with the exception of Diclofenac, the concentrations necessary to induce lethality or malformation were several orders of magnitude higher than those observed in the STPE or the surface waters. The LC50 and EC50 of Diclofenac was 480±50μg/l and 90±20 μg/l, respectively. Diclofenac concentrations detected in STPE and surface waters range between 0.05-1.6 μg/l and 0.015-0.5 μg/l, respectively. In view of the 9-12 fold longer hatching period of embryos of feral fish species when compared to the zebrafish embryos, detrimental effects (malformation) in feral fish embryos resulting from exposure to environmental concentrations of Diclofenac appears a distinct possibility.

711 ONTOGENY OF HEAT SHOCK PROTEIN RESPONSE IN THE TELEOST, MEDAKA (ORYSIAS LATIPES).

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Different developmental stages of fish and other organisms vary in their susceptibility to toxic compounds and other physiological stressors. In part, this has been linked to the individual's ability to express certain proteins , i.e., stress proteins or cytochrome P450 monooxygenases, after exposure. It is important to understand whether there are developmental landmarks which must be reached before expression may occur. This study used the transparent chorion of a well-characterized fish model to time initiation of heat stress to coincide with particular landmarks of development. Responses, induction of hsp70, hsp60 and hsp90 and developmental defects, were and are being characterized in eight stages of the teleost, medaka, including embryo (11, 19, 23, 28 and 35), larvae (24-36 hours after hatch), sexually immature juveniles (1 month old) and sexually mature adults. Fish were heat-shocked at 40° C for 30 minutes, and subsamples were preserved after 1, 2 3, 6, 12 and 24 hours. Western blotting techniques were used to analyze stress proteins, and developing embryos were examined daily. Hsp70 was not inducible in stage 11 embryos, and these embryos subsequently showed developmental defects. In all other stages, hsp70 levels increased after heat-shock, and embryos developed normally. Although larvae, juveniles and adults were able to mount a stress protein response, they proved more susceptible to heat-shock than embryonal stages (with the sole exception being stage 11). Additional hsp70 isoforms were present in Medaka larvae, juveniles and adults. Constitutive levels of hsp70 were elevated just before (stage 35) and after hatching (larvae). Results suggest that the ability to express hsp70 confers a degree of protection on the individual. Further work will involve exposure to xenobiotics, where timing of exposure may affect toxicity results.

712 APOPTOSIS AS A POTENTIAL MECHANISM OF IMMUNE SUPPRESSION CAUSED BY EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD).

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The immune system is very sensitive to the toxic effects of TCDD. The mechanism by which TCDD suppresses immune function is currently unknown. Apoptosis plays a critical role in the normal immune response during lymphocyte maturation, activation, effector function and down regulation. We hypothesized that one potential mechanism by which TCDD suppresses immune function is by the induction or augmentation of apoptosis in responding immune cells. We have previously reported that in response to anti-CD3, TCDD increased the percentage of $CD4^+$ and $CD8^+$ T cells undergoing late apoptosis/necrosis but not early apoptosis. Apoptosis was measured by phosphatidylserine (PS) exposure on the cell surface, an early marker of apoptotic cells. From these results, we were interested in testing our hypothesis in a model previously shown to be suppressed by exposure to TCDD, the P815 tumor allograft model. Male C57Bl/6J mice were gavaged with vehicle or TCDD (15μg/kg) one day prior to intraperitoneal injection of P815 allogeneic tumor cells ($1x10^7$/ mouse). The spleens were harvested on days 1-7 after P815 injection and analyzed for the apoptotic phenotype by flow cytometry using FITC-Annexin V, 7-AAD, PE-CD4, and Red613-CD8. When compared to vehicle-treated mice, TCDD exposure significantly decreased the percentage of $CD4^+$ and $CD8^+$ T cells that expressed the early apoptotic phenotype. This effect was significant on days 5-7 in $CD4^+$ cells and in $CD8^+$ cells on all days tested. TCDD treatment had no consistent effect on T cell Fas expression or relative intensity of the stain on any of the days tested. These results suggest that apoptosis is not a mechanism by which TCDD causes suppression of the P815 response. However, this conclusion should be tempered by the fact that the data from this study represent a measure of cells with the apoptotic phenotype in the entire population of T cells and not exclusively the antigen-specific cells. (This work is supported by NIEHS grants ES07060 and ES00040.)

713 MECHANISTIC DIFFERENCES IN APOPTOSIS OF PULMONARY NEUTROPHILS FOLLOWING IN VIVO EXPOSURES TO CRYSTALLINE SILICA OR TITANIUM DIOXIDE PARTICLES.

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Apoptosis, or programmed cell death has been reported to occur in macrophages in vitro following exposures to fibrogenic dusts such as silica or asbestos. This study was undertaken to test the hypothesis that the inability to resolve the silica-induced inflammatory response via apoptosis is associated with its fibrogenic potential. Groups of rats were exposed by intratracheal instillation to varying doses of crystalline silica (Si) or pigment-grade titanium dioxide (TiO_2) particles and evaluated over several postexposure time periods. Following exposure, the BAL cells of control and exposed rats were assessed for apoptosis using four different techniques 1) cell death ELISA assay, 2) DNA ladder; 3) apoptotic morphology; and 4) TUNEL assay. Silica induced a significant dose-related increase in inflammation and apoptosis of pulmonary inflammatory cells. The inflammation was sustained but the apoptotic effects declined over time. TUNEL assay studies confirmed that the vast majority of apoptotic cells were neutrophils and not alveolar macrophages. In contrast, at high doses, TiO_2 produced lung inflammation but only a weak apoptotic response. The results from this in vivo study show that the PMN is the primary inflammatory cell-type which undergoes apoptosis. In addition, the magnitude of apoptosis induced by Si was significantly larger than that induced by TiO_2 at doses causing similar degrees of inflammation, indicating mechanistic differences between the cellular responses to the 2 dusts. Finally, it seems likely that the apoptotic clearance mechanisms to resolve pulmonary inflammation were overwhelmed in silica-exposed rats.

714 CHARACTERIZATION OF CI-994 INDUCED APOPTOSIS IN HUMAN LEUKEMIA (HL60) CELLS.

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CI-994 (acetyldinaline) is an investigative oral anticancer drug with an unknown mechanism of action. In previous in vitro toxicity studies, CI-994 was shown to induce apoptosis in rat blood lymphocytes and HL60 cells based on changes in cell morphology and DNA fragmentation. To identify the molecular pathways involved in this response, HL60 cells were incubated with CI-994 at concentrations of 0, 3, 10, or 30 μ M for up to 24 hours, and immunoblotting techniques were used to evaluate changes in various proteins involved in apoptosis. Apoptosis was confirmed by flow cytometry analysis using Hoechst 33342 and Annexin V stains. CI-994 did not effect protein levels of bax, bcl-2, cyclin D1, CDK-2, or p21/WAF1. However, p27KIP1 levels were increased following CI-994 treatment. The inactive form of caspase-3 was decreased at 30 μ M CI-994, and the caspase-3 substrate poly(ADP-ribose) polymerase (PARP) was cleaved into the signature 89kd fragment at 10 and 30 μ M CI-994, suggesting that caspase-3 was activated during CI-994-induced apoptosis. Interestingly, induction of PARP, which can recognize and bind to DNA strand-breaks, occurred at 3 μ M CI-994, indicating the presence of DNA damage. These results suggest that CI-994-induced apoptosis involves a caspase-3 mechanism which may be precipitated by DNA damage.

715 2,3,5-*TRIS*-(GLUTATHION-*S*-YL)HYDROQUINONE DEPLETES CELLULAR GLUTATHIONE, STIMULATES SPHINGOMYELIN TURNOVER, AND INDUCES APOPTOSIS IN HL-60 CELLS: ROLE OF REACTIVE OXYGEN SPECIES AND NUCLEAR FACTOR-κB.

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Quinol-thioether metabolites are present in the bone marrow of rats and mice cotreated with hydroquinone and phenol. Several of these metabolites, including 2,3,5-*tris*-(glutathion-*S*-yl)-hydroquinone [TGHQ], are hematotoxic in rats and induce apoptosis in human promyelocytic leukemia (HL-60) cells. The mechanism governing this process is unknown. We now report that TGHQ (200 μM) inhibits γ-glutamyl transpeptidase *in vitro* and depletes cellular glutathione (GSH) in HL-60 cells in a reactive oxygen species-(ROS) independent manner. GSH is a physiological inhibitor of neutral, magnesium-dependent sphingomyelinases *in vitro*. Consequently, TGHQ

also induced sphingomyelin turnover in HL-60 cells, leading to a >300% increase in intracellular ceramide. Ceramide, a lipid second-messenger, mediates a variety of biological effects, including activation of caspase-3, and generation of ROS *via* inhibition of the mitochondrial respiratory chain. Similarly, TGHQ- induced activation of caspase-3 and TGHQ-mediated apoptosis were partially inhibited by the caspase-3 inhibitor, DEVD-CHO, and completely blocked by catalase. Consistent with an increase in ROS, increased NF-κB binding activity was also observed. Cotreatment of TGHQ-treated cells with a cell-permeable peptide (SN50), corresponding to the nuclear localization sequence (VQR**KRQK**LM) of NF-κB, completely blocked TGHQ-induced DNA fragmentation. Surprisingly, catalase did not appear to block the induction of NF-κB. We are therefore currently investigating the precise role of NF-κB in TGHQ-mediated apoptosis. (ES 07247).

716 CATECHOL AND HYDROQUINONE METABOLITES OF BENZENE AND REMOXIPRIDE, TWO COMPOUNDS ASSOCIATED WITH APLASTIC ANEMIA, INDUCE APOPTOSIS IN HUMAN BONE MARROW PROGENITOR CELLS.

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The antipsychotic agent remoxipride ((S-(-)-3-bromo-N-[(1-ethyl-2-pyrrolidinyl) methyl]2,6-dimethoxybenzamide) was introduced to the market in 1992 and withdrawn due to induction of aplastic anemia in a small number of patients. Metabolism of remoxipride generates both pyrrolidine and benzene ring oxidation products. In humans pyrrolidine ring oxidation predominates although phenol, catechol and hydroquinone derivatives are also generated. Benzene has also been associated with aplastic anemia in humans. Induction of apoptosis in human CD34+ bone marrow progenitor cells represents a potential mechanism underlying aplastic anemia and the benzene metabolites, catechol and hydroquinone, but not phenol, induce marked apoptosis in CD34+ cells. Remoxipride metabolites were examined for their ability to induce apoptosis in both HL-60 cells and in CD34+ human bone marrow progenitor cells. Remoxipride and its pyrrolidine ring oxidation products were inactive while catechol and hydroquinone derivatives of remoxipride induced marked apoptosis in both HL-60 cells and in human CD34+ cells. Interestingly, phenol metabolites of remoxipride were inactive at inducing apoptosis in either HL-60 or CD34+ cells. These data demonstrate marked structural similarities between metabolites of benzene and remoxipride that are capable of inducing apoptosis in human hematopoietic progenitor cells and provide insight into cell-specific toxic effects that may be associated with aplastic anemia. (Supported by Astra AB.)

717 THE ROLE OF DMBA METABOLITES IN PREB CELL APOPTOSIS.

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PAH, including 7,12-dimethylbenz[a]anthracene (DMBA), are immunosuppressive. Comparable or superior immunosuppression with PAH metabolites has been taken as evidence of a role for metabolism in PAH immunotoxicity. DMBA immunotoxicity may be manifest as apoptosis induction in a preB cell line (BU-11) maintained on cloned bone marrow stromal cells (BMS2). Apoptosis was dependent on the aryl hydrocarbon receptor/transcription factor (AhR) or AhR-regulated genes. Since PAH metabolism is dependent on AhR-regulated cytochrome P-450 (CYP) enzymes, the present study evaluated the role of metabolism in preB cell apoptosis. The AhR antagonist and CYP1A inhibitor α-naphthoflavone (α-NF) blocked DMBA-induced apoptosis. While a general CYP inhibitor completely ablated CYP1A activity in control Hepa-1 cells, it had no affect on DMBA-induced apoptosis in BMS2/BU-11 cultures. Indeed, BMS2 cells expressed little or no constitutive or inducible CYP1A1 mRNA or protein. They did express CYP1B1 mRNA and protein. Since CYP1B1 metabolizes DMBA to DMBA 3,4-dihydrodiol in BMS2 cells, the potential for this metabolite to induce apoptosis and/or circumvent a requirement for the AhR was evaluated. Indeed, DMBA 3,4-dihydrodiol was as potent as the parent compound in inducing BU-11 cell apoptosis. Surprisingly, apoptosis induced with DMBA 3,4-dihydrodiol was blocked with either α-NF or another AhR antagonist, galangin. Furthermore, DMBA 3,4-dihydrodiol induced relatively little apoptosis when added to co-cultures of BU-11 cells and bone marrow stromal cells from $AhR^{-/-}$ mice, suggesting a role for the AhR even after initial oxidation of the PAH substrate. Finally, DMBA-3,4 dihydrodiol was shown to bind to and activate the AhR. Collectively, these data suggest a feedback loop in which DMBA metabolism, possibly mediated by CYP1B1, may augment AhR signaling leading to preB cell apoptosis. (Supported by PHS grants RO1-ES06086 and 1P42ES 07381.)

718 VISUALIZATION OF INTRACELLULAR SIGNALS BEFORE AND DURING DEXAMETHASONE-INDUCED APOPTOSIS OF NB2 RAT LYMPHOMA CELLS.

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Synchronized (G_0/G_1) Nb2 rat lymphoma cells were fixed in 3.7% formalin-0.9% NaCl and spotted on to slides after incubation with 100 nM dexamethasone (Dex) or vehicle for 0, 1, 2, 4, 6, and 8 hr. Apoptosis was monitored by the TUNEL assay. Glucocorticoid receptor (GR), IkB-α , and NFkB were visualized with ABC immunoperoxidase assay. The percentage of cells stained by TUNEL or ABC was determined by counting 400 to 1600 cells from photomicrographs. A significant ($p<0.05$) increase in apoptotic cells was first observed 8 hr after Dex (20±2%)* vs control (10±2%)*, which is later than the 4 hr previously reported with DNA laddering or diphenylamine (LaVoie and Witorsch, PSEBM 209: 257, 1995). Synchronized cells revealed similar percentages of immunostaining for GR (42±1%)*, IkB-α (39±0%)*, and NFkB (43±1%)*. Up to 8 hr Dex exposure produced no significant change in the percent GR, IkB-α , or NFkB cell staining. The percent GR+ cells is consistent with the magnitude of Dex-induced cell death at 24 hr (44±4%)* vs control (16±3%)*. The similar percentage of cell staining for GR, IkB-α , and NFkB is consistent with their functional associations. (Supported by the Jeffress Trust of NationsBank and CAAT of Johns Hopkins Univ.) * Mean±SEM from 3 experiments.

719 GROUP II CASPASES (CASPASES-3, -7, -2) ARE NOT INVOLVED IN APOPTOSIS INDUCED BY GLUCOCORTICOIDS AND CYTOKINE DEPRIVATION ON LYMPHOID CELLS.

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Group II caspases (casp-3, -2, -7) cleave key proteins possessing a DExD (asp-glu-x-asp) amino acids consensus sequence during the apoptotic process. Among these caspases, casp-3 has been shown in numerous models to be a key executionner of apoptosis, and is partially or totally responsible for the proteolytic cleavage of key proteins. We investigated the role of different caspases in dexamethasone (dex) and cytokine withdrawal-induced apoptosis on murine interleukin-2 (IL-2)-dependent lymphocytes, CTLL-2 and HT-2 cells. The implication of caspases in the apoptotic process in these cells was evaluated using caspase inhibitors (z-VAD-fmk, ubiquitous inhibitor of caspases; z-DEVD.fmk, selective inhibitor of group II caspases; Ac-VEID-CHO, inhibitor of casp-6) and cell cycle analysis (subG1 peak), cell morphology, annexin V staining and DNA fragmentation. Caspase activities were also measured using specific fluorometric or colorimetric substrates for casp-3, casp-6 and casp-2. All the caspases tested were activated in our cells whatever was the stimulus used. Our results showed that VAD (since 20 μM) but not DEVD (up to 200 μM) or VEID (up to 100 μM) reduced the subG1 peak. VAD was also able to stop the apoptotic process were chromatin margination was observed but not cellular or nuclear fragmentation. This was not the case for DEVD. Neither inhibitors were capable of inhibiting oligonucleosomal DNA fragmentation. Therefore it appears that the inhibition of group II caspases or casp-6 in IL-2-dependent cell lines doesn't prevent apoptosis induced by dex or cytokine withdrawal in our model.

720 OXIDATIVE STRESS AND THE FAS PATHWAY IN APOPTOSIS OF CULTURED HUMAN RETINAL PIGMENT EPITHELIAL CELLS.

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Age-related macular degeneration (AMD) is the leading cause of blindness in elderly Americans. During the early phase of this disease, loss of retinal pigment epithelial (RPE) cells occurs. The mechanism for the death of RPE cells is not understood clearly. Previous studies from our laboratory have shown that the oxidant t-butylhydroperoxide (tBH) induces apoptosis in cultured human RPE cells. In the present study, the molecular mechanism

involved in tBH-induced apoptosis was investigated. As the Fas/FasL system is a key cellular apoptosis pathway and oxidants can up-regulate Fas and FasL expression, we examined whether the Fas pathway mediates apoptosis in RPE cells and whether this is the initial signaling in tBH-induced apoptosis. By using RT-PCR, flow cytometry, and Western blot analyses, we found that RPE cells constitutively expressed Fas. Treatment of RPE cells with soluble FasL or the agonistic anti-Fas antibody CH-11 triggered apoptosis. In the presence of the antioxidants glutathione and *N*-acetyl-$_L$-cysteine, apoptosis induced by soluble FasL was inhibited. When the expression of Fas was measured in RPE cells undergoing apoptosis after tBH treatment, no change in Fas expression was observed. Moreover, pretreatment of RPE cells with the antagonistic anti-Fas antibody ZB4 (that blocks the interaction between Fas and FasL) had little effect on tBH-induced DNA cleavage, phosphatidylserine exposure and subsequent cell death. Our data suggests that the Fas pathway can mediate apoptosis in RPE cells with reactive oxygen intermediates acting as mediators. However, in tBH-induced apoptosis, the Fas pathway is not directly involved.

721 MERCURY SUPPRESSES CD95/FAS-MEDIATED APOPTOSIS: A SEARCH FOR THE MECHANISM.

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Exposure to inorganic mercury (Hg^{2+}) is associated with autoimmune disease but the molecular mechanisms underlying Hg^{2+}-mediated immunopathology are poorly understood. Since disregulation of CD95/Fas-mediated apoptosis has been implicated in autoimmune disorders, one hypothesis explaining how Hg^{2+} contributes to autoimmune disease could be by blocking the CD95 death pathway in autoreactive lymphocytes. Using the Jurkat T-cell line we have established that 5-10 μM $HgCl_2$ prevents anti-CD95-mediated inhibition of DNA synthesis, induction of cell death, and activation of caspase-3. Here we are pursuing the molecular component(s) that Hg^{2+} interacts with to attenuate apoptosis. Mercury attenuates anti-CD95-mediated DNA damage, a late event in apoptosis, and phosphatidylserine externalization, a relatively early event in apoptosis. Mercury does not down regulate cell surface expression of CD95, nor does it prevent the anti-CD95 agonist from binding to the CD95 receptor. When the human antioxidant enzyme glutaredoxin, which prevents H_2O_2-mediated apoptosis, was overexpressed in Jurkat cells, Hg^{2+} still suppressed anti-CD95-mediated apoptosis, suggesting that Hg^{2+} does not interfere with caspase activation via the production of H_2O_2 as has been suggested previously. Our studies indicate that a functional molecular component of the CD95 death pathway is targeted by Hg^{2+} and that this component is upstream of caspase-3, not shared by other death receptor pathways (e.g., TNF-R), and downstream of the CD95 receptor. This study is the first to correlate Hg^{2+}exposure with disregulation of the CD95 death pathway and represents a model to elucidate the mechanism whereby Hg^{2+} modulates peripheral tolerance leading to autoimmune dysfunction. (Supported by P30-ES06639.)

722 ROLE OF TCDD-INDUCED APOPTOSIS AS A MECHANISM OF IMMUNOTOXICITY IN PERIPHERAL T CELLS ACTIVATED THROUGH THE T CELL RECEPTOR.

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Previous studies from our laboratory have shown that treatment with TCDD inhibits the responsiveness of antigen-activated but not naive T cells from C57BL/6 +/+ mice. In the current study, we investigated whether this decreased responsiveness of such T cells resulted from increased apoptosis following restimulation. To this end, C57BL/6 +/+ or C57BL/6 *gld/gld* (Fas ligand defective) mice were injected with TCDD (50 mg/kg body weight, i.p.) and with anti-CD3 mAbs into the footpads. Five days later, axillary lymph nodes cells (source of naive T cells) and popliteal lymph nodes cells (source of activated T cells) were cultured *in vitro* with anti-CD3 mAbs and cell proliferation was measured. Also, such cells were studied for their ability to undergo apoptosis upon *in vitro* culture. The data demonstrated that activated but not naive T cells from TCDD-treated wild-type (+/+) mice showed a significant decrease in their responsiveness to anti-CD3 mAbs. Also such cells exhibited increased levels of apoptosis. In contrast, both naive and activated T cells from C57BL/6 *gld/gld* mice showed normal responsiveness to anti-CD3 stimulation. Also, the activated T cells from TCDD-treated gld mice failed to exhibit increased apoptosis. The current study demonstrates that the immunotoxic effects of TCDD on activated T cells may be mediated through Fas ligand-based apoptosis. (Supported in part by grants from EPA and NIH).

723 ROLE OF CASPASES AND FAS LIGAND IN THE INDUCTION OF APOPTOSIS IN THYMOCYTES BY TCDD.

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Recent studies from our laboratory demonstrated that TCDD induces apoptosis and thymic atrophy in C57BL/6 +/+ but not in Fas-deficient (*lpr/lpr*) or Fas-ligand defective (*gld/gld*) mice. In the current study, we further investigated the role of Fas ligand (FasL) in TCDD-induced apoptosis. TCDD-administration at a dose of 50 mg/kg body weight in C57BL/6 +/+ mice caused significant upregulation of FasL in thymocytes. Furthermore, sera from TCDD-treated mice contained increased levels of soluble FasL. This was shown by their ability to induce apoptosis in Fas^+ targets such as thymocytes from +/+ mice or L1210 Fas^+ transfectants and inability to induce apoptosis in Fas^- targets including thymocytes from *lpr/lpr* mice or L1210 Fas^- transfectants. The TCDD-induced apoptosis in thymocytes *in vitro* was inhibited by caspase inhibitors such as YVAD-CMK and DEVD-CHO. Also, injection of YVAD-CMK significantly inhibited the TCDD-induced apoptosis in thymocytes of C57BL/6 +/+ mice. Together, the current study demonstrates that TCDD-induced upregulation of FasL and activation of caspases may constitute an important mechanism by which TCDD mediates immunotoxicity. (Supported in part by grants from EPA and NIH.)

724 DIETHYLSTILBESTROL DOES NOT DIRECTLY, INDUCE APOPTOSIS OF THYMOCYTES UNLIKE DEXAMETHASONE: COMPARISON OF MULTIPLE ASSAYS FOR HORMONE-INDUCED APOPTOSIS.

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Endocrine disrupting agents, dexamethasone (DEX) and diethylstilbestrol (DES) have been shown to induce atrophy of thymus, a major T cell developmental organ. DEX has been shown to directly induce apoptosis of thymocytes. To definitively ascertain whether DES also directly induces apoptosis of thymocytes, many common assays of apoptosis were performed on thymocytes exposed to various doses of DES or DEX. An additional aim was to critically evaluate common apoptosis techniques, by examining kinetics of induction of apoptosis in dexamethasone (DEX)-exposed thymocytes at 2, 4, 8, 12, 26-28 and 48-50 hours of culture. Apoptotic thymocytes were identified by six methods, including flow cytometric examination of 7-aminoactinomycin D (7-AAD), propidium iodide (PI), or annexin V-FITC stained cells, changes in cell diameter/size by cell-size analyzer and forward/side scatter, and microscopic cytopathologic examination. After 4 hours of DEX exposure, apoptosis was evident by 7-AAD, annexin and cytopathological assays, but no cells with sub-diploid DNA content were evident by PI analysis. Maximal apoptosis was evident by all the above flow cytometric techniques at 12 hours after DEX exposure. The 7-AAD and annexin assays, which allow discrimination between early apoptosis and late apoptosis/necrosis, were comparable and identified similar apoptotic populations. Appearance of a $FS^{lo}/SS^{increased}$ population was evident only after 12 hours of DEX exposure. DEX induced a decrease in cells of the 4.9-6.2 μm diameter range after 12 hours. Importantly, apoptosis could not be detected by any of the above assays in thymocytes exposed to various doses of DES, suggesting that these two synthetic hormones have different mechanisms of thymic atrophy *in vivo*. (Supported by NIEHS/NIH - RO1 ES0843.)

725 MK886, A 5-LIPOXYGENASE ACTIVATING PROTEIN (FLAP) INHIBITOR, INDUCES FLAP-INDEPENDENT APOPTOSIS IN FL5.12 CELLS.

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The ability of various inhibitors of lipoxygenase (LOX) enzymes and FLAP to induce apoptosis has implicated these pathways as being important in the mechanisms of this form of cell death. However, although FLAP plays an important role in 5- LOX activity, this protein is found at high levels in cells lacking LOX suggesting it may mediate other effects. Furthermore, the con-

centration of MK886, a FLAP inhibitor, required to induce apoptosis is 100x more than that required to stimulate LOX. We recently reported that MK886 induces apoptosis in an IL3-dependent pro-B lymphocytic cell line expressing FLAP but lacking LOX [*J. Biol. Chem.* **273**: 1998 (in press)]. The mechanism of this effect may be related to the release of free arachidonic acid after MK886 treatment. In support of this concept, arachidonic acid treatment (50 μM) induced ~25% apoptosis after 24 h. The role of FLAP in MK886-induced apoptosis was directly examined by decreasing its expression by incubation with 10 μM of an antisense oligonucleotide. After 48 h, FLAP expression was decreased by more than 50%. This change was not accompanied by any increase in apoptosis. FLAP antisense oligo-treated cells exhibited about 30% apoptosis 8 h after treatment with 10 μM MK886. This level of apoptosis was similar to that seen in control cells. These data suggest that MK886-induced apoptosis may not be directly associated with FLAP. This conclusion is further supported by the fact that a more specific FLAP inhibitor, MK591 (supplied by Merck Frosst, Canada), was unable to induce significant apoptosis in this cell line. (Supported by HL51005 and Center Grant ES07784.)

726 POLY(ADP-RIBOSE) POLYMERASE INHIBITION PREVENTS OXIDANT-INDUCED ONCOSIS AND PERMITS APOPTOSIS IN ENDOTHELIAL CELLS.

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Reactive oxygen species (ROS) are mediators of cell death, especially in toxic processes involving inflammation where the endothelium is a significant tissue of injury. In endothelial cells (EC), DNA appears to be a critical target of ROS, which cause DNA strand scissions. In response to DNA breaks, the nuclear enzyme, poly(ADP-ribose) polymerase (PARP), is activated resulting in NAD^+ depletion, a decline in cellular energy and cell death. Based on the premise that apoptosis is an active process, we hypothesize that following lethal oxidant injury, oncosis predominates as a result of the consequences of PARP activation. However, if PARP is inhibited and cellular energy sustained, oncosis is prevented and apoptosis is allowed to proceed. Biochemical and morphological indicators of apoptosis were used to investigate the mode of EC death after an oxidant stress (H_2O_2). Following H_2O_2 exposure, EC began to lose membrane integrity between 2-3 hr, which was preceded (~1 hour) by a significant rise in intracellular free calcium ($[Ca^{2+}]_i$). Inhibition of PARP during the oxidant stress delayed both the rise in $[Ca^{2+}]_i$ and cell lysis. Caspase-3-like activity, a hallmark of apoptosis, was negligible in oxidant-treated EC. However, when PARP was inhibited, caspase-3-like activity was dramatically increased at all H_2O_2 concentrations tested. The increased caspase-3-like activity after inhibiting PARP was confirmed by Western analysis showing extensive apoptotic PARP cleavage by caspase. Furthermore, PARP inhibition just prior to the onset of oxidant-induced EC lysis (at 2 hours), reversed the elevated $[Ca^{2+}]_i$ to normal, delayed the loss of membrane integrity and increased caspase-3-like activity. Fluorescence microscopy with acridine orange showed hallmark nuclear features of apoptosis in oxidant-stressed EC when PARP was inhibited, and oncosis when EC were treated with H_2O_2 alone. In addition, maintenance of energy-dependent processes in oxidant-stressed EC following PARP inhibition was demonstrated by the energy-dependent lysosomal concentration of acridine orange and maintenance of low $[Ca^{2+}]_i$. These results show that the mode of oxidant-induced EC death is switched from oncosis to apoptosis when PARP is inhibited and suggest that PARP activity must be suppressed for apoptosis to occur. Therefore, modulation of PARP activity following oxidant-induced DNA damage would appear to provide a mechanism to manipulate this injury into a mode of apoptotic cell death that may ultimately be controlled.

727 N-ACETYL CYSTEINE (NAC) PARTIALLY BLOCKS FLUDARABINE-INDUCED APOPTOSIS IN CLL CELL LINES BY ACTING DOWNSTREAM OF CASPASE-3 ACTIVATION.

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B-cell chronic lymphocytic leukemia (B-CLL) is the most common and prevalent form of leukemia. Fludarabine, an adenine nucleoside analog, is being used extensively to treat this disease. Fludarabine inhibits DNA synthesis and induces apoptosis. However, its pro-apoptotic mechanism is unclear. Two human B-CLL cell lines (I-83 & WSU) and another human B cell leukemia cell line (JVM-2) were used to study the mechanism of action of fludarabine. Apoptosis, detected by ELISA for cytosolic histone-associated DNA fragments, was increased 3- and 5-fold relative to controls 18 h after treatment with 50 μM fludarabine in I-83 and JVM-2 lines, respectively. Fludarabine (10 μM) also produced a 10-fold increase in apoptosis in WSU cells after 18 h. All fludarabine-treated cell lines showed significant activation of caspase-3 at the times apoptosis was assessed. Since the analogous nucleoside cytosine arabinoside is known to induce apoptosis via oxidative stress, these three cell lines were co-treated with 15 mM NAC to assess any protective effect. There was 40 and 47% less apoptosis in NAC-treated WSU cells 12 and 18 h after fludarabine, respectively. NAC also partially protected (~50%) JVM-2 and I83 cells treated with fludarabine for 18 and 24 h. Surprisingly, NAC was unable to prevent caspase-3 activation under these conditions. It seems possible that NAC blocks apoptosis at a step downstream of caspase-3 activation. (Supported by HL51005 and HL48035.)

728 DICHLOROACETONITRILE INDUCES OXIDATIVE STRESS AS A MEDIATOR OF APOPTOSIS OR NECROSIS IN MOUSE PERITONEAL MACROPHAGES (MPM).

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The water disinfectant byproduct Dichloroacetonitrile (DCAN) is a direct acting mutagen and known to induce DNA strand breaks in cultured human lymphoblastic cells. The effect of DCAN in cultured mouse peritoneal macrophages (MPM, RAW 264.7) was explored. MPMs were exposed to various concentrations (100μ M- 400μ M) of DCAN for 4h. The phagocytic activation of MPMs was characterized by the production of reactive oxygen intermediates (ROI) and secretion of TNF- α . The ratios of intracellular GSH/GSSG were assessed and used as an indicator of oxidative stress. Electrophoretic detection of DNA degradation and light microscopy were utilized for the characterization of DCAN-induced apoptosis. LDH leakage and trypan blue exclusion were used as markers of the necrotic effects of DCAN. Following treatment with DCAN, GSSG was increased (135% of control, $P< 0.05$). DCAN activation of MPMs was observed by elevated levels of ROI (190-250% of control, $P<0.05$) and increased secretion of TNF-α (450% of control, $P<0.05$). Elecrophosresis of DNA of treated MPMs indicated a dose dependent increase in the degradation of genomic DNA. This information combined with morphological studies indicated that exposure of MPMs to lower levels of DCAN (100μ M and 200μ M) incites apoptic cell death. At 400μ M DCAN cellular necrosis was observed as indicated by increased LDH leakage and decreased viability (45% of control, $P< 0.05$). These studies suggest that the dose dependent DCAN-induced apoptosis or necrosis in MPMs is due to the disturbance in GSH/GSSG ratio and initiation of ROI mediated oxidative damage mechanisms. (Supported by USEPA.)

729 ALLOPURINOL INHIBITS POLY(ADP-RIBOSE) POLYMERASE AND CONVERTS OXIDANT-INDUCED ENDOTHELIAL CELL DEATH FROM ONCOSIS TO APOPTOSIS.

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Allopurinol (AP) has been shown to limit tissue damage associated with reperfusion following ischemia, putatively by inhibiting xanthine oxidase (XO), a source of reactive oxygen species (ROS). This study was undertaken to investigate the ability of AP, as an inhibitor of poly(ADP)-ribose polymerase (PARP), to modulate the mode of endothelial cell (EC) death following exposure to ROS. In response to oxidative induced DNA strand scissions, the nuclear enzyme PARP is activated, resulting in NAD^+ depletion, a decline in cellular energy and cell death. We hypothesize that part of the mechanism by which AP protects against oxidant damage is its ability to inhibit PARP, thus preserving cellular energy status and switching the mode of EC death from oncotic necrosis to apoptosis. Since, in vivo, inflammation is associated with necrosis and not with apoptosis, conversion of oncotic necrosis to apoptosis may be of benefit. Bovine pulmonary artery EC were used as a model target cell for oxidant stress, which was produced by exposing cells to H_2O_2. The potential contribution of secondary oxidative stress by endogenous XO was eliminated by growing EC in the presence of 50 μ M tungsten, which replaces the molybdenum in XO thus blocking XO activity. When EC, grown with or without the presence of tungsten, were exposed to H_2O_2 and AP the time-course of oxidant-induced loss of plasma membrane integrity was delayed. Caspase-3-like activity, a hallmark of apoptosis, was negligible in H_2O_2-treated EC, but was significantly increased in oxidant-stressed EC treated with AP. In addition, apoptotic PARP cleavage by caspase was evident only in oxidant-stressed EC treated with AP. Nuclear morphology in EC treated with H_2O_2 plus AP was consistent with apoptosis.

Fluorescence micrographs demonstrated globular, condensed and fragmented DNA, whereas, micrographs of EC treated with H_2O_2 alone illustrated an oncotic necrosis. The biochemical and morphological results obtained from EC treated with H_2O_2 in combination with AP mirror those obtained with EC treated with H_2O_2 and a known PARP inhibitor, 1,5-dihydroxyisoquinoline. These results suggest that, in addition to its action as a XO inhibitor, the effects that are observed with AP in models of oxidative stress may be a result of AP's ability to inhibit PARP and convert oxidant-induced oncotic necrosis to apoptosis.

730 HYDROGEN PEROXIDE INDUCES TWO TYPES OF PROGRAMMED CELL DEATH IN NORMAL HUMAN DIPLOID FIBROBLASTS.

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Cell death occurs in normal human cells as a result of aging, diseases and chemical or radiation therapy. The mechanism of normal human cell death is largely unknown. Since oxidants have been suggested as a cause of aging and many diseases, we test whether H_2O_2 can induce apoptosis or programmed cell death in normal human diploid fibroblasts. Apoptosis was observed with low concentrations of H_2O_2 (≤ 150 μM) after exponentially growing cells were treated for 2 hr. The apoptotic cells showed detachment, cell shrinkage, nuclear condensation and S-phase distribution (type I cell death). These cells elevate p53 protein and express a soluble bax protein. Introducing human papillomavirus (HPV) protein E6 results in reduction in p53 protein and apoptosis within 24 hr after H_2O_2 treatment. However, E6 cells appeared to show fragmented nuclei and cell death at 2-3 days after the treatment (type II cell death). Introducing HPV E7 protein did not result in this phenotypic change. Both types of cell death involved activation of caspase-3 and were dose or time dependent. To identify the difference in the molecular programs of the two types of cell death, we measured the level of cyclin B1 and cdc-2 kinase. Western blot showed an elevation of cyclin B1 and phosphorylation of cdc-2 at 48 hr after H_2O_2 treatment in E6 but not wild type or E7 cells. This suggests the possibility that E6 cells exit at G2/M phase to undergo cell death. Our results indicate that H_2O_2 can cause two morphologically different types of cell death in normal human cells. The difference is attributed to the presence p53, cyclin B elevation, cdc-2 phosphorylation and cell cycle position.

731 ANTIOXIDANT GRAPE SEED PROANTHOCYANIDIN EXTRACT (GSPE) AND A DNA REPAIR MODULATOR 3-AMINOBENZAMIDE (3-AB) PROTECT DOORUBICIN (DOX)-INDUCED CARDIOTOXICITY *IN VIVO.*

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Cardiotoxicity of DOX is a major limiting factor for clinical application of this important anticancer drug. Since, DNA is the primary target of DOX *in vivo*, this study was designed to investigate whether GSPE via its antioxidant properties, and 3-AB through its DNA repair capabilities [by influencing PARP: poly (ADP-ribose) polymerase activity], can negate DOX-induced DNA damage and prevent cardiotoxicity. To assess these events, male ICR mice (Fec, 30-40g) were either pre-exposed to GSPE (100 mg/kg for 7 days, po) followed by DOX (20mg/kg,ip), or DOX followed by a post-exposure, all the animals were sacrificed and their serum CPK activities were determined. Hearts were also collected for histopathology and DNA analysis. Results indicate that DOX alone caused significant heart injury, whereas, preexposure to GSPE or post-exposure of 3-AB significantly reduced DOX-induced cardiotoxicity, and prevented DNA damage. Histoathological evaluation of PAS and H&E stained heart sections indicated that GSPE and 3-AB successfully intervened DOX-induced cardiotoxic events and protected cells from both apoptotic and necrotic deaths. In addition to 3-AB, GSPE's ability to abrogate DOX-induced DNA damage in the cardiac tissue may suggest that it can modulate PARP activity and protect cells from toxic assaults. Similarly, 3-AB possibly mimics GSPE's antioxidant property and plays a role in detoxifying toxic intermediates formed during redox-cycling of DOX. (Supported by Inter-Health Nutraceuticals, Concord, CA.)

732 MECHANISM OF MENADIONE TOXICITY IN A LYMPHOBLAST LINE.

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The cell death seen with menadione (MEM) toxicity is presumably due to oxidant stress. Yet it is not clear how this stress causes death. In the current study we have examined various indices of apoptosis in a control lymphoblast line to determine whether the toxicity seen in these cells could be due to enhanced programmed cell death. We find that incubations of the cells with MEM (5-50 μ M) for 24hrs leads to a concentration dependent inhibition of cell proliferation. At 20 μ M the inhibition was 30%. There was an associated 100% increase in DNA fragmentation as determined by the fluorescent assisted cell sorter (FACS)-TUNEL assay and an 800% increase in caspase 3 activity as determined by proteolysis of the synthetic peptide DVED. Paradoxically, at 50 μ M MEM the caspase 3 activity was lower than that observed at 20 μ M. The increase in caspase 3 activity seen at 20 μ M was not associated with changes in the cellular contents of BCL-2, BCL-X, BAX or P53, but there was proteolysis of PAPR as determined by western blot. At 20 μ M leucine incorporation was completely inhibited within 4 hrs of incubation. The complete inhibition of caspase 3 activity by Ac-DEVD-CHO had no effect on any of these indices except PAPR cleavage. These data suggest that while various indices of apoptosis increase in lymphoblasts incubated with MEM, the decrease in proliferation is due to some other mechanism. It is unclear what role the profound decrease in protein synthesis may have in this toxicity.

733 INHIBITION OF DOXORUBICIN-INDUCED APOPTOSIS IN METALLOTHIONEIN OVEREXPRESSING TRANSGENIC MOUSE HEART.

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Previous studies using transgenic mice have demonstrated that metallothionein (MT), a low molecular weight and cysteine-rich protein, protects the heart from oxidative injury by doxorubicin (DOX). To investigate possible mechanisms of this protection, the effect of MT on DOX-induced apoptosis in the heart was examined. Seven-weeks-old transgenic mice overexpressing MT specifically in the heart about 10-fold higher than normal, along with their non-transgenic littermates, were treated with DOX by i.v. injection at 20 mg/kg. Twelve hours after the treatment, the hearts were isolated for analysis of apoptosis by a TUNEL assay, which was further evaluated by an electron microscopic examination. Results showed that DOX induced apoptosis in the mouse heart and MT overexpression markedly suppressed the effect of this drug. Furthermore, neonatal mouse cardiomyocyte cultures were established. The transgenic cardio-myocytes contained higher concentrations of MT than the non-transgenic cells. Other antioxidant activities were comparable between the two cell types. Studies using this cell culture system further confirmed the *in vivo* findings. In addition, it was revealed that preceding the programmed cell death, a delta isoform protein kinase C (PKC_δ) was activated by DOX. MT inhibited this activation, an event that was likely involved in the MT inhibition of the drug-induced apoptosis. (Supported in part by NIH grant CA68125, and an Established Investigator Award from the American Heart Association (9640091N).)

734 THE 'APOSOME' A LARGE (~700 KDA) CASPASE ACTIVATING COMPLEX.

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It is now widely recognised that apoptotic cell death requires the activation of aspartate specific cysteine proteases or caspases, which are related to interleukin converting enzyme (ICE). This enzyme was identified as the first mammalian homologue of CED-3, a protease that is essential for programmed cell death in Caenorhabditis elegans and is activated by CED-4, and regulated by CED-9. The caspases, Apaf-1 and Bcl-2 family members are the mammalian homologues of CED-3, CED-4 and CED-9 respectively. Recent evidence has shown that Apaf-1.dATP and cytochrome c activate caspase-9 which then processes downstream caspases to initiate the caspase cascade. Caspase activation requires proteolytic cleavage at specific tetrapeptide sites to yield small (~10 kDa) and large (~20 kDa) subunits to form the active ~ 60 kDa heterotetramer. However, we now show that in THP.1

cells, activated caspases are associated with a very large ~700 kDa multimeric protein complex, the 'aposome'. This complex has a constitutive protease (IETDase) activity, capable of cleaving the IETD↓ S motif necessary for effector caspase activation. The aposome normally has a very low effector caspase (DEVDase) activity, however during apoptosis or in vitro, dATP dependent caspase activation there is a marked increase in the DEVDase activity of the aposome. This is accompanied by the recruitment of Apaf-1, procaspase-8 and Bcl-X_L to the complex. In addition, procaspases-2, -3 and -7 are also recruited and processed to their active forms. Thus, the aposome is a large multimeric protein complex, which plays a very important role in the processing and activating of the caspases.

735 PROTEASOME INHIBITION INDUCES HSP70 AND PROMOTES APOPTOSIS WHICH IS DECREASED BY *BCL-X_L* OVEREXPRESSION IN MURINE PRO-B LYMPHOCYTIC (FL5.12) CELLS.

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Proteasome is a multi-subunit 700 kDa complex that recognizes and degrades various proteins including polyubiquitinated, misfolded and short-lived regulatory proteins. Previous studies report that inhibitors of proteasome activity, which are currently being used in drug development, promote apoptosis. *bcl-xL* and hsp70 proteins can protect against apoptosis induced by a variety of stimuli. In this study, control and *bcl-xL*-overexpressing IL-3-dependent FL5.12 cell lines were treated with 500 nM MG132, a peptide aldehyde inhibitor of proteasome activity. 24 h after MG132 treatment, apoptosis in control and *bcl-xL* cells, measured by acridine orange/ethidium bromide, was ~35% and ~22% of total cells, respectively. Concomitantly, there were ~7- and ~3.5-fold increases in caspase-3 activity in control and *bcl-xL* cells, respectively. Necrotic cells were a maximum of 7%. Inhibiting caspase-3 activity with 50 M Ac-DEVD-CHO prior to MG132 treatment resulted in a marginal reduction in apoptosis in both cell lines by 24 h. Immunoblot data revealed similar levels of hsp70 in untreated control and *bcl-xL* cells. hsp70 protein levels increased ~3-fold by 8 h following MG132 treatment in both control and *bcl-xL* cells. This increase was maintained up to 24 h. The current data indicate: (1) an inhibitor of proteasome stimulates apoptosis and induces hsp70, perhaps by preventing the degradation of misfolded or key pro-apoptotic regulator proteins; (2) *bcl-xL* overexpression lessens the pro-apoptotic but not the hsp70-inducing effect(s) of proteasome inhibition; and (3) proteasome inhibitor-induced apoptosis involves a caspase-3-independent mechanism. (Supported by HL51005 and Center Grant ES07784.)

736 DEATH OR SURVIVAL IN NORMAL HUMAN FIBROBLASTS IS DETERMINED BY BAX β OR P21 GENE EXPRESSION.

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Normal human cells in culture provide a relevant model for elucidating toxin produced molecular changes inside our body. Oxidants have been found to mediate toxicity of many physical or chemical insults. When normal human fibroblasts were treated with sub-necrotic doses of oxidants for 2 hr, a small proportion of cells underwent apoptosis while the rest of cells survived but developed premature senescence after the treatment. Apoptotic cells detached before plasma membrane leakage occurred and showed caspase-3 activation, nuclear condensation, cell shrinkage but not DNA ladder. The ratio of apoptotic versus surviving cells increased with high doses of H_2O_2 and reached 1:1.5 with 200 μM H_2O_2. Since apoptotic cells detach while surviving cells remain attached to the culture matrix, we can easily separate the two populations and identify the difference in the molecular programs leading to the two different cell fates. The p53 tumor suppressor protein showed super elevation in apoptotic cells but some elevation in surviving cells. Inhibiting p53 elevation by human papillomavirus E6 protein reduced apoptosis. The p21 cyclin dependent kinase inhibitor protein was absent in apoptotic cells but was strongly induced in surviving cells. Apoptotic cells expressed a soluble bax protein while surviving cells did not. RT-PCR experiments indicated a reduction of bax α mRNA, which encodes a nuclear membrane protein, but an increase of bax β mRNA, which encodes a cytosolic protein, in apoptotic cells but no change of bax α or β mRNA in surviving cells. Both apoptotic and surviving cells elevated bcl-2 protein with time. Therefore our data support that while expression of bax β is a driven force of apoptosis, expression of p21 is a surviving factor.

737 PRECURSORS DECREASE CONSTITUTIVE LEVELS OF ACTIVATED NFkB AND GRP78 AND PROTECT CELLS AGAINST APOPTOSIS.

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The natural detergent, sodium deoxycholate (NaDOC), induces apoptosis through oxidative stress and DNA damage. NaDOC (0.5mM) treated Jurkat cells show classic features of apoptosis under light and transmission electron microscopy. To determine the effects of NAD^+ precursors on NaDOC induced apoptosis, cells were incubated in 10 mM nicotinic acid (NA) or 10 mM nicotinamide (NAM) for 24 hours before treatment with 0.5 mM NaDOC. Levels of apoptosis were reduced by 60% in cells treated with NA and by 40% in cells treated with NAM. Jurkat cells grown in NA or NAM for four days showed no significant difference between the growth rate of the untreated versus treated cells. Jurkat, HT-29, and HCT-116 cells incubated in the presence of NA or NAM for 24 hours, then fixed and stained for *activated* NFκ B, show significantly (p=0.0001) reduced levels of *activated* NF-κ B in the nuclei of these cells. Effects of NA and NAM on GRP78 levels in Jurkat cells were determined using a polyclonal antibody to GRP78. Digital image analysis showed that NA significantly (p=0.0001) reduced nuclear levels by 40% and cytoplasmic levels by 20%, while NAM significantly (p=0.0007) reduced cytoplasmic levels. Jurkat cells treated with NAM for 24 hours, then fixed and reacted with and antibody to GAPDH, show increased intracellular levels of the protein. HepG2 cells, transfected with 13 different promoter/response element CAT reporter constructs, were treated with 10mM NAM or 10 mM NA. NAM did not activate any of the 13 promoters or response elements of the genes associated with cellular stresses. NA produced only marginal induction of grp78 (1.1 fold induction) and hsp70 (1.1 fold induction). The NAD^+ precursors, NA and NAM, may, therefore, protect against apoptosis by multiple mechanisms that help reduce oxidative stress.

738 p53 SUPPRESSES APOPTOSIS IN ALVEOLAR MACROPHAGES EXPOSED TO BLEOMYCIN *in vivo*.

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Chronic inflammation in the lung leading to the development of pulmonary fibrosis is a common response to environmental pollutants and cancer chemotherapeutics such as bleomycin. We speculate that pulmonary fibrosis may be initiated by bleomycin-induced apoptosis of alveolar macrophages resulting in impaired clearance of apoptotic cells and/or release of inflammatory cytokines. Here we report that bleomycin stimulated apoptosis in alveolar macrophages from C57/Bl6 mice exposed to bleomycin *in vivo* or *in vitro*. Cell death was associated with oligonucleosomal DNA fragmentation and was inhibited by the caspase inhibitor ZVAD-fmk. Analysis of p53 localization demonstrated translocation of wild-type p53 into the nucleus following exposure to bleomycin *in vivo* but not *in vitro*. Strikingly, alveolar macrophages from homozygous p53-deficient mice were 2 fold more sensitive to bleomycin than those obtained from wild-type mice following exposure *in vivo*. Similarly, TUNEL analysis demonstrated that lung epithelial cells were 4-5 fold more sensitive to bleomycin-induced apoptosis than were wild-type cells. However, there was no difference in levels of apoptosis in wild-type and $p53^{-/-}$ macrophages exposed to bleomycin *in vitro*. Together, our results demonstrate that wild-type p53 and factors within the lung microenvironment play critical roles in modulating the effects of bleomycin within the lung.

739 MECHANISTIC STUDIES OF PUVA INDUCED APOPTOSIS.

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A combination of psoralens and ultraviolet-A (320-400 nm) radiation (UVA), commonly referred to as "PUVA", is being widely used in the treatment of psoriasis, a skin disorder that affects between 2 and 8 million per-

sons in the U.S. Although it is a highly efficacious treatment for psoriasis, studies have shown that PUVA is a potent mutagen and a carcinogen. PUVA is highly effective in killing hyperproliferative psoriatic keratinocytes but its mechanism of action has not been fully elucidated. Psoralen binds to DNA and forms monofunctional adducts and interstrand DNA crosslinks once it is photoactivated by UVA. It is likely that such damage initiates cell death by apoptosis. *In vitro* studies were undertaken to investigate the molecular mechanisms by which PUVA induces cell death. Immortalized mouse keratinocyte cells (JB-6) were treated with 10 μ g/ml of 8-Methoxypsoralen (8-MOP) for 15 min at 37° C and then irradiated with 10 kJ/m² of UVA radiation, using filtered Sylvania F15T8/BLB lamps. As controls, cells were either untreated or treated with 8-MOP or UVA alone. At various time points after treatment (3, 6, 12, 24, 48, and 72 hr), the cells were quantitatively analyzed for apoptosis by propidium iodide staining. Apoptosis in treated cells was also evaluated by morphological and biochemical assays such as TEM, TUNEL, and DNA ladder formation. In addition, the induction of a variety of apoptosis-related proteins such as p53, p21$^{Waf/Cip1}$, mdm2, Bcl-2, Bax, caspase-3, and PARP were analyzed by western blot analysis. Our results indicate that PUVA inhibits proliferation and causes cell cycle arrest at G1 in JB6 keratinocytes. Results from the morphological and biochemical assays reveal that the cells also undergo apoptosis following treatment with PUVA. p53 is induced in a time-dependent manner beginning at 6 hrs, with peak levels occurring at 24-48 hrs post-treatment. p21 levels were elevated at 6-24 hrs post-treatment. In contrast, there was no evidence of apoptosis or induction of p53 or p21 in cells treated with 8-MOP or UVA alone. These preliminary studies lend credence to our hypothesis that PUVA induces apoptosis involving the p53 pathway.

740 IDENTIFICATION OF THE PREDOMINANT SITE OF COVALENT BINDING OF ACRYLONITRILE TO RAT HEMOGLOBIN.

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Acrylonitrile (AN) is a vinyl monomer used in the production of synthetic fibers and plastics. Previous work [Benz et al. (1997) *Fundam. Appl. Toxicol.* **36**: 149-156] has shown that, following sc administration to rats, the extent of AN covalent binding to protein in different tissues varied by nearly 10 fold. Blood was found the most reactive tissue and hemoglobin (Hb) the most highly labeled protein. The current experiments established the primary site of covalent binding of AN to rat Hb. Globin was prepared from a male Sprague-Dawley rat treated sc with [2,3-^{14}C]-AN at a dose of 115 mg/kg. The alpha and beta chains were separated by reverse phase HPLC. More than 98% of the recovered radioactivity was on the beta chains. Electrospray ionization mass spectrometry (ESI-MS) yielded theoretical masses for the alpha chains whereas the masses of the beta chains were 53 Da greater than expected theoretically. This mass increase is consistent with the binding of one molecule of AN to the beta chains. A Staphylococcus V8 protease digest of the beta chains was prepared and the resulting peptides were separated by reverse phase HPLC. A single radioactive peptide was detected that contained 90% of the radioactivity injected on the column. Amino acid sequence analysis of this peptide was consistent with beta 122-146. The theoretical mass for this fragment is 2616.04 Da, however the mass determined by ESI-MS was 2669.96 Da, also a 53 Da increase. Further analysis of this peptide by MS-MS revealed that AN was bound to Cys-125. The data indicate that beta Cys-125 is the dominant site of AN covalent binding to rat Hb *in vivo*. (Supported by NIEHS 06141 and NIH S10 RR11368.)

741 CHARACTERIZATION OF HUMAN SERUM ALBUMIN REACTED WITH TRANS,TRANS-MUCONALDEHYDE, A HEMATOTOXIC BENZENE METABOLITE.

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Trans,trans-muconaldehyde (muconaldehyde, MUC), a six carbon diene dialdehyde, is a ring-opened hematotoxic metabolite of benzene. Reaction of MUC with proteins is of interest since characterization of protein modification by MUC is important in understanding the cellular toxicity of MUC and could potentially lead to the development of cumulative biomarkers of benzene exposure based on protein adducts of ring-opened metabolites. Human serum albumin (HSA) was incubated with 10-1000 μM MUC in PBS, pH 7.4, at 37°C for 16 hr. Control and MUC-reacted HSA was analyzed for the following: (i) available primary amino groups using the fluorescamine assay; (ii) carbonyl group content using reaction with 2,4-dinitrophenylhydrazine; (iii) the presence of pyrrole moieties using Ehrlich's reagent; (iv) number of MUC molecules bound using mass spectrometry, and (v) amino acid modification using amino acid analysis. Incubation of HSA with 10, 50 and 100 μM MUC lead to a 4, 17 and 27% decrease of available primary amino groups, respectively. Reaction of HSA with 10 and 100 μM MUC resulted in a 2.6 and 22.7 fold increase in carbonyl content, respectively. Reaction of MUC-reacted HSA with Ehrlich's reagent indicated the presence of pyrroles detected by UV absorption spectra. MALDI-TOF mass spectrometry suggests that HSA reacted with 1, 10, 100 and 1000 μM MUC contains on average 1, 2, 11 and 30 molecules MUC per molecule HSA, respectively. Amino acid analysis of HSA reacted with 10, 100 and 1000 μM MUC and reduced with $NaCNBH_3$ indicates that 3, 6 and 21 lysine residues are modified, respectively. These studies suggest that amino group modification of proteins by MUC could result in pyrroles, a structural moiety previously identified to be formed in the reaction of MUC with N-acetyl-l-lysine methyl ester, a model nucleophile for lysine in proteins. (Supported by NIH grant ES02558 from the National Institute of Environmental Health Sciences and NIEHS Center grant ES05022.)

742 NITRIC OXIDE (NO) INHIBITS HEME OXYGENASE-2 (HO-2) THROUGH BINDING TO HEME REGULATORY MOTIFS: HO-2 IS A SUICIDE SINK FOR NO.

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Heme oxygenase-2 (HO-2) is the constitutive cognate of HSP32 family. These proteins catalyze oxidative cleavage of heme to CO and biliverdin and release Fe. HO-2 is a hemoprotein that binds heme at "heme regulatory motifs" (HRMs) with a conserved Cys-Pro pair. Two copies of HRMs are present in HO-2. HRMs are neither present in HO-1 nor are they involved in HO-2 catalytic activity (McCoubrey et al., J Biol Chem 272, 12568, 1997). Presently we have examined spectrophotometrically, using NO donors, reactivity of the hemoprotein with NO and the effect of NO donors on activity of purified *E. coli* expressed HO-2. A red shift of Soret band (405 nm→ 415 nm) was observed when wild-type HO-2 was treated with sodium nitroprusside (SNP), S-nitroglutathione (GSNO), S-nitroso-N-acetylpenicillamine (SNAP), or 3-morpholinosydnonimine (SIN-1). Purified *E. coli* expressed mutant form of HO-2 (HO-2 mut), in which Cys264 and Cys281 of HRMs were substituted with Ala, did not exhibit a Soret band. Treatment of hematin with NO donors also caused similar red shift. SIN-1, in addition, decreased HO-2 Soret absorption, which correlated with a decrease in the pyridine hemochromogen absorption spectrum of the hemoprotein; the decrease was abolished in the presence of superoxide dismutase. The NO donors inhibited HO-2 activity and ranked: GSNO<SNAP≤ SIN<<SNP in potency to inhibit the enzyme. HO-1 or HO-2 mut were not inactivated by NO donors. Trapping of NO with hydroxocobalamin protected HO-2 against inactivation by NO donors. Also the inhibition was reversed partially by dithiothreitol. The decreased HO-2 activity appeared not related to disruption of HO-2 protein integrity, as suggested by Western blot analysis. Data suggest that inactivation of HO-2 requires HRMs and likely involves interation of NO with heme bound the motifs. We propose HO-2 is "suicide sink" for NO, and as such that this hemoprotein can control the level of both signaling molecules, CO and NO. (Supported by NIEHS ES03968, ES04391, ES001247 and ES07026.)

743 LIPIDS AND SERUM-DERIVED PROTEINS SEQUESTER NITRIC OXIDE.

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Nitric oxide is an important biological mediator, acting to regulate such diverse processes as neuronal transmission and programmed cell death. This reactive free radical is relatively short-lived in biological systems and its physiological effects are believed to be dependent on the immediate presence of specific target molecules. In macrophages nitric oxide is produced by the high-output cytokine-inducible isoform of the enzyme nitric oxide synthase (NOS2). Nitric oxide release from macrophages contributes to tumor cell cytostasis and non-specific host defense. Using a self-referencing nitric oxide sensor we found that RAW 264.7 macrophages release nitric oxide following prolonged treatment (12-24 hr) with gamma-interferon. Nitric oxide activity could be detected for 3-4 cell diameters. Following the

addition of cationic liposomes, or bovine serum albumin, activity of nitric oxide decreased 3-4 fold. These effects were rapidly reversed (<2 min) following removal of the trapping agents indicating that neither the liposomes nor the albumin altered NOS2 activity. Increased solubility of nitric oxide in the liposomes and albumin appears to account for the decrease in activity of nitric oxide. We speculate that the microenvironment surrounding activated macrophages is an important factor mediating the physiological effects of nitric oxide. (Supported by NIH grants ES 03647, ES 06897 and RR 01395.)

744 THE NOVEL TUMOR PROMOTER PALYTOXIN ACTIVATES P38 THROUGH DIFFERENT PROTEIN KINASE CASCADES IN COS7 AND HELA CELLS.

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We previously showed that the potent skin tumor promoter palytoxin activates c-Jun NH2-terminal kinase/stress-activated protein kinase (JNK) through an SEK1 (MKK4/JNKK)-dependent protein kinase cascade. SEK1 has also been implicated in the regulation of the stress-activated protein kinase p38. Therefore, we investigated whether palytoxin activates p38 through an SEK1-dependent pathway. Expression of a dominant negative mutant of SEK1 inhibited the activation of p38 by palytoxin in COS7 cells, but not in HeLa cells. The kinases MKK3 and MKK6 specifically phosphorylate and activate p38 but not JNK. Our results indicate that in COS7 cells, palytoxin activates SEK1 and MKK6 with kinetics that correspond to the activation of p38, whereas in HeLa cells palytoxin activates MKK3, in addition to SEK1 and MKK6. Finally, expression of a dominant negative mutant of Ras blocked activation of p38 by EGF in both COS7 and HeLa cells, but did not affect activation of p38 by palytoxin. Altogether, these results indicate that palytoxin activates p38 by both SEK1-dependent and SEK1-independent pathways. Furthermore, these studies indicate that the palytoxin-stimulated protein kinase cascades which couple to p38 differ depending on the cell type.

745 THE TEMPORAL EFFECTS OF THE DETOXIFICATION ENZYME INDUCER, BENZYL ISOTHIOCYANATE: ACTIVATION OF C-JUN N-TERMINAL KINASE PRIOR TO THE TRANSCRIPTION FACTORS AP-1 AND NFKB.

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Benzyl isothiocyanate (BIT), a microconstituent found in cruciferous vegetables, is known to be a potent inducer of the detoxification enzyme, NAD(P)H: quinone reductase (QR). QR catalyzes a two-electron transfer to a wide variety of quinones and other redox-cycling species, thereby preventing the mutation of DNA and reducing cancer risk. The 5' promoter region of the human QR gene contains the *cis*-acting AP-1 and NFkB transcription factor binding sites. The upstream signaling mechanisms that lead to the induction of QR through this promoter region remain unclear. When HT29 human colon cells were exposed to 25μM BIT, a physiologically relevant dose, AP-1 binding increased, first at 3 hours and again at 16 hours indicating the possibility of increased *de novo* c-Jun synthesis at the later time point as a result of the initial activation of JNK. NFkB binding also increased with a narrow peak around 6 hours. BIT also activated c-Jun N-terminal kinase (JNK), which phosphorylates and transactivates c-Jun, a component of AP-1. JNK was activated 6-fold over controls, beginning at 60 minutes and continuing through 8 hours. The temporal sequence of these events, JNK activation, followed by increased AP-1 and NFkB binding, resulting in increased QR activity, supports the premise that JNK is involved in the induction of QR. (Supported by the Cancer Research Foundation.)

746 AH RECEPTOR AND NF-κB INTERACTION: FUNCTIONAL DOMAINS INVOLVED IN THE CROSS-MODULATION.

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The Ah receptor (AhR) mediates many of the toxic responses induced by polyhalogenated and polycyclic aromatic hydrocarbons (PAHs) which are ubiquitous environmental contaminants causing toxic responses in human and wildlife. NF-κB is a pleiotropic transcription factor controlling many physiological functions adversely affected by PAHs, including immune suppression, thymus involution, hyperkeratosis and carcinogenesis. We have recently shown physical interaction and mutual functional repression between AhR and NF-κB. These interactions connect the two important signal transduction pathways and may provide an underlying mechanism for many hitherto poorly understood dioxin-induced toxic responses. In this study, by using transient transfection and deletion mutagenesis, we demonstrated that the transactivation domains of AhR is responsible for the observed suppression of the activity of NF-κB RelA, while the bHLH-PAS domain alone is ineffective in the suppression. Reciprocally, the activation domain of the NF-κB RelA is essential in suppression of activity of DRE-driven reporter gene (pGudLuk 6.1). Interestingly, while the full length of the NF-κB RelA markedly suppresses the activity of dioxin-induced pGudLuk 6.1 in Hepa1c1c7 cells, expressing the Rel homology domain (RHD) of the NF-κB RelA superinduces the activity of pGudLuk 6.1. The results of this study defined the functional domains that are involved in the AhR/ NF-κB interaction. Supported in part by NIEHS Center Grant #ES05002.

747 REGULATION OF CONSTITUTIVE GENE EXPRESSION THROUGH AhR COMPLEX - Sp1 PROTEIN INTERACTIONS.

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The -145 to -119 region (CD/L) of the cathepsin D gene promoter contains a GC-rich motif that binds Sp1 protein and an adjacent pentanucleotide (CACGC) corresponding to the core sequence of a dioxin responsive element (DRE) that binds the aryl hydrocarbon receptor (AhR)/AhR nuclear translocator (Arnt) complex. This Sp1$(N)_4$DRE (core) motif has been identified in promoters of several genes in which Sp1 plays an important role in basal gene expression. In transient transfection assays in MCF-7 human breast cancer cells using wild-type pCD/L and constructs mutated in the core DRE (pCD/L_{m1}) and Sp1 (pCD/L_{m2}) sites, it was shown that both motifs were required for maximal basal activity. The requirements for AhR/Arnt interactions with Sp1 protein for maximal activity of pCD/L was confirmed in wild-type MCF-7 and Hepa 1c1c7 cells and Arnt-deficient Hepa 1c1c7 cells using antisense Arnt and Arnt expression plasmids. The functional interactions of Sp1-AhR/Arnt were paralleled by physical interactions showing that AhR/Arnt and Sp1 proteins were coimmunoprecipitated and AhR/Arnt enhanced Sp1-[^{32}P]CD/L binding in electrophoretic mobility shift assays. The physical and functional interactions of Sp1 with AhR/Arnt proteins bound to the Sp1$(N)_4$DRE(core) motif were also dependent on proximity of these sites and both activity and Sp1-DNA binding decreased as the number of intervening nucleotides increased from 4 to 20. These studies show that regulation of basal expression of some genes by Sp1 may also require interactions with AhR/Arnt. (NIH ES04176)

748 SELECTIVE INDUCTION OF SP1 DNA ACTIVITY IN THE MOUSE BRAIN BY METHAMPHETAMINE.

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Methamphetamine, a derivative of amphetamine is a CNS stimulatory drug, that is widely abused. Repeated administration of methamphetamine causes long lasting depletion of dopamine, a decrease in the number of dopamine transport pumps, a decrease in the activity of tyrosine hydroxylase and degeneration of dopaminergic nerve terminals. These long lasting changes by methamphetamine may be mediated through alterations in gene expression. In an effort to elucidate the mechanisms of methamphetamine toxicity, we examined the effect of methamphetamine on a series of transcription factors: SP1, AP1 and NFkappaB. Mice were administered (i.p.) four repeated doses of methamphetamine (2.5, 5.0, and 10.0 mg/kg) at 2 hour intervals and sacrificed 48 hrs. later. The frontal cortex, caudate nucleus and hippocampus were dissected and analyzed for SP1, AP1 and NfkappaB using the electrophoretic mobility shift assay (EMSA). SP1 DNA-binding was induced in all the brain regions examined and at all doses tested. The greatest induction was observed at the 2.5 mg/kg dose. The pattern of AP1 and NFkB DNA-binding was not significantly altered. SP1 is a member of the zinc finger family of transcription factors that is involved in eukaryotic gene regulation and is induced by various stimuli including differentiation and growth signals. This data suggest SP1 may play a role in the long term cellular response to metamphetamine-induced insults. (Funded by Grant from NIEHS #521140.)

749 ROLE OF HISTONE TAIL MOBILITY IN DNA REPAIR.

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In eukaryotes, DNA is complexed with histone proteins to form nucleosomes and the DNA repair machinery must deal with DNA in this context. Since the N-terminal tails of core histones are positively charged and interact with the negatively charged backbone of the DNA, it is important to determine the energy of these DNA/histone interactions in order to understand how repair enzymes overcome these forces. To study this we used H2A mutated to cysteine at position 2. After covalently attaching a spin label through the sulfhydryl of the Cys, H2A tail mobility was observed at several temperature and at 250, 150, 50 and 0.05 mM NaCl. Under low salt and low temperatures the spin label was relatively immobile indicating that the tail spent most of its time complexed with the DNA. As the salt concentration and temperature increased, the level of mobility increased so that at physiological conditions the tails were poised for dissociation. These results suggest that access of repair enzymes to DNA damage sites may depend strongly on whether the alterations in DNA structure strenghthens or weaken the interactions with core histones. (Supported by US DOE contract DE-ACO6-76RLO 1830.)

750 EFFECT OF A SINGLE UV-INDUCED LESION ON DNA REPLICATION IN EXTRACTS OF NORMAL AND CANCER-PRONE HUMAN MUTANT CELLS.

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The conversion of unrepaired chemical and environmental DNA damage to mutations provides the initiating step in many cancers. To understand the biochemical and genetic basis of this process, we have investigated the human skin cancer-prone disease Xeroderma Pigmentosum (XP) Variant. Unlike XP groups A-G, removal of UV-induced DNA damage by the nucleotide excision repair system is normal in XPV cells. XPV is characterized by altered DNA replication and hypermutability after UV irradiation. To investigate the molecular basis of this phenotype, we use cell-free extracts to carry out replication of a set of double-stranded plasmids containing a single pyrimidine dimer or (6-4) photoproduct, on either the leading or lagging strand. Compared to normal extracts, XPV extracts have a reduced capacity to carry out complete replication of DNA containing either lesion, particularly if the lesion is on the leading strand. Both extracts retain some lesion-bypass capacity *in vitro*, but compared to normal extracts, bypass of a pyrimidine dimer is reduced in XPV extracts. The proportion of newly synthesized DNA molecules arrested at the lesion site is increased in XPV extracts, directly demonstrating that XPV extracts have a defect in replication past both major UV-induced lesions, in particular when the lesion is on the leading strand. The present approach should allow further characterization of the proteins involved in the conversion of DNA damage to mutations in human cells. (Supported by ACS-IRG-189 and NIH R29 ES08073-01 to MPC.)

751 THE INVOLVEMENT OF THE ESTROGEN RECEPTOR IN THE DIFFERENTIAL EXPRESSION OF METALLOTHIONEIN-1E IN ESTROGEN RECEPTOR-POSITIVE AND -NEGATIVE HUMAN BREAST CANCER CELL LINES.

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Several studies have shown a correlation between immunohistochemically determined metallothionein (MT) expression and disease prognosis in breast cancer. Because these methods do not discriminate between the large number of MT isoforms found in humans, the prognostic value of metallothionein detection could be increased if expression could be correlated to specific metallothionein gene products. As an initial step in determining the possible significance of MT isoform gene expression in human breast cancer, MT-1E was found to be expressed preferentially in estrogen receptor-negative breast cancer cell lines and not expressed in estrogen receptor-positive cell lines as determined by RT-PCR. In order to assess the possible involvement of the estrogen receptor in the expression pattern of MT-1E, the total MT isoform expression pattern was determined in an estrogen receptor negative cell line (MDA-MB-468) that was transfected with the estrogen receptor gene. MT expression was not altered by the presence of the estrogen receptor in this cell line, indicating that the estrogen receptor does not directly influence the expression of MT-1E in breast cancer cell lines.

752 MECHANISM OF ESTROGEN-MEDIATED INDUCTION OF DNA POLYMERASE GENE EXPRESSION IN MCF-7 HUMAN BREAST CANCER CELLS.

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17β -Estradiol (E2) induces DNA synthesis and proliferation of MCF-7 human breast cancer cells and the molecular mechanism of DNA polymerase α induction by E2 has been investigated by promoter analysis. pDNAP1, pDNAP2 and pDNAP3 are constructs containing -258/+1, -120/+1, and -66/+1 bp inserts from the DNA polyerase α gene promoter linked to a luciferase reporter gene; 10 nM E2 induced luciferase activity in MCF-7 cells transiently transfected with pDNAP1 and pDNAP2 (>4-fold), whereas no induction was observed with pDNAP3. These results suggested that enhancer elements required for E2-responsiveness were upstream from -66 and further analysis demonstrated that the -147 to -100 region of the promoter (pDNAP4) were E2-responsive. This region of the promoter contains an upstream estrogen responsive element half-site (ERE½, -147) and downstream E2F (-135) and GC-rich (-106) sites. Mutational analysis of each site showed that the GC-rich site was required for E2-induced transactivation and gel electrophoretic mobility shift assays confirmed that Sp1 protein bound to this region of the promoter. DNA polymerase α represents another E2-responsive gene induced via ER/Sp1 interaction, and current studies are focused on aryl hydrocarbon receptor-mediated inhibition of the hormone-induced transactivation. (Welch Foundation and NIH CA76636)

753 MOLECULAR ANALYSIS OF ESTROGEN RECEPTOR α (ERα) AND ERβ-Sp1-MEDIATED TRANSACTIVATION: ROLE OF ACTIVATION FUNCTION 1.

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Estrogen receptor α (ERα) and ERβ physically interact with the nuclear Sp1 protein and bind GC-rich enhancer sequences; however, only ERα/Sp1 results in transactivation after treatment with 17β -estradiol (E2). Human ERα and ERβ proteins differ primarily in the activation function (AF-1) domain where there is only 27% amino acid identity. Differences in ERα/Sp1 and ERβ/Sp1 action were investigated using chimeric proteins that interchange the AF-1 domains; hERα/β contains the AF-1 domain of hERα and the DNA binding and ligand binding domains of hERβ and hERβ/α contains the AF-1 domain of hERβ and the DNA binding and ligand binding domains of hERα. In GST-pulldown assays, both chimeric proteins interact with the transcription factor Sp1 and in gel shift assays, both chimeric proteins bind estrogen responsive element (ERE) and enhance Sp1 binding to a GC-rich motif. In MDA-MB-231 human breast cancer cells, the chimeric proteins induced chloramphenicol acetyltransferase (CAT) activity from an ERE-CAT reporter construct after treatment with E2. E2 also induced CAT activity (3-fold) in cells transiently transfected with hERα/β or ERα and an Sp1-CAT reporter construct; however, hERα/β or hERβ were inactive. Similar results were observed in other cell lines (LnCaP, MCF-7 and Ishikawa) suggesting that the AF-1 domain of ERα is required for E2-induced transactivation via ER/Sp1 interactions at GC-rich elements. (Welch Foundation and NIH CA76636)

754 MOLECULAR CLONING OF THE REPTILIAN, *ANOLIS CAROLINENSIS*, ESTROGEN RECEPTOR LIGAND BINDING DOMAIN: COMPARATIVE AMINO ACID SEQUENCE ANALYSIS TO KNOWN ESTROGEN RECEPTORS.

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The estrogen receptor (ER) ligand binding domain (LBD; D, E and F domains) of the anole, *Anolis carolinensis*, was cloned using degenerate PCR primers coupled with the 3' RACE (rapid amplification of cDNA ends) strategy. Two forward degenerate PCR primers complementary to the DNA binding domain (C domain) and a reverse degenerate PCR primer complementary to a highly conserved region of the E domain were derived from a consensus sequence generated from a multiple species sequence alignment of 10 known ER sequences. Using successive rounds of PCR, an 800 bp fragment representing approximately 75% of the region of interest was

amplified and sequenced. Based on this sequence, anole ER specific primers were generated and used in conjunction with the oligo dT primer to clone the remaining 3' end of the cDNA. The ER LBD amino acid sequence of the anole exhibits a high degree of sequence identity (79%) and similarity (83%) when compared to the human ERα, as determined from sequence comparisons using the GCG suite of programs. The boundaries of the individual D, E and F domains for the anole ER were determined from a primary sequence alignment to the human ERα LBD. The E domain displayed the highest degree of sequence identity (89%) when compared to the human ERα, followed by the D (57%) and F (32%) domains. Relative to other species, the anole ER LBD was intermediate in terms of its sequence identity when compared to the human α (100%), mouse α (90%), chicken (82%), Xenopus (70%) and rainbow trout (40%) ER LBDs. This is the first complete reptilian ER LBD sequence reported and will be used to prepare recombinant chimeric anole ER LBD proteins to identify and assess chemicals and complex mixtures for estrogenic activity that may be specific to reptilian species

755 MUTATIONAL ANALYSIS OF ALU SEQUENCES IN DES-INDUCED HAMSTER KIDNEY TUMORS.

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Diethylstilbestrol (DES) is a renal carcinogen. The mechanism by which DES causes cancer is not known . In this study we have examined the mutations in cancer cells genome by RAPD compared to control cells . Alu repeat is one of the most common repeat element in the human as well as in hamsters genome . In an effort to examine the status of Alu sequence in DES - induced hamster kidney tumor, genomic DNA isolated from 15 kidney tumors and age- matched control kidney were digested with Alu I restriction enzyme . Kidney tumors were induced by subcutaneous implant of DES . After 7 months of treatment animals were killed to excise the kidney . Amplifications were performed using random primer with the digested as well as undigested DNA . Amplification from undigested DNA did not reveal any difference between control and tumors. Amplifications of Alu digest using random primer revealed that there was a loss of 0.7 kb and gain of 0.4 kb fragments in tumor DNA compared to genome from control .Finding of this study suggest that DES induced mutation in the Alu sequences may be involved in the induction of tumor.

756 11-DEOXY-16,16-DIMETHYL PROSTAGLANDIN E2 PROTECTS AGAINST RENAL TOXICITY IN RATS AND INDUCES SPECIFIC PROTEINS VIA A PUTATIVE THROMBOXANE RECEPTOR IN LLC-PK1 CELLS.

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Prostaglandin E2 and 11-deoxy-16,16-dimethyl prostaglandin E2 (DDM-PGE2) protect LLC-PK1 cells against 2,3,5-*tris*-(glutathion-*S*-yl)hydroquinone (TGHQ)-induced cytotoxicity via a PKC-coupled receptor that exhibits properties different from those of currently classified PGE receptor (EP) subtypes. The ability of the thromboxane agonist, U-46619, to protect LLC-PK1 cells against chemical insult and the antagonism of this response by the thromboxane antagonist, SQ29,548, suggests that DDM-PGE2 may act via a putative thromboxane receptor. DDM-PGE2 cytoprotection is associated with the increased synthesis of a number of proteins, the most prominent being a ~230 KD protein. U-46619 also induces synthesis of the same ~230 KD protein. Using 35S-methionine labeling, SQ29,548 (1 M) cotreatment completely blocks induction of the ~230 KD protein following DDM-PGE2 (1 M; 24 hr) exposure, consistent with a thromboxane-like receptor-mediated response. To validate the *in vitro* model, the effects of DDM-PGE2 on TGHQ-mediated nephrotoxicity in Fischer 344 rats was investigated. Administration of DDM-PGE2 (75 g/kg, s.c.) 24 and 0.5 hr prior to, and 6 hr after, TGHQ (5 mol/kg, i.p.) decreased TGHQ-mediated elevations in the urinary excretion of glutathione *S*-transferase and glucose by 51% and 56%, respectively, 18 hr after TGHQ treatment. These results suggest that DDM-PGE2 possesses cytoprotective properties *in vivo* and that cytoprotection *in vitro* involves the participation of a thromboxane-like receptor. (GM56321, ES07247).

757 EXTENSIVE DEGRADATION OF DNA IN NUCLEAR EXTRACTS PREPARED FROM ATAXIA TELANGIECTASIA CELLS MAY BE REPONSIBLE FOR THE DECREASE OF THE FIDELITY OF DOUBLE-STRAND BREAK REPAIR IN THIS CELL LINE.

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Ataxia telangiectasia (A-T) is a human genetic disorder characterized by neurodegeneration, hypersensitivity to ionizing radiation (IR) and increased cancer risk. Cultured fibroblasts and lymphoblasts from A-T patients exhibit a normal rate of repair of IR-induced DNA double-strand breaks (DSB), despite their sensitivity to killing by IR. However, several papers have reported that in A-T cells the fidelity of DSB repair was decreased leading to an increase in the frequency of IR-induced deletion mutations. The underlying mechanism is unclear. We have established an in vitro DSB repair system in order to characterize the defect in DSB repair in A-T cells. We have used the PUC18 vector cut by EcoRI within the lacZ gene to evaluate the DSB-repair efficiency and fidelity of nuclear extracts. The repair efficiency was evaluated by the restoration of the transformation capacity of the vector and the fidelity of break rejoining was measured by the frequency of lacZa mutants. We found that the frequency of lacZa mutants was more than two-fold higher when the vector which is cut by EcoRI and then treated with phosphatase to remove 5' phosphate was incubated in extracts from A-T compared to extracts from normal cells. Southern blotting of DNA products generated during incubation of the cut DNA in cells extracts revealed more extensive degradation of the vector DNA in A-T extracts. These data suggest that A-T cells are defective in protecting DSB ends from nuclease digestion.

758 TELOMERES AND RECOMBINATION: END GAMES.

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Sponsor: B Lehnert.

Telomeres are specialized structures at the ends of linear eukaryotic chromosomes composed of tandom arrays of repetitive DNA sequence plus associated proteins. They protect chromosomal ends against exonucleolytic attack and preserve genomic stability by preventing undesired end-joining reactions. Paradoxically, the vertebrate $(TTAGGG)_n$ sequence is reportedly a hotspot for certain types of genetic recombination. Furthermore, DNA double-strand break repair proteins bind to telomeres where they are thought to limit illicit acts of recombination - a proposed role directly opposed to their known function in non-homologous end joining. We are employing a strand-specific fluorescence *in situ* hybridization technique (CO-FISH) to visualize recombination within telomeric repeats as a means of quantifying this activity. Preliminary evidence indicates that telomeres are indeed dynamic structures showing recombination rates 2 - 3 orders of magnitude above the genomic average. Through the use of DNA repair mutants, we plan to examine the impact of repair genes on telomeric recombination. Other studies will determine whether interstitial repeats are also recombination hotspots, and whether this behavior is confined to telomeric sequences or is a general property of repetitive sequences. It is hoped that these studies will help define the rules governing the paradoxical behavior of chromosome ends.

759 AN INVESTIGATION OF THE STRUCTURAL REQUIREMENTS FOR EFFICACY AND MECHANISM OF ACTION OF A SHORT TELOMERE MIMETIC PHOSPHOROTHIOATE OLIGODEOXYNUCLEOTIDE (S-ODN).

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A structure activity study was performed on a series of short telomere mimetic S-ODNs. These compounds were able to inhibit the telomerase enzyme and were lethal to OMA-BL1 (Burkitt's Lymphoma) cells. A cytotoxicity assay was used to screen for active compounds based on length and reading frame. It was found that shorter compounds (6-9 bases) were almost as efficacious, but exhibited a more dose dependent relationship than longer compounds (10-24 bases). Studies to evaluate the influence of reading frame on cytotoxicity concluded 5'-d(GGGTTAGGG)-3' (TAG 9-2) was the most effective reading frame. This compound was then used to test the contribution of hydrogen bonding and the 3' end to cytotoxicity and telomerase inhi-

bition. Blocking hydrogen bonding decreased the efficacy of the compound in both the cytotoxicity assay and the Telomerase Repeat Amplification Protocol (TRAP) assay, a measure of telomerase activity. The 3' end of this compound was blocked by the addition of a glycerol molecule during synthesis, as before this compound was less efficacious in both the cytotoxicity assay and the TRAP assay. The putative mechanism of cytotoxicity was investigated for the TAG 9-2 compound. The telomeric regions of chromosomes contain 50% guanine nucleotides on the leading strand, making them an ideal target for reactive oxygen species (ROS). The interaction of ROS with the telomere could generate a short single stranded fragment having similar properties to the TAG 9-2 compound. The endogenous telomeric fragments may be signals to the cell of oxidative stress. We hypothesize that the TAG 9-2 compound is mimicking these fragments and inducing apoptotic pathways. To test this hypothesis we have treated OMA-BL1 cells with radical generating compounds and then performed a fragment capture assay to detect the endogenous fragments. We were also able to abrogate the generation of fragments by concurent treatment with radical scavengers. In these studies we have investigated the stuctural requirements for activity and also a putative mechanism of action for short, telomere mimetic, S-ODNS.

760 A NOVEL ASSAY FOR MITOCHONDRIAL DNA SYNTHESIS.

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Mitochondria are a target organelle for a large number of toxic chemicals, and as such parameters of mitochondrial number, respiration, and membrane integrity are often examined to investigate mechanism of toxicity. Mitochondria also possess their own genome and unique RNA transcription, protein translation, and DNA replication activities. We have developed a simple assay that allows quantitative measurement of mitochondrial DNA synthesis, especially in response to toxic events. The assay takes advantage of the large discrepancy in size between the circular mitochondrial genome (16,000 base pairs) and mammalian chromosomes (several million base pairs) and makes use of methodology developed for analysis of chromosomal structure. Control and treated cells are allowed to incorporate radiolabeled thymidine, then are harvested and embedded in agarose plugs. The plugs serve to protect genomic DNA from mechanical shear in subsequent steps. After a detergent and proteinase digestion step, the plugs are embedded in a low percentage agarose gel and the mitochondrial DNA separated from genomic DNA by electrophoresis. Using this technique chloramphenicol was found to specifically inhibit mitochondrial DNA synthesis in H4IIe rat liver hepatoma cells in a dose-dependent fashion, while ethidium bromide at low concentrations appeared to increase mitochondrial DNA synthesis.

761 MULTIPLEX PCR COUPLED WITH RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) AS A NOVEL METHOD TO DETECT MITOCHONDRIAL DNA POLYMORPHISMS IN HUMAN.

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Recently, the search for mitochondrial DNA (mtDNA) defects in tumor tissues has drawn attention. Most studies have used PCR or multiplex PCR to detect the mutation of mtDNA. The sensitivity of these methods is limited. In the present study, we have developed an effective multiplex PCR/RAPD method to detect the polymorphims of mtDNA in human tissues. Total DNA were isolated from tumor tissues and normal tissues. Using specific primers to the mitochondrial genome, multiple fragments were amplified. Multiplex PCR did not reveal any mutation in the mitochondrial genome of tumor tissues compared to controls. The fragments of multiplex PCR products were then eluted from agarose gel and used as template for RAPD analysis. The profile of amplification using random primers in tumor tissues was significant different from normal tissues. In summary, the multiplex PCR/RAPD revealed mutation in mitochondrial genome, but not multiplex PCR alone. This method may be useful in detecting mutation in both mitochondrial and nuclear genomes from cancer and age-related disorders.

762 DISRUPTION OF THE E-CADHERIN/CATENIN COMPLEX BY OXIDATIVE STRESS CORRELATES WITH PHOSPHORYLATION OF TYROSINE BUT NOT SERINE.

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E-cadherin, the predominate mediator of cell-cell adhesion in epithelium, regulates adhesion via a Ca^{++}-dependent, homophilic mechanism. β-catenin binds the cytoplasmic domain of E-cadherin and is in turn bound by α-catenin, which links the complex to the actin cytoskeleton. E-cadherin function is regulated by post-translational modification. Therefore, we hypothesized that chemically-induced oxidative stress would disrupt the E-cadherin/catenin complex in precision-cut mouse liver slices via phosphorylation. Diamide (DA), a sulfhydryl depleting agent, and tert-butylhydroperoxide (tBHP) disrupted the protein interactions β-catenin with its adhesion partners. Although a significant decrease in β-catenin:α-catenin interactions was observed, the binding of β-catenin to E-cadherin was also affected. This disruption was associated with a significant increase in the tyrosine phosphorylation of β-catenin. However, no effect on the serine phosphorylation of this protein was observed. Taken together, these results suggest that chemically-induced oxidative activates tyrosine kinases, or inhibits tyrosine phosphatases, that regulate the post-translational modification of β-catenin. Importantly this is associated with a loss of biochemical function of the E-cadherin complex. (Supported by NIEHS Center Grant (T30 ES06694), American Cancer Society Institutional Grant (IRG110T) and an SOT-Colgate Palmolive Fellowship.)

763 TAU PHOSPHORYLATION BY DIISOPROPYL PHOSPHOROFLUORIDATE (DFP)-TREATED HEN BRAIN SUPERNATANT INHIBITS ITS BINDING WITH MICROTUBULES: ROLE OF Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II IN TAU PHOSPHORYLATION.

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Diisopropyl phosphorofluoridate (DFP) produces organophosphorus ester-induced delayed neurotoxicity (OPIDN) in hen, human, and other sensitive species. This is characterized by mild ataxia which progresses to severe ataxia or paralysis in a few days. Ultrastructurally, OPIDN is associated with the degeneration of axons in central and peripheral nervous system. Bacterially expressed longest human tau protein (htau40) was phosphorylated by DFP-treated hen brain supernatant in a shorter time than control brain supernatant to show significant decrease in microtubule binding. The decrease in htau40-microtubule binding observed on phosphorylation of htau40 by recombinant. Ca^{2+}/calmodulin (CaM)-dependent protein kinase II (CaM kinase II) α-subunit showed that CaM kinase II present in brain supernatant could participate in tau phosphorylation even in the absence of Ca^{2+}/CaM and decrease tau-microtubule binding. In addition, use of htau40 mutants, htau40ml (Ala^{416}) and htau40m6 (Asp^{416}), showed that replacement of Ser^{416} by neutral or acidic amino acid produced change in htau40 conformation and exhibited lesser binding with microtubules on phosphorylation by brain supernatant in the presence of EGTA. The change in conformation produced by Ser^{416} phosphorylation, however, was different from that produced by mutants in that only htau40 isoform showed significant decrease in binding with microtubule on phosphorylation by recombinant CaM kinase II in the presence of Ca^{2+}/CaM compared to that obtained by phosphorylation in the presence of EGTA. This study showed that enhanced Ca^{2+}/CaM-dependent protein kinase activity in DFP-treated hen brain supernatant may cause decreased tau-microtubule binding and destabilization of microtubules, leading to axonal degeneration in OPIDN. (Supported in part by NIEHS Grant ESO 5154.)

764 THE ROLE OF cAMP SIGNALING ON INHIBITION OF IL-2, CREB, AND NF-κB BY CANNABINOL.

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Ligand binding to CB1 or CB2 cannabinoid receptors inhibits the cAMP signaling pathway in splenocytes and thymocytes. Cannabinol (CBN), a CB2 receptor selective ligand, has been shown to inhibit IL-2 expression and the activation of CREB and NF-κB following stimulation of thymocytes with phorbol ester plus calcium ionophore (PMA/Io). Cross-talk between PKA and PKC has been previously demonstrated by the potentiation of the cAMP pathway following PKC activation. Therefore, the objective of the present

studies was to determine the role of cAMP and PKA in the regulation of IL-2, CREB and NF-κB in PMA/Io activated thymocytes. Dibutyryl cAMP (DBcAMP), a cAMP analog, was used to examine the involvement of cAMP. Stimulation of thymocytes with DBcAMP (10 or 100 M) for 60 min induced CREB phosphorylation. However, the inhibition of CREB phosphorylation produced by CBN in PMA/Io stimulated thymocytes could not be reversed by co-stimulation with DBcAMP (100 M). DBcAMP was also not able to reverse the CBN-mediated inhibition of CRE or κB DNA binding activity. Furthermore, DBcAMP (1 or 5 M) failed to reverse the dose-dependent inhibition of IL-2 protein secretion by CBN. To investigate the role of PKA in the inhibition produced by CBN, the PKA inhibitor H89 was used. Induction of CREB phosphorylation and CRE binding by DBcAMP was inhibited by H89 (50 or 500 nM). Pretreatment of thymocytes with H89 (10-500 nM) produced minimal inhibition of PMA/Io-induced CREB phosphorylation and CRE binding activity. H89 had no effect on protein binding to a κB motif and did not prevent the degradation of IκB-α. Additionally, a modest inhibition of IL-2 protein secretion was detected with H89 at 50 and 100 nM. These results support a modest involvement of the cAMP/PKA pathway in CREB activation and IL-2 regulation following PMA/Io stimulation of thymocytes and further suggest that ligand binding to the CB2 receptor modulates additional signaling pathways in activated T-cells. (This work was supported by NIDA Grants DA07908 and DA09171.)

765 PIVOTAL ROLE OF MITOCHONDRIA IN ARSENIC ACID INDUCED INTERLEUKIN-1α PRODUCTION IN MURINE KERATINOCYTES.

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In humans, inorganic arsenicals are skin carcinogens and induce hyperproliferation and hyperkeratosis of the palms and soles and hyperpigmentation/hypopigmentation of the trunk. It has been recently demonstrated that arsenic induces overexpression of keratinocyte-derived growth factors, which are likely to play a significant role in arsenic-induced skin hyperkeratoses and cancer. The mechanism(s) involved in this induction are, however, still elusive. The purpose of this study was to investigate the early intracellular events that follow in vitro treatment with arsenic acid in a murine keratinocyte cell line (HEL30) and that leads to interleukin-1α (IL-1α) overproduction. First, we observed that sodium arsenate (As) induced a dose dependent IL-1α production both intracellular and released, at concentrations as low as 2 μM. Increased intracellular IL-1α could be detected as early as 1 h following treatment. Electron microscopy analysis revealed that As-induced a drammatic alteration in mitochondria, which could be prevented by rotenone pretreatment, suggesting a possible role of mitochondria-derived reactive oxygen species. Arsenic indeed induced a dose and time dependent increase in cellular oxidative activity, which is followed by activation of redox sensitive transcription factors, such as NF-κB and AP-1, essential for IL-1α neosynthesis. Pretreatment with rotenone or prolonged treatment with ethidium bromide, an inhibitor of mitochondrial DNA and RNA synthesis, to deplete cells from functional mitochondria, completely abrogated As-induced IL-1α production, indicating the pivotal role of these organelles in As-induced keratinocyte growth factors. A role of calcium could be ruled out since BAPTA, a calcium chelator, did not affect As-induced reactive oxygen species or IL-1α production, indicating that the first intracellular event following As treatment is the binding at the mitochondria followed by ROS generation, transcription factors activation and finally IL-1α neosynthesis. (Partially funded by UNIPRO.)

766 INDUCTION OF THE MITOCHONDRIAL PERMEABILITY TRANSITION ALTERS THE DISTRIBUTION OF F-ACTIN AND MITOCHONDRIA IN L929 FIBROBLASTS.

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Treatment of L929 fibroblasts with tumor necrosis factor (TNF) and cycloheximide (CHX) induces the mitochondrial permeability transition (MPT), thereby causing the death of the cells. The MPT and the cell killing are prevented by pretreatment with cyclosporin A (CyA) and aristolochic acid (ArA). The current study was designed to study changes in cellular organization that could link the MPT to cell death. L929 cells exhibit a fine meshwork of short F-actin fibers. With TNF and CHX after six hours, there was less fibrillar actin in the cytoplasm and more at the cell periphery. CyA and ArA prevented these alterations. With the mitochondrion-specific dye MitoTracker Green, untreated cells displayed diffuse staining that extended throughout the cytoplasm, although not always to the cell periphery. After four hours with TNF and CHX, there was obvious translocation of the mitochondria to the perinuclear region. The staining also became more punctate, suggesting that the mitochondria are clumping. CyA plus ArA did not prevent this redistribution of mitochondria. We hypothesize that the MPT interferes with a crucial interaction between the mitochondria and the cytoskeleton, leading to a disruption of cytoskeletal architecture. Loss of viability due to induction of the MPT, as evidenced by loss of the permeability barrier function of the plasma membrane, could result from alterations in the structure of the membrane due to loss of the interaction with the cytoskeleton.

767 EFFECT OF VOMITOXIN (DEOXYNIVALENOL) ON THE BINDING ACTIVITY OF A NEGATIVE TRANSCRIPTION FACTOR FOR IL-2 BY IN MURINE EL-4 THYMOMA CELLS.

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The gene expression of IL-2 is superinduced by the trichothecene mycotoxin vomitoxin (VT, deoxynivalenol) in murine T-cells. It has been recently reported that negative regulatory regulatory element (NRE) plays a important role in the silencing of IL-2 expression. Here, the *in vitro* effect of VT on NRE binding activity was studied using EL-4 thymoma cell by electrophoretic mobility shift assay. When cells were treated with phorbol 12-mystate 13-acetate plus ionomycin, NRE binding activity was reduced. Co-treatment of VT caused synergistic reduction of the NRE binding activity in a dose dependent manner. The inhibition of NRE binding activity by VT was shown from 2 hr to 48 hr. The inhibition of the NRE binding activity by VT alone was also found in unstimulated cells. Interestingly, the inhibition of NRE binding activity was shown only at the slower migrating band which has been known to be more sensitive to acid phosphatase than the faster migrating band. These data suggest that IL-2 superinduction by VT and other trichothecenes might be caused in part, through inhibition of the binding activity of a negative transcription factor for IL-2.

768 AKT PHOSPHORYLATION OF p47^{PHOX} PRODUCES ACTIVIATION OF THE NADPH OXIDASE IN A CELL-FREE SYSTEM COMPARABLE TO AMPIPHILE ACTIVATION.

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The leukocyte NADPH oxidase catalyzes the 1-electron reduction of oxygen to superoxide at the expense of NADPH. Extensive phosphorylation of p47^{PHOX} has been demonstrated to occur during the assembly and activation of the oxidase. While expression of various serine mutants of p47^{PHOX} no longer support oxidase activity in the whole cell, they are still functional in the amphiphile-activated system. This suggests that activation of the NADPH oxidase is a phosphorylation dependent event. Through the use of kinase inhibitors, the PKC and PI-3 kinase pathways have been implicated in the activation of the oxidase. While p47^{PHOX} phosphorylated with PKC is capable activating the oxidase in a cell free system, the rate is not of the same amplitude as the amphiphile-activated system. Although phosphorylation of p47^{PHOX} has been observed during activation of PI-3 kinase, we have found p47^{PHOX} not to be a direct target. We investigated the activation of p47^{PHOX} by Akt, a direct target of PI-3 kinase. Akt phosphorylated p47^{PHOX} supports NADPH oxidase activity of equal amplitude to amphiphile activation. Fewer serines are phosphorylated by Akt further suggesting that several serines act to restrict activity of p47^{PHOX}. Akt activation of p47^{PHOX} demonstrates the first kinase dependent activation of NADPH oxidase comparable to amphiphile-activation of the oxidase.

769 CONSTRUCTION AND CHARACTERIZATION OF A COLITOSE NEGATIVE MUTANT IN *V. CHOLERAE* 0139.

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Sponsor: K S Squibb.

Vibrio cholerae secretes several major toxins, the most important is cholera toxin that elicits fulminant diarrhea in its hosts. In 1992 a new strain appeared which had only two major differences from any other epidemic-causing strain. It was not an 01 strain, and had a capsular polysaccharide (CPS) encoded by 36 kb of novel DNA. It was designated serotype 0139

Bengal. In bacteria, polysaccharides are considered essential virulence determinants and antigens that play important roles in the pathogenicity of the organism. Our lab has determined the chemical and 3 dimensional structure of the CPS, and has sequenced the entire 36 kb. capsular cassette. The capsule region has 35 open reading frames (orfs), many of which have homology to biosynthesis and processing genes in other bacteria. The capsule structure is composed of six sugars, four of which look very similar to Lewis B blood group tetrasaccharide. In particular colitose, an unusual sugar, appears to be encoded by orfs *wbfJ, H, I*, is known to play an immunodominant role in polysaccharides and is found in other gram-negative bacteria such as *E. coli* 011, 055 and several *Salmonella ssp*. The colitose residues in 0139 are found on the sides of the polysaccharide structure and deletion of them is not believed to affect the integrity of the polymer. In order to elucidate if colitose is an immunologically important epitope of the CPS, the *wbfJ, H, I* genes were genetically deleted by allelic exchange. A 4509bp fragment was amplified by PCR and cloned into pUC18. By restriction digests and re-ligation, the above orfs were replaced with an in-frame 840bp kanamycin cassette that lacked a promoter. This construct in pCVD442 was mated into *V. cholerae* 0139. Kanamycin selection allowed for screening of positive clones and PCR and sequencing were used for confirmation. The colitose negative mutant can now be used to determine immunological and pathogenic effects.

770 MOLECULAR PLAYERS IN INCREASED THIOACETAMIDE-HEPATIC INJURY AND DECREASED MORTALITY FOLLOWING DIET RESTRICTION.

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Earlier studies (TAP, **150,** 12-21, 1998 and Tox. Sci, 45: 233-241, 1998) revealed highly increased liver injury (>6-fold at low dose and >2.5-fold at lethal dose) of thioacetamide is accompanied by up regulation of compensatory liver regeneration response that rescues the diet restricted (DR) rats from a lethal dose of thioacetamide (TA). The objective of this study was to investigate molecular mechanisms for the increased liver injury and liver tissue repair responses. We tested two hypotheses: increased liver injury is due to induction of CYP2E1 and increased liver regeneration due to upregulation of RAS-MAPK pathway involving epidermal growth factor (EGF) and its receptor (EGFr). Male SD rats (225-275 g) maintained on *ad lib* diet regimen were pretreated with isoniazid (INZ) or pyridine (PYR), known inducers of liver microsomal CYP2E1, and liver injury (assessed by ALT and histopathology) of TA (50 mg/kg, ip) was compared. Total CYP450 content was measured and CYP2E1 activity by radioactive HPLC method for chlorzoxazone hydroxylation specific for CYP2E1. Liver EGFr mRNA levels were measured by RT-PCR. EGFr tyrosine phosphorylation and total EGFr density were measured by immunoblotting and bands quantified by imaging densitometer. DR (65% of AL, three weeks) resulted in increased total CYP450 content as well as 150% increase in CYP2E1 activity suggesting CYP2E1 induction by DR. RT-PCR studies indicated that although DR alone did not affect EGFr mRNA levels, TA challenge to DR rats significantly increased the levels at 12 hr compared to AL rats. Investigation into EGFr tyrosine phosphorylation and total EGFr density revealed no change by DR or DR+TA treatment. Results of liver injury indicate the possible mediation of TA bioactivation by CYP2E1. Further, liver tissue repair studies indicate that although EGFr transcriptional activation occurs, this is not accompanied by translation in DR rats treated with TA. We conclude that while increased liver injury is likely due to induction of CYP2E1 as hypothesized, additional studies are needed to test the hypothesis on liver tissue repair. (Supported by ES-08604.)

771 COMPETITIVE BINDING OF POSITIVE- AND NEGATIVE-ACTING TRANSCRIPTION FACTORS TO THE HUMAN *CYP1A1* NEGATIVE REGULATORY ELEMENT.

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CYP1A1 is a phase I detoxifying enzyme which is involved in the bioactivation of several polycyclic aromatic hydrocarbons. *CYP1A1* aberrant expression has been correlated with greater risk for developing bronchogenic carcinoma and poor prognosis in breast cancer. We have identified a 275 bp region (position -833 to -558) in the *CYP1A1* promoter that is involved in modulating inducible expression and is likely involved in the aberrant expression of this gene. Further characterization of this negative regulatory element (NRE) revealed two binding regions for an approximately 100 kDa protein, which we termed the negative regulatory factor (NRF). The upstream higher-affinity binding site has been characterized by *in vitro* DNA binding assays and is composed of a CCAAT box separating an 8 bp inverted repeat centered at position -784. EMSA supershift assays reveal that the ubiquitous, positive-acting CCAAT box transcription factor, NF-Y, also binds competitively to this site. The downstream lower-affinity NRF binding site is not as well characterized, but has been localized to a 68 bp region (position -656 to -588). We have shown by EMSA supershift assay that a binding site for the ubiquitous positive- or negative-acting transcription factor, YY-1, is also present within this fragment. Although the binding sites for NRF and YY-1 do not appear to overlap, they also appear to bind competitively. We propose a competitive binding model in which positive- and negative-acting factors contribute to the observed activity of this region. (Supported in part by PHS grant no. ES03832.)

772 USE OF DIFFERENTIAL DISPLAY FOR IDENTIFICATION OF PCN INDUCIBLE GENES IN RAT LIVER.

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The cytochromes P450 of the CYP3A gene superfamily is a group of microsomal hemoproteins prominent in human liver involved in the metabolism of clinically important drugs such as cyclosporin, nifedipine and in the bioactivation of such carcinogens as aflatoxins and benzo(a)pyrene. Cytochrome P450 3A23 in rat liver is induced by such glucocorticoids as dexamethasone (DEX) and by Pregnenolone-16-alpha-carbonitrile (PCN) an anti-steroid compound, through a non-classical mechanism mediated by PXR, a nuclear receptor which binds both with DEX and PCN and acts through a DNA sequence distinct from the Glucocorticoid Response Element (GRE). To learn the identity of other genes that may be under the control of the PXR receptor, we used the method of Differential Display of liver RNA from Female Sprague-Dawley rats treated for 24hrs with 80mg/kg of DEX or PCN or vehicle alone. The total liver RNA was isolated, transcribed to cDNA, amplified by PCR, submitted to parallel lanes of electrophoresis on DNA sequencing gels. We extracted those amplified cDNA fragments indicating PCN induction and reamplified them for hybridization with total RNA for induced and untreated animals. Cloning and DNA sequence analysis revealed that of several selected bands induced by PCN, one could be identified as MHC class II RT1.BBI. That this gene was inducible in rat liver by PCN within 24 hrs. of treatment was confirmed by Northern blot analysis. We conclude that this reverse gentic approach is invaluable for defining the domain of genes under the control of the PXR/PCN system.

773 EFFECTS OF MICROSOMAL ENZYME INDUCERS ON RAT MULTIDRUG RESISTANCE PROTEIN 2 (Mrp2).

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Previous studies have shown that certain microsomal enzyme inducers (phenobarbital [PB] and pregnenolone-16α-carbonitrile [PCN]) cause an increase in bile flow and biliary excretion of xenobiotics in rats, while other inducers (3-methylcholanthrene [3MC] and 3,4-benzpyrene [3,4-BP]) do not cause the same effects. Within the past four years, a protein that localizes on the canalicular membrane of hepatocytes and that transports organic anion conjugates from liver to bile, has been cloned, and termed the multidrug resistance protein 2 (Mrp2). The purpose of this study was to determine whether microsomal enzyme inducers increase Mrp2 expression in rat liver, and whether the increase might correlate with previously demonstrated effects on bile flow and biliary excretion. Male Sprague-Dawley rats (250-350 g) were injected *ip* for 4 days with PB (75 mg/kg), PCN (75 mg/kg), 3MC (20 mg/kg), 3,4-BP (20 mg/kg), or control vehicle (saline or corn oil). On the fifth day, rat livers were removed. Mrp2 protein expression was detected using the polyclonal antibody EAG15 by western-blot analysis. Liver samples from EHBR rats lacking Mrp2 were used as negative controls for protein analysis. PB treatment increased Mrp2 protein expression by 183%, while PCN treatment increased Mrp2 protein expression by 288%. Neither 3MC nor 3,4-BP treatments altered Mrp2 protein expression. In conclusion, PB and PCN increase Mrp2 protein expression while 3MC and 3,4-BP do not. There is a good correlation between the ability of the microsomal enzyme inducers to increase Mrp2, and increase bile flow and biliary excretion of xenobiotics. This suggests that induction of Mrp2 may be important in the chemical regulation of bile flow and biliary excretion of xenobiotics.

(Supported by American Heart Association grant KS-98-GS-11 and NIH grant ES-07079.)

774 FLAVIN-CONTAINING MONOOXGENASE 2: POLYMORPHISM CHARACTERIZATION AND DETERMINATION OF ALLELIC FREQUENCY IN AFRICAN AMERICANS, CAUCASIANS, AND ASIANS.

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The flavin-containing monooxygenases (FMO) are a family of drug metabolizing enzymes that are expressed in a species- and tissue-specific manner. *FMO2* is expressed in the nasal mucosa and type II and Clara cells of the lung. Two point mutations have been reported in the C-terminal end of the human FMO2 cDNA, a C to T transition at position 1475 and a thymidine insertion at position 1650. The transition mutation results in a premature stop codon, eliminating the activity of the enzyme due to truncation. The insertion mutation results in a frameshift, altering the last 6 amino acids of the protein, as well as extending the reading frame. Whether or not these mutations represent true polymorphisms or rare alleles was not known. To address this question, DNA samples from 180 unrelated African Americans, 52 Caucasians, and 100 Asians were genotyped. A region containing both mutations was amplified using gene specific primers and PCR amplification. The amplicons were slot blotted, and allele specific oligonucleotide probes used to identify the presence or absence of the specific sequences. In the African American population tested, the 1475C allele occurred at an overall allelic frequency of 13%; however, all of the Caucasians and Asians tested were homozygous for the 1475T allele. The frameshift mutation occurred at a frequency of 6.9% and 17.4% in the tested African Americans and Caucasians, respectively. The Asian DNA samples are presently being analyzed for the frameshift mutation. A preliminary analysis of those individuals heterozygous for both mutations suggests the 1650T insertion occurs secondarily to the C1475T transition. These data demonstrate that a functional FMO2 enzyme occurs in a small percentage of the population. Given the role of this enzyme in xenobiotic biotransformation, these individuals may be protected from or more susceptible to certain environmental toxicants. (Supported in part by PHS grant CA53106.)

775 MOLECULAR MECHANISMS FOR THE TISSUE-SPECIFIC EXPRESSION OF THE RABBIT FLAVIN-CONTAINING MONOOXYGENASE 1 (*FMO1*) GENE.

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The flavin-containing monooxygenases (FMO) are a family of microsomal xenobiotic-metabolizing enzymes differentially expressed in several tissues. Because of their roles in detoxification and bioactivation of exogenous compounds, it is important to understand their tissue-specific expression at the molecular level to fully understand the tissue-targeting toxicities of their substrates. In rabbit, *FMO1* has been identified as the major isoform in liver and is not expressed in lung. We previously identified a dual promoter system that directs the transcription of rabbit *FMO1*. Several sequences corresponding to the consensus binding site for HNF3 were located upstream of both promoters. There are also several other sequences corresponding to the consensus binding sites for HNF1, HNF4 and C/EBP. Electrophoretic mobility shift assays (EMSA) have been used to study these potential hepatic regulatory elements. One region spanning from position -737 to -664 was of particular interest. It can form two specific protein-DNA complexes when incubated with nuclear extract from HepG2 cells, a human hepatoblastoma cell line. However, one of the protein-DNA complexes was absent when incubated with nuclear extract from H441 cells, a human lung papillary adenocarcinoma cell line. DNA methylation interference identified the binding sites for both complexes at position -694, corresponding to the consensus binding site for HNF3β. Besides this region, sequences from position -157 to -37 and from position -412 to -333 also formed specific DNA-protein complexes present in HepG2 cells but not in H441 cells. Our data support the hypothesis that combinatorial regulation involving these factors and associated co-regulatory factors are responsible for the tissue-specific expression of rabbit *FMO1*. (Supported in part by PHS CA53106.)

776 ISOLATION OF A FLAVIN-CONTAINING MONOOXYGENASE CDNA SEQUENCE FROM THE TELEOST RAINBOWTROUT (ONCORHYNCHUS MYKISS).

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Flavin-containing monooxygenases (FMOs) are microsomal enzymes that play an important role in xenobiotic metabolism and bioactivation. In mammals, FMO(s) catalyze the oxidation of endogenous sulfur-containing compounds as methionine and cysteine. Several lines of evidence suggest an osmoregulatory role for FMOs in euryhaline fish. Since very few non-mammalian FMO sequences are available, the primary goal of this study was to isolate gene(s) encoding FMO(s) from the euryhaline teleost rainbow trout. Total RNA isolated from rainbow trout liver was subjected to reverse transcription-polymerase chain reaction (RT-PCR) using degenerate primers derived from conserved regions of FMO1 proteins (between amino acid residues 27-32 and 304-309). PCR amplification resulted in a 842 bp electrophoretic band. The amino acid sequence deduced from this fragment demonstrated closest identity to rabbit hepatic FMO1 (96%) and pig FMO1 (85%). Using another pair of primers derived from the conserved FAD- and NADP- binding domains, a 400 bp band was obtained. The deduced amino acid sequence was 100% identical to the overlapping region of the 842 bp fragment and 95% homologous to rabbit FMO1. Attempts to amplify the full length cDNA using 5' and 3' rapid amplification of cDNA ends are currently underway. (EPA grant # R-823450-01-0.)

777 TRANSCRIPTIONAL REGULATION OF THE RAT HYDROXYSTEROID SULFOTRANSFERASE (SULT2-40/41) GENE BY GLUCOCORTICOIDS.

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We previously reported that glucocorticoid treatment induces hydroxysteroid sulfotransferase expression in rat liver and primary cultured rat hepatocytes, and that this induction shares features with glucocorticoid-inducible CYP3A gene expression. Current evidence suggests that glucocorticoid-inducible CYP3A transcription is mediated through a *cis*-acting element containing an ATGAACT direct repeat. Sequence analysis of the SULT2-40/41 5'-flanking region revealed an ATGAACT half-site at bp -184 to -178. To begin examining whether this half-site has a functional role in mediating glucocorticoid-inducible SULT2-40/41 expression, we performed transient transfection studies in primary cultured rat hepatocytes using SULT2-40/41-luciferase reporter constructs containing ~1.9 Kb of the 5'-flanking region, either intact or lacking a 70 bp fragment delimited by *Pst*I sites at -227 and -158 (Δ-227 to -158). In hepatocytes transfected with the SULT2-40/41 construct containing the intact 5'-flanking region, 24 h treatment with 10^{-7} M or 10^{-5} M dexamethasone produced an ~6- or ~10-fold increase in luciferase activity, while treatment with 10^{-5} M betamethasone, triamcinolone acetonide, or hydrocortisone produced an increase of ~9-, ~6-, or ~3-fold, respectively. In contrast, steroid treatment failed to induce luciferase activity in hepatocytes transfected with the Δ-227 to -158 construct. These data support a role for the ATGAACT half-site in mediating glucocorticoid-inducible SULT2-40/41 gene transcription. (Supported by NIH Grant ES05823 and NIEHS Center Grant ES06639.)

778 INHIBITION OF RECOMBINANT HYDROXYSTEROID SULFOTRANSFERASES AND MICROSOMAL ARYLSUFATASE-C BY PENTACHLOROPHENOL.

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Pentachlorophenol (PCP) is a ubiquitous environmental pollutant known to inhibit phenol sulfotransferases. A recent report indicates that PCP treatment elevated plasma levels of dihydroxyepiandrosterone (DHEA) in rats infused with the steroid (Toxicol. and Appl. Pharmacol. 151:105-109,1998). This unexpected action was attributed to elevation of hepatic PAPS but could also be explained by other actions such as inhibition of enzymes hydrolyzing DHEA-SO_4. Consequently, we evaluated the action of PCP on arylsulfatase-C (STS) and recombinant forms of the three hydroxysteroid sulfotransferases in rat, HST_{20}, HST_{40} and HST_{60}. In addition, the action of PCP on human hydroxysteroid sulfotransferase hDHEA-ST (SULT-2A1) was examined. Recombinant enzymes in vectors pET17b or PKK233 were expressed in E.

coli. Arylsulfatase-C was measured using microsomes isolated from rat liver and brain. STS was measured using either methylumbelliferyl sulfate (MUS) or estrone sulfate (ES) as substrate (10-80 μM) in the presence of increasing concentrations of PCP. Half-inhibition of hydrolysis was observed with approximately 5 μM PCP. Activities of the recombinant HSTs were measured using either DHEA (0.5 μM) or pregnenolone (5 μM) as substrates. Half-inhibition (IC_{50}) of HSTs was observed at much higher concentrations of PCP (50-200 μM) than required to inhibit STS. Thus, PCP may elevate circulating steroid sulfates by inhibiting STS in liver and target tissues at concentrations significantly below those required to inhibit HSTs. Collectively, these results suggest a novel mechanism of endocrine disruption by PCP. (Supported in part, by USPHS grants TWO2194 and ES707148.)

779 DIFFERENTIAL SUBCELLULAR LOCALIZATION OF ENDOGENOUS AND TRANSFECTED SOLUBLE EPOXIDE HYDROLASE IN MAMMALIAN CELLS: EVIDENCE FOR ISOZYME VARIANTS.

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Soluble epoxide hydrolase (sEH; EC3.3.2.3) is thought to catalyze the hydrolysis of a variety of toxic and carcinogenic epoxides to their corresponding diols. Previous studies suggest that sEH exists in the cytosol as well as within peroxisomes of mammalian cells. The dual localization of this enzyme has been attributed to the slow uptake of sEH into peroxisomes from its site of synthesis in cytosol due to an impaired peroxisomal targeting signal, Ser-Lys-Ile (SKI), located at the C terminus of the nascent sEH. In this study, immuno-fluorescence microscopy was used to evaluate the subcellular compartmentation of mouse and rat sEH in cultured mammalian and plant cells. Application of mouse sEH antibodies to mouse NIH3T3 cells revealed an exclusive peroxisomal localization of endogenous sEH. However, when wild-type mouse sEH was transiently expressed in NIH3T3 or HeLa cells, it accumulated in the cytosol. In contrast, when Ile at the C terminus of sEH was replaced with Leu to generate sEH-SKL-COOH, the protein was imported into peroxisomes of NIH3T3 and HeLa cells. Similar results were obtained in Chinese hamster cells stably expressing rat sEH appended to the green fluorescent protein (GFP), i.e., GFP-sEH-SKI-COOH was not imported into peroxisomes, whereas GFP-sEH-SKL-COOH was imported. That wild-type mouse sEH-SKI could indeed be sorted to peroxisomes was demonstrated in plant cells. Biolistic transformation of tobacco BY-2 cells with either the wild-type sEH (SKI-COOH), or the modified sEH (SKL-COOH), resulted in the import of both proteins into peroxisomes. We suggest the Ser-Lys-Ile on sEH is a non-functional rather than an inefficient peroxisomal targeting signal, and that two isoforms of sEH exist; a peroxisomal form and a cytosolic form.

780 PROFILE OF GLUTAMINE SYNTHETASE (GS) ACTIVITY IN METHYLMERCURY (MeHg)-EXPOSED ASTROCYTES.

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The glial-specific enzyme GS plays a key role in the maintenance of glutamate homeostasis in the CNS. This report presents data on the dosage effect of methylmercury (MeHg) on the enzymatic activity of GS and its expression in cultured astrocytes. Duplicate sets of astrocyte cultures (3-4 weeks) were exposed to MeHg (50 nM to 8 μM) for 72 or 96 hours. Astrocytes exposed to 50 nM MeHg for 72 hours exhibited a 30% increase in GS activity, but the activity was diminished by 40% at 200 nM MeHg. After 96 hour exposure to MeHg (400 nM), GS activity was reduced by ~50%. However, the GS activity was recovered in astrocytes treated with higher concentrations of MeHg (0.8-6 μM). In parallel to GS activity assays, RNA (RT-PCR and A_{260}) and GS protein level profiles (Western blots) were also obtained. In general, these results corroborated the data of GS enzyme activity. The GS protein profile from this study is consistent to the results of Western blotting analysis from our laboratory (abstract by Allen et al, 1999). The decrease in GS activity at 200-400 nM MeHg may be due to initial inhibition of GS, and the overall restoration of GS activity at later time points is likely due to enhanced expression of the GS gene. (Partially supported by NCRR NIH 2P20RR 22 583.)

781 THE DEVELOPMENTAL TOXICITY OF TOBACCO SMOKE.

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Today, tobacco use continues in about 25% of pregnancies, with additional gestational and childhood exposures to environmental tobacco smoke. Maternal smoking has been linked to low birth weight, increased perinatal morbidity and mortality, SIDS, and persistent deficits in learning and behavior. This symposium presents recent studies on effects of prenatal or early postnatal tobacco smoke (TS) exposure. Risks associated with maternal smoking as well as maternal and/or paternal exposure to second-hand smoke will be discussed. Recent studies which reveal lasting effects of prenatal and/or postnatal TS exposure on cognitive development in children, including reading and language, will be presented. Associations between maternal smoking and incidences of specific congenital malformations have been analyzed, including gene-environment interactions which may lead to increased risk of TS-induced birth defects. These studies will be reviewed and summarized. Animal models of developmental TS exposure will be reviewed, and new studies on the effects of perinatal environmental TS on lung development and airway reactivity in rats will be discussed. Nicotine in a known neuroteratogen. The effects of nicotine on the development of neurotransmitter systems and brain development in rats will be discussed in the context of TS-induced morbidity and SIDS in humans. Finally, these talks will be summarized in a risk assessment framework.

782 ANALYSIS OF RISK TO BIRTH DEFECTS ASSOCIATED WITH SMOKING: CONSIDERING THE ROLE OF GENE-ENVIRONMENT INTERACTIONS.

T H Beaty. *Dept. Of Epidemiology, School of Hygiene & Public Health, Johns Hopkins University, Baltimore, MD, USA.* Sponsor: J M Rogers.

Exposure to cigarette smoke during pregnancy is widely recognized as a risk factor for adverse reproductive outcomes, primarily low birth weight, perinatal mortality, and certain birth defects. There are inherent limitations in measuring the strength of associations between an environmental risk factor such as smoking and rare outcomes, such as birth defects. Furthermore, it is very likely that the response to such exposure depends on the genotype of the developing fetus and possibly that of the mother, i.e., there may be gene-environment interaction influencing risk associated with smoking. A number of common epidemiologic study designs are available to test for gene-environment interaction, including case-only, case-control, and case-parent trios. Each has its strengths and limitations. The application of these different study designs will be discussed with particular focus on the effects of maternal cigarette smoking and risk to non-syndromic oral clefts, a common birth defect influenced by both genetic and environmental factors. A number of candidate genes have shown sometimes conflicting evidence of being associated with non-syndromic oral clefts (including transforming growth factor alpha and transforming growth factor beta 3, among others), and frequently the association appears to be dependent on exposure to maternal smoking. (Supported by NIDR grant R01-DE-10293.)

783 ENVIRONMENTAL TOBACCO SMOKE AND PERINATAL AIRWAY REMODELING.

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Epidemiologic and experimental studies suggest that passive exposure to cigarette smoke during the perinatal period may have an adverse effect on airway function which can persist into adulthood. However, the mechanisms leading to this process are unknown. We hypothesized that neuroendocrine mediated events due to smoke exposure in the lungs lead to a persistent increase in airway reactivity. Timed pregnant dams were exposed to aged and diluted sidestream cigarette smoke (ADSS) at a particulate concentration of 1 mg/m3 for 6 h/day, 7 days/wk. Following birth, mothers and neonates continued ADSS exposure to 21 days of age. Animals were subsequently maintained in filtered air until 60 days of age. Animals were examined at gestational days (GA) 19 and 21, and postnatal days (PN) 1, 7, 21,

and 60. Airway whole mounts and paraffin sections were prepared to examine the distribution of neuroendocrine cells (NECs) in the airways. A significant increase in the number of NECs were noted at GA 19 and 21 as well as at PN 1 and 7. Isolated perfused lungs and lung slice videomicrometry were used to measure airway response to methacholine challenge at PN 60. Significant increases were noted in airway narrowing by lung slice videomicrometry as well as a significant increase in airway resistance in the isolated perfused lung 5 weeks following the cessation of exposure to ADSS. We conclude that exposure to ADSS during the perinatal period (1) significantly increases NEC number in the airways and (2) is associated with a persistent and significant alteration in airway hyperreactivity to methacholine challenge. These changes in airway function may be due to early developmental changes in NEC differentiation.

784 MATERNAL SMOKING DURING PREGNANCY: ARE THERE CIGNIFICANT LONG TERM COGNITIVE EFFECTS IN OFFSPRING?

P A Fried. *Dept. of Psychology, Carleton Univ., Ottawa, Canada.* Sponsor: J M Rogers.

The Ottawa Prenatal Prospective Study (OPPS), an ongoing, longitudinal investigation underway since 1978, has been studying the effects of cigarettes and other drugs used during pregnancy. The subjects are offspring of primarily middle class, low risk women who entered the study early in their pregnancy. Extensive demographic and lifestyle information was gathered several times during pregnancy and after the birth. The offspring have been assessed repeatedly during the neonatal period, at least annually until the age of 6 and less frequently thereafter. Data will be presented describing these children from birth until early adolescence. The outcome measures to be presented in this symposium include a variety of age appropriate standardized global measures as well as a large series of neuropsychological tests attempting to assess discrete functioning within particular domains including language development, memory, visual/perceptual functioning, components of reading and sustained attention. Data have been collected on the same children for their entire lives allowing for the examination of continuity of observations and making the ascribing of cause and effect somewhat less problematic. Across all ages of assessment, a dose dependent association, that remains after controlling for potential confounds (including second hand smoke), was noted between prenatal cigarette exposure and lower global IQ with tests of auditory/verbal functioning showing the maximum vulnerability. (Supported by NIDA.)

785 DEVELOPMENTAL TOXICITY OF NICOTINE.

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Despite extensive publicity, tobacco use continues in one-fourth of all pregnancies. The societal cost of maternal smoking is seen in underweight newborns, higher rates of perinatal morbidity, mortality and SIDS, and persistent deficits in learning and behavior. Animal models and in particular, nicotine infusion paradigms that, like the nicotine transdermal patch, produce nicotine exposure without the confounds of other components of tobacco or episodic hypoxic - ischemic insult, have demonstrated conclusively that nicotine is a neurobehavioral teratogen. Nicotine targets specific neurotransmitter receptors in the fetal brain, eliciting abnormalities of cell proliferation and differentiation and attendant shortfalls in the number of cells and eventually in synaptic activity. Because of the close regulatory association of cholinergic and catecholaminergic systems, adverse effects of nicotine involve multiple transmitter pathways and influence not only the immediate developmental events in fetal brain, but also the eventual programming of synaptic competence and plasticity. Accordingly, behavioral defects may appear after a prolonged period of apparent normality. Comparable alterations in the competence of peripheral autonomic systems can be demonstrated in animal models of nicotine exposure and probably contribute to perinatal morbidity and SIDS in babies born to smokers. In light of the role of nicotine in the physiological and behavioral deficits associated with maternal smoking, factors such as dose, route of administration, and critical periods of development need to be considered before using nicotine substitution during pregnancy. (Supported by the Smokeless Tobacco Research Council.)

786 REGULATION OF GENE-EXPRESSION VIA THE ELECTROPHILE RESPONSE ELEMENT.

K S Ramos. *Center for Environmental and Rural Health and Department of Physiology and Pharmacology, Texas A&M University, College of Veterinary Medicine, College Station, TX, USA.*

Transcriptional activation and repression events are responsible for coordinate regulation of gene expression at all levels of biological organization. Because transcriptional events are susceptible to toxicant interference, and chemical injury often involves disruption of signal transduction to the nucleus, toxicologists have become interested in defining cis-acting elements and DNA-protein/protein-protein interactions which govern these processes. The multiplicity of transrciptional and signaling events triggered by oxidants and electrophiles are not yet fully understood. Of relevance within this context, are studies focusing on redox regulation of xenobiotic-responsive genes mediated by the Antioxidant/Electrophile Response Element (ARE/EpRE). The first two presentations in this symposium will discuss advances in the euclidation of molecular mechanisms by which reactive oxygen species activate the rat glutathione transferase and quinone reductase genes, and the putatinve transcriptional factors that mediate these responses. The third speaker will discuss the role of Nrf1 and Nrf2, and the other leucine zipper proteins, in the activation of ARE-regulated genes. This will be followed by two presentations focusing on the pathophysiological consequences of ARE/EpRE activation as it relates to the identification of a neural-specific ARE/EpRE sequence involved in neuroprotection, and the study of promoter-specific patterns of gene regulation via the ARE/EpRE following oxidant/electrophile injury to vascular cells.

787 TRANSCRIPTIONAL REGULATION OF THE GLUTATHIONE S-TRANSFERASE A2 GENE.

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Transcripional activation of the rat liver glutathione S-transferase A2 gene by xenobiotics is mediated by two cis-acting regulatory elements, The ZRE and ARE, found in the 5' flanking region of this gene. The XRE contains a core sequence 5'TNGCGTG 3' that mediates an inducible response to planar aromatic compounds (PAC). The ARE is a unique cis-acting element responsive to metabolize PAC, phenolic antioxidants and oxidants. Using point mutations and transfection inducibility in HepG2 cells, was identified as 5'-GTGACAAAGC-3'. The core sequence is recognized with high affinity by a nuclear protein in vitro. Using a ligation-mediated polymerase chain reaction technique we have found that the core sequence interacts with a nuclear protein in vivo. More recently we have been trying to identify the nuclear protein which interacts with the ARE core consequence. Based on the similarity in sequence between the ARE and the Jun-Fos binding site, we investigated whether members of the AP-1 family of transcriptional factors bind to the ARE. Using in vitro transcription and translation of Jun and Fos coupled with gel shift assays we found that these transrciptional factors did not bind to the ARE. Similar experiments with the maf family of transrciptional factors involve in high affinity binding to the ARE. However, recent gel shift data suggest that nrf-maf heterodimers can bid with high affinity to the core ARE sequence. Current studies are aimed at elucidating how these transcriptional factors are modified by oxidative stress to activate the transcription of genes that contain an ARE.

788 ANTIOXIDANT REGUALTION OF GENES ENCODING ENZYMES THAT DETOXIFY XENOBIOTICS AND CARCINOGENS.

A K Jaiswal and V Radjendirane. *Dept. of Pharmacology, Baylor College of Medicine, Houston, TX, USA.* Sponsor: K S Ramos.

Detoxifying enzymes including quinone oxidoreductases (NQO1 and NQO2) and glutathione S-transferases (GSTs) catalyze metabolic detoxification of xenobiotics, drugs and carcinogens and, thus, protects the cells against redox cycling and oxidative stress. Genes encoding the various detoxifying enzymes are ubiquitiosly expressed and coordinately induced in response to antioxidants and xenobiotics. Deletion mutagenesis and transfection assays have identified antioxidant response element (ARE) in the promoters of the various detoxifying enzyme genes, which regulate the expression and coordinated induction of detoxifying enzyme genes. ARE is a unique cis-element that contains two AP1/AP1 like elements followed by a 'GC' box. Band and supershift assays were used to demonstrate that

nuclear transcription factors Nrf1, Nrf2, Jun, Fos, and Fra bind to the NQO1 and GST Ya genes ARE. Overexpression of Nrf1 and Nrf2 individually in human hepablastoma (Hep-ge2) cells significantly increased the ARE-mediated genes expression and induction by ß-napthoflavone (ß-NF) and tert-butyl hydroquinone (t-BHQ). Nrf2 containing one mutated leucine in its leucine zipper region was more efficient in upregulation of ARE-mediated gene expression, as compared to nrf1 with two mutated leucines. Immunoprecipitation assays demonstrated that Nrf1 and Nrf2 heterodimerize with Jun (c-Jun, Jun-B and Jun-D) proteins that bind to the ARE. Overexpression of Nrf and Jun combinations led to superactivation of ARE-mediated gene expression in transfected Hep-G2 cells. These results suggested that ARE-regulated expression of the NQO1 and other detoxifying enzyme genes and their coordinated induction by antioxidants and xenobiotics are mediated by nuclear factors Nrf (Nrf1 and Nrf2) and Jun (c-Jun, Jun-B, and Jun-D) proteins.

789 FUNCTIONAL INTERACTION OF PROTEINS WITH THE ELECTROPHILE RESPONSIVE ELEMENT.

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Sponsor: K S Ramos.

The Electrophile Responsive Element (EpRE) mediates induction of phase II detoxifying enzymes by tert-butyl hydroquinone and other redox-cycling compounds. Most of the trans-acting factors involved in transcriptional activation through the EpRE are unknown. Although candidate factors like Nrf-2 have been identified, functional mechanistic studies need to be done to identify the contributory roles of Nrf-2 and its functional homologues as well as the other factors which interact with the EpRE. Our current efforts involve: i) efforts to purify ARE-BP-1, a 160 kDa transcription factor that has been functionally implicated in mediating the oxidant stress response, ii) studies of reconstituted, in vitro transcription using a synthetic promoter containing an EpRE which confers EpRE-enhanced transcription with nuclear extracts from HeLa cells, and iii) studies of EpRE-mediated transcription in intact HepG2 cells using direct nuclear injection of antibodies, and competitive oligonucleotides and peptides, and iv) collaborative studies to determine the degree of naturally-occurring sequence polymorphism in EpRE elements which regulate transcription of environmentally relevant human genes. Results from these studies illustrating the functional interactions of regulatory molecules at the EpRE will be presented.

790 NAD(P)H:QUINONE OXIDOREDUCTASE 1 (QR1) AND THE ANTIOXIDANT/ELECTROPHILE RESPONSIVE ELEMENT (ARE/EpRE) IN CELLS OF NEURONAL ORIGIN.

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The ARE/EpRE is an enhancer element found in the 5'-flanking region of QR1. Others have shown that pretreatment with tert-butylhydroquinone (tBHQ) and increased QR activity protects rodent neuroblastoma cells from glutamate-mediated cytotoxicity. Since induction of the QR1 gene by tBHQ is mediated through the ARE/EpRE, hQR1-ARE/EpRE-luciferase construct was transiently transfected into IMR-32 human nueroblastoma cells and treated with tBHQ. hQR1-ARE/EpRE-luciferase expression was increased 23-fold and subsequent studies defined an expanded core sequence containing a complete 5' palindrome necessary for maximal activation in IMR-32 cells. In addition, the recent finding that women on hormone replacement therapy have a reduced incidence of Alzheimer's disease has lead us to investigate whether estradiol or estradiol metabolites activate the ARE/EpRE in neuroblastoma cells. Estradiol did not activate the ARE/EpRE, but catechols of both estradiol and estrone increased hQR1-ARE/EpRE-luciferase expression and QR1 protein (5- to 10-fold). ARE/EpRE induction by tBHQ and the catechol estrogens is estrogen receptor-independent. Pretreatment with tamoxifen or estradiol had no effect on tHBQ- or catechol-mediated ARE/EpRE-activation suggesting an estrogen receptor-independent mechanism. Furthermore, activation of the ARE/EpRE by catechol estrogens and tBHQ was not blocked by pretreatment with N-acetylcysteine, glutathione, or glutathione ethyl ester. Finally, the hydroquinone-based tyrosine kinase inhibitors lavendustin A, erbstatin and tyrophostin AG957 also activate the ARE/EpRE and increase QR1. These data imply that the mechanism of activation of the ARE/EpRE in IMR-32 cells is not dependent on generation of reactive electrophiles or reactive oxygen species, but results from an effect on tyrosine kinase activity. Finally, ARE/EpRE activation correlates with neuroprotection, but a direct link remains to be determined. (Funded by NIEHS 08089 and Burroughs Wellcome New Investigator in Toxicology Award.)

791 CELL AND PROMOTER SPECIFIC PATTERNS OF GENE REGULATION VIA THE ELECTROPHILE RESPONSE ELEMENT.

K S Ramos, Y-H Chen, M T Holderman, and K P Miller. *Center for Environmental and Rural Health and Department of Physiology and Pharmacology, Texas A&M University, College of Veterinary Medicine, College Station, TX, USA.*

Recent efforts in this laboratory have focused on the study of c-Ha-ras and GST-Ya gene regulation via Electrophile Response Element (EpRE). Four plasmids containing GST-Ya regulatory sequences linked to a chloramphenicol acetyltransferase reporter gene were transfected into murine vascular smooth muscle cells (vSMCs) or HepG2 cells to evaluate transactivation profiles following challenge with benzo(a)pyrene (BaP) (0.3-30 (M), TCDD(0.1-10 nM), or H2O2 (12.5-50 (M0 for 24 hr. The indication patterns by BaP in vSMCs were AhRECAT>1.6CAT=EpRECAT=-164CAT. In contrast, induction profiles in HepG2 cells were AhRECAT = EpRECAT>1.6CAT=-164CAT. In vSMCs, the induction profiles by TCDD differed from those in HepG2 where AhRECAT>1.6CAT=EpRECAT=-164CAT. BaP and TCDD transactivated c-Ha-ras via the EpRE, but not the AhRE, showing that response profiles via EpRE are promoter specific. Interestingly, H2O2 did not transactivate the GST-Ya promoter, but increased EpRE induction of the c-Ha-ras gene in vSMCs. Cross-linking experiments with a BrdU-substituted oligonucleotide EpRE probe identified several specific complexes of Mr 80, 60, 55, 23, 21 kDa in vSMCs. We conclude that gene regulation via EpRE exhibits cell and promoter specific patterns that may be relevant to the spectrum of toxic responses elicited by BaP and related chemicals. (Supported in part by NIH Grants ES 04849 and ES 09106.)

792 TRICHLOROETHYLENE ACCELERATES AN AUTOIMMUNE RESPONSE IN MRL+/+ MICE AT DOSES SIMILAR TO HUMAN EXPOSURE LEVELS.

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Trichloroethylene is a volatile organic solvent that is primarily used as a metal degreaser. With its widespread use, trichloroethylene has become a major environmental contaminant. Recently, trichloroethylene has been shown to accelerate an autoimmune response in MRL+/+ mice at relatively high doses. We have previously shown that trichloroethylene exposure in the drinking water is capable of accelerating an autoimmune response at an average dose of 450 mg/kg/day. However, the relationship between trichloroethylene exposure at levels similar to occupational exposure and the exacerbation of autoimmunity has not been established. Consequently, we conducted experiments designed to examine the effects of trichloroethylene at more relevant doses. Autoimmune prone MRL+/+ mice were treated with 0, 0.1, 0.5 or 2.5 mg/ml trichloroethylene in the drinking water for 4 weeks. The average dose received per mouse during the 4 weeks was 0, 22, 99 and 400 mg/kg/day, respectively. At these doses, trichloroethylene was metabolized to a reactive intermediate that covalently bound to protein. Serum levels of the autoimmune markers antinuclear antibodies and total IgG and IgM were significantly increased following treatment. It has previously been shown that $CD4^+$ T cells play a role in the development of an autoimmune response and in this experiment there was a dose-dependant and significant increase in the secretion of IFN-γ by activated $CD4^+$ T cells from treated mice, while the level of IL-4 was unchanged. Taken collectively, the results show that MRL+/+ mice treated with trichloroethylene at doses relevant to human exposure levels develop an accelerated autoimmune response in association with the activation of $CD4^+$ T cells that secrete Th_1 cytokines.

793 INITIATION OF DRUG-INDUCED LUPUS BY DISRUPTION OF POSITIVE SELECTION OF T CELLS IN THE THYMUS.

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Drug-induced lupus is a systemic autoimmune disease that can be acquired as a side effect of therapy with more than 40 medications. Procainamide poses the greatest risk for development of this syndrome with an annual inci-

dence of 20 % among treated patients. Much of our work to elucidate the underlying mechanisms of this disease has been driven by the hypothesis that systemic autoimmunity results from a failure of self-tolerance during the generation of T cells in the thymus. The mouse model we developed was designed to test this notion and to determine its cellular and molecular basis. We previously demonstrated that two intrathymic injections of procainamide-hydroxylamine (PAHA, a reactive metabolite of procainamide) into B6D2 mice caused the appearance of chromatin-reactive T cells and anti-chromatin antibodies. We now determined how thymic PAHA exposure results in the release of autoreactive T cells. In reaggregate cultures of thymic epithelial cells with $CD4^+CD8^+$ double positive cells derived from TCR-AND mice, the emerging transgenic $CD4^+$ cells expressing a T cell receptor specific for pigeon cytochrome c (PCC) are normally unresponsive to PCC. However, if 20 μM PAHA was present during selection, the T cells subsequently exhibited a 40-fold increase in proliferation in response to PCC. In contrast, when single positive thymocytes were used in the reaggregate, PAHA presence did not result in PCC-responsive T cells. These observations suggest that PAHA affects double positive thymocytes undergoing selection. To determine whether PAHA interferes with negative selection, we measured deletion of $V_\beta3$-expressing PCC-specific transgenic T cells as a result of exposure to PCC during selection. Presence of PAHA did not prevent the deletion of the transgenic T cell population. An effect on positive selection was tested using DPK cells, a double positive thymocyte cell line from a TCR-AND mouse, which mature to $CD4^+$ cells when exposed to fibroblasts presenting PCC. When selected on [PCC] < 2 μM, the emerging cells were unresponsive to PCC. Addition of 20 μM PAHA to this system resulted in a 4-fold increase in IL-2 production upon challenge with PCC. These studies suggest that PAHA induces autoimmunity by preventing acquisition of unresponsiveness to self peptides during positive selection of T cells in the thymus.

794 IMMUNOSTIMULATORY EFFECTS OF ANTI-DEPRESSANTS ON REPORTER ANTIGEN RESPONSE IN THE MODIFIED PLNA: ROLE OF SEROTONERGIC ACTIVITY.

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The list of pharmaceuticals with autoimmunogenic potential is extensive, and includes drugs of the category of anti-depressants, in particular the serotonin-reuptake blockers. The immunomodulatory effect of these reuptake inhibitors may partly be caused by their capacity to act as hapten or induce neo-antigen formation generating non-cognate T cell help with subsequent determinant spreading to a (true) auto-antigen specific immune response. In addition, these compounds may cause inflammation and thus generate costimulatory signals that stimulate an immune response against an auto-antigen. The aim of this study was to discern whether anti-depressants were able to (non-)cognate help and to assess whether the serotinergic activity of these drugs might be involved in their immunostimulatory potential. To this end, we utilized the PLNA modified to read-out the characteristics of the response by using well defined reporter antigens. A number serotonin-reuptake blockers (clomipramine, imipramine, maprotiline, nortryptyline, zimeldine, paroxetine, fluvoxamine) and also serotonin itself were injected together with the T cell-independent TNP-Ficoll (requiring non-cognate T cell help) into the hind paw of a BALB/c mouse. In a next series of experiments, zimeldine, imipramine or serotonin were co-injected with the T cell-dependent antigen TNP-OVA (requiring cognate T cell help for IgG1). The immune reponse was monitored in the draining popliteal lymph node by using an TNP- and IgG1-specific ELISPOT assay. Results demonstrated that all drugs tested stimulated the IgG1-response against TNP-OVA and TNP-Ficoll whereas serotonin stimulated the response to TNP-OVA only. In addition, it was found that the 5HT2R-antagonist ketanserin caused partial decrease of the stimulatory effect of serotonin and zimeldine to TNP-OVA. Results indicate that the anti-depressive drugs can stimulate both cognate and non-cognate T cell help and that part of the cognate help may arise from their serotonergic activity.

795 POPLITEAL LYMPH NODE RESPONSE TO STREPTOZOTOCIN IS UNDER TYPE-1 CD8+ T-CELL CONTROL.

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The mechanism of chemical-induced autoimmune reactions is not known. The popliteal lymph node (PLN) assay has been proposed to predict the sensitizing potential of xenobiotics, but a better understanding of the mechanisms involved is absolutly needed to establish its value for preclinical safety evaluation. In this respect, the cytokine profile of PLNs was analyzed after the injection of streptozotocin (STZ). We used different groups of mice to assess the role of the CD4+ and CD8+ T-cells subtypes in the occurrence of positive responses: C57 BL/6 mice, CD4+ T-cells and CD8+ T-cells depleted mice, MHC-class I deficient mice and MHC-class II deficient mice. PLNs of 6-8 week-old mice were removed at day 7 after the injection of 0.25mg STZ (right footpad), or saline (left footpad) and the weight indices were calculated. After treatment for RNA extraction, the expression of mRNAs of interleukin (IL)-4, IL-6, IL-10, and gamma interferon (IFN-γ), was assessed in the PLNs by a quantitative RT-PCR method, using the coamplification of an internal plasmid. Naive or saline treated mice expressed few or no gamma-IFN, IL-4, IL-6, and IL-10. The prominent feature of PLNs from STZ treated C57 BL/6 mice was a high increase in IFN-γ mRNA (2900 copies), and in IL-6 mRNA (200 copies). No significant increases in IL-4, nor IL-10 mRNA were noted. CD8+ T-cells depleted mice and MHC-class I deficient mice, deficient in CD8+ T-cells, exhibited no positive PLN response, nor increase in the secretion of IL-6 or IFN-γ . On the contrary CD4+ T-cells depleted mice and MHC-class II deficient mice, deficient in CD4+ T-cells, exhibited a normal PLN response, with a cytokine profile equivalent to non-depleted mice.

These results suggest that positive responses to STZ are mediated by CD8+ T-cells restricted to MHC class-I, with a high production of IFN-γ .

796 TDI-SPECIFIC ANTIBODY GENERATION IS ALTERED IN TUMOR NECROSIS FACTOR R1/R2 DOUBLE KNOCKOUT MICE FOLLOWING EXPOSURE TO THE CHEMICAL.

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Toluene diisocyanate (TDI) is a widely used industrial chemical causing respiratory sensitization in some exposed individuals. In spite of intensive research, understanding of the mechanism of chemical allergy is unclear. B and T lymphocyte activation, T helper differentiation and tissue pathology, largely controlled by the release of cytokines, are required for an organism to mount an effective immune response. The pleitropic, potent proinflammatory cytokine TNF may contribute to the induction +/or illicitation phase of TDI sensitization. To address this hypothesis TNF R1/R2 -knockout mice (C57 black) were sensitized (sc, 20 μ l, day 1) boosted (sc, 5 μ l, days 4, 11) and repeatedly challenged (inhal., 100 ppb, days 21, 23 and 25) with TDI (80:20, 2,4:2,6 TDI). TDI sensitized and challenged wild type mice were included in the study as positive controls. Twenty-four hours following the last inhalation challenge the mice were sacrificed and their blood was collected for analysis of total IgE and TDI-specific IgG and IgE. Lungs were collected for RT-PCR measurement of IL-4, IL-5 and INF-γ mRNA expression and histopathologic evaluation of the airways. Total IgE was significantly increased in the knock-out mice compared with the wild type controls. In contrast, the titers of TDI-specific IgG and IgE were reduced in the TNF R1/R2 mice. Histopathology indicated differences in the trachea, nasal area and formation of germinal centers of regional lymph nodes. Similar results were obtained in normal mice receiving TNF neutralizing antibody prior to TDI exposure. The results of this study suggest that the proinflammatory cytokine TNF participates in the murine, immune response to TDI including TDI-specific antibody production and class switching. (Supported by NIEHS # 05651.)

797 QUANTITATION OF IGE ON THE SURFACE OF BASOPHILS USING ANTI-IGE AND QUANTITATIVE FLUORSCENCE.

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The binding of IgE to high affinity receptors on the surface of basophils plays an important role in allergic reactions in man. The number of IgE molecules expressed on the surface of a basophil has been shown to correlate with the serum concentration of IgE, based on measurements made by eluting the IgE from a known number of basophils, measuring the amount of IgE, and then back calculating for the number of IgE molecules/basophil. The purpose of this study was to verify that a 2-color staining method analyzed by flow cytometry could be used to determine number of IgE molecules. Both whole blood and separated lymphocyte preparations were compared. The cells were stained with CD45 FITC and anti-IgE Biotin with a strepavidin PE second step. Basophils exhibit a $CD45^{DIM}/IgE^{BRIGHT}$ phenotype that allows secondary gating on the IgE^{+} population to determine mean fluorescence intensity (MFI). The number of IgE molecules bound to the surface of the basophils was determined using Becton Dickinson's QuantiBRITE system. This system consists of a set of four pre-calibrated bead levels in the form of a lyophilized pellet to calibrate the FL2 axis in terms of PE molecules. Preliminary results of 7 donors (4 with allergies and 3 with no known allergies) are as follows. In whole blood, the range of %total IgE was 0 to 1.15%, the MFI range was 0 to 936.7 and the range of the # of IgE molecules/basophil was 0 to 11490.5. The ranges for the separated lymphocytes were: %total IgE 0 to 1.5%, MFI of 0 to 1418.9 and the # of IgE molecules/basophil was 0 to 17458.8. There appeared to be a correlation between the number of IgE molecules/basophil and allergic status of the donor. Based on the preliminary data, there is no notable difference between the analysis of whole blood preparation verses the separated lymphocyte preparation regarding # of IgE molecules/basophil. The whole blood staining procedure is a rapid and reproducible means for determining the number of IgE molecules/basophil without enriching for basophils.

798 EXPERIENCE WITH A MOUSE INTRANASAL TEST FOR THE PREDICTIVE IDENTIFICATION OF RESPIRATORY SENSITISATION POTENTIAL OF PROTEINS.

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The predictive identification of respiratory allergenic potential is an important primary step in the safety evaluation of (novel) proteins, such as the enzymes used in a range of consumer laundry products. In the past this has been achieved by assessing the relative ability of proteins to give rise to the formation of anaphylactic antibody in the guinea pig. Recently, it has been suggested that the same endpoint can be obtained by measuring the formation of specific IgG1 antibody in a mouse intranasal test (MINT). This procedure has undergone successful initial intralaboratory and interlaboratory assessment. In the present work, the MINT has been evaluated in a more thorough intralaboratory program. The data generated with a reference enzyme protein, Alcalase™, showed great reproducibility, results from 15 separate studies expressed as mean (μ g protein/dose/animal) ± standard deviation were 0.208 ± 0.079 using a statistically based curve comparison method. However, analysis of a range of 8 other proteins demonstrated that while there was reasonable correlation with historical guinea pig data for some, for others there was considerable divergence. When compared to the reference Alcalase, two proprietary enzymes were ~3x more antigenic and a third was of similar antigenicity as assessed in both MINT and guinea pig. Three other proprietary enzymes of similar antigenicity to Alcalase in the guinea pig were all substantially less antigenic in the BDF1 mouse. Another enzyme together with ovalbumin which were both significantly more antigenic in the guinea pig were also less antigenic in the MINT. The variance with data derived from guinea pig/human experience may in part be due to use of a surrogate marker for IgE in the MINT protocol (IgG1 is also regulated by IL-4) and the use of an inbred mouse strain (BDF1), where some antigens may not be recognised (due to differences in immunological repertoire). In consequence, it is concluded that the MINT is at present not suitable as a replacement for the guinea pig models currently used.

799 THE ADJUVANT ACTIVITY OF PARTICULATE POLLUTANTS IN THE PLN-ASSAY WITH REPORTER-ANTIGEN TNP-OVA.

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The prevalence of respiratory allergy is increasing in industrialized countries. Environmental pollutants may play a role by acting as adjuvants, stimulating Thelper 2 (Th2) immune responses to common allergens. In vivo studies already showed that diesel exhaust particles (DEP) enhance immunoglobulin E (IgE) production in mice and man, but neither the adjuvant activity of other particulate pollutants nor their mode of action has been determined. In these studies, the modulating effects of DEP, silica and carbon black (CB) on the immune response to TNP-OVA (2,4,6 trinitrophenylphosphate-ovalbumin) as a defined reporter antigen were determined in a modified popliteal lymph node (PLN) assay. DEP, CB and silica were coinjected with TNP-OVA into the hind footpad of female BALB/c mice (6-8 weeks), four weeks later an intranasal challenge with TNP-OVA alone was given. Five days later the number of TNP-specific antibody forming cells (AFC) (IgG1, IgE, IgG2a) was determined in the PLN, spleen, bone marrow and parabronchial lymph nodes (PBLN) by ELISPOT assay. ELISA was performed on the sera. In DEP and CB treated animals the number of IgG1 and IgE producing AFC was significantly higher than in silica treated animals, while the latter possessed more IgG2a producing AFC. Serum titers were in accordance with the AFC numbers. TNP-specific antibodies could not be detected when the reporter antigen was injected alone. From these studies it can be concluded that the three particles possess intrinsic adjuvant activity. However, they modulate the immune response to the reporter antigen differently, resulting in Th2-like isotypes being produced in presence of DEP and CB, while the response to silica tends more to a Th1 response. At the moment, T cell cytokines and costimulatory molecules are assessed in the PLN assay.

800 ORAL SENSITIZATION TO FOOD PROTEINS; A BROWN NORWAY RAT FOOD ALLERGY MODEL.

L M J Knippels, A H Penninks and G F Houben. *TNO Nutrition and Food Research Institute, Zeist, The Netherlands.* Sponsor: B Kulig.

We developed an oral sensitization protocol for food proteins for the rat. In addition, we investigated several immune mediated effects after an oral challenge and specific protein recognition was studied to determine whether a comparable spectrum of proteins is recognized by the rat and human immune system. Young Brown Norway (BN) rats were exposed to 1 mg ovalbumin (OVA) by daily gavage dosing during 42 days without the use of an adjuvant. OVA-specific IgE and IgG responses were determined by ELISA. Upon an oral challenge with OVA effects on the respiratory system, blood pressure, and permeability of the gastro-intestinal barrier were studied. In addition, BN rats were orally exposed to a total hen's egg white-protein (HEW) extract and cow's milk (CM) and the specificities of induced antibody responses were compared with the specificities of antibodies in sera from egg and milk allergic patients using immunoblotting. Animals orally exposed to the allergens developed specific IgE and IgG antibodies which recognized the same proteins compared to antibodies from egg or CM allergic patients. After an oral challenge, gut permeability was increased. Some animals demonstrated a temporary decrease in breathing frequency or systolic blood pressure. We show that the Brown Norway rat provides a suitable animal model for inducing specific IgG and IgE responses upon daily intra gastric dosing of OVA without the use of an adjuvant. Moreover, local immune mediated effects upon oral challenge are observed. In addition we showed that enterally exposed BN rats and food allergic patients demonstrate antibody responses to a comparable selection of proteins upon exposure to different protein mixtures and further support that the BN rat may provide a suitable animal model for food allergy research and to study the allergenicity of (novel) food proteins.

801 LOCAL LYMPH NODE ASSAY : HAZARD IDENTIFICATION AND POTENCY ASSESSMENT.

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The murine local lymph node assay (LLNA) has been used extensively for the prospective identification of skin sensitization hazard. The method assesses contact sensitization potential as a function of proliferative activity induced in lymph nodes draining the site of topical exposure to test chemical. In the current investigations we have examined the use of the LLNA for the evaluation of relative potency of skin sensitizing chemicals. A range of materials of differing sensitizing potency has been examined using extended dose responses to allow the mathematical derivation of the estimated concentration of chemical required to induce a threefold increase in lymphocyte proliferation (EC3 value). Materials were ranked as strong (2,4-dinitrochlorobenzene, DNCB; Kathon CG; *p*-phenylenediamine, PPD), moderate (formaldehyde, isoeugenol) or weak sensitizers (eugenol) and non-sensitizers (*p*-aminobenzoic acid, PABA) on the basis of sensitization in humans. Each of the sensitizers stimulated a threefold increase in ^{3}H-thymidine incorporation, compared with concurrent vehicle controls, at one or more test concentrations; all materials would be classified therefore as contact allergens according to current LLNA criteria. The non-sensitizer PABA failed to induce signifciant proliferation at any concentration tested (maximum dose, 10%). Derivation of EC3 values revealed a ranking order of potency as follows : DNCB > PPD > Kathon CG > formaldehyde > isoeugenol > eugenol. These data suggest that in addition to hazard identification, the LLNA may be useful for the evaluation of relative skin sensitizing potency as a first step in risk assessment.

802 EVALUATION OF TRANSDERMALLY-DELIVERED DRUGS USING THE LOCAL LYMPH NODE ASSAY.

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Continuous exposure of skin to exogenous chemicals can lead to a condition of chemical hypersensitivity, or allergic contact dermatitis. As a predictive measure, guinea pig tests have been considered the model of choice. More recently, the Local Lymph Node (LLN) assay has been shown to be a reliable indicator of moderate to strong sensitizing activity. The purpose of this study was to establish, validate, and test the LLN assay for use in assessing the contact sensitizing potential of transdermally-delivered drugs. CBA/J mice were exposed daily for 3 consecutive days to chemical applied to the dorsal portion of both ears. Five days after the initial dose, radiolabeled thymidine was administered via the tail vein and nodes harvested 5 hours later. In the validation phase, the chemicals tested included 2,4-dinitrochlorobenzene (DNCB) and oxazolone (potent sensitizers), para-aminobenzoic acid (pABA, non-sensitizer), and the irritant, sodium lauryl sulphate (SLS). Oxazolone induced an average stimulation index (SI) of greater than 3-fold at every concentration tested and was dose responsive (0.05%,19.7; 0.1%, 33.5; 1.0%,77.2). DNCB produced a similar response (0.1%,5.7; 0.5%,41.5; 1.0%,45.9), but was less potent even at higher concentrations. SLS induced an SI above 3 that was consistent only at the highest concentration tested (20%), whereas pABA (10%) induced no response as expected. Vehicles tested included acetone:olive oil (4:1), dimethylsulfoxide, and dimethylformamide, with moderate increases observed in DNA radiolabel incorporation as compared to naïve control animals. Based on reports of potential human sensitization, commercially available estradiol and scopolamine were evaluated in the test phase. Concentrations tested included levels above and below human pharmacologic doses for both chemicals. Neither estradiol (0.025-0.4%) nor scopolamine (0.5-8.0%) induced a SI of 3-fold or greater. These results support the utility of the LLN assay in characterizing the sensitizing potential of transdermally-delivered drugs.

803 A MODIFIED MURINE LOCAL LYMPH NODE ASSAY: A TWO-TIERED APPROACH TO IDENTIFY CONTACT (PHOTO)ALLERGENIC POTENTIAL.

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To improve the predictive differentiation of contact allergy (CA) and photoallergy (CPA) from irritancy (IR) and phototoxicity (PT), induced by low molecular weight compounds, we developed a modified, two-tiered protocol of the murine local lymph node assay (LLNA). During tier I testing groups of female BALB/c mice received compound solution or vehicle (40% Dimethylacetamid, 30% Acetone, 30% Ethanol) alone on the dorsum of both ears on three consecutive days. Immediately after compound application indicated groups of mice were exposed to a UVA light-dose of 10J/cm^2. Auricular lymph nodes draining the ear tissue were excised 24 hr following the last exposure. Both the contact allergens oxazolone (OXA) and 2,4-dinitrochlorobenzene (DNCB) and the contact photoallergens 3,3',4',5-tetrachlorosalicylanilide (TCSA) and chlorpromazine (CLPZ) caused a concentration dependent increase in ear tissue-draining lymph node weights and cell counts pointing to an inflammatory process in the lymph nodes. However, the same effect was found following application of the irritant croton oil and the phototoxic compounds 8-methoxypsoralene (8-MOP) and acridine. To differentiate between allergic and non-allergic reactions, mice were sensitised with a selected tier I concentration of chemicals or treated with vehicle on the shaved back on 3 consecutive days with and without UVA exposure and challenged 12 days later on the ears as described above in order to investigate the expression of relevant cytokines at two stages of the evolution of an allergic response, the induction and the challenge phase (tier II analysis). Cytokine analysis on the gene expression level in freshly prepared total lymph node cells (LNC) by quantitative reverse transcription-polymerase chain reaction (RT-PCR) and on the protein level in vitro following restimulation of magnetically purified LN CD4 T cells via T cell receptor crosslinking revealed an increased expression of IL(Interleukin)-4 and IL-10 during the challenge phase of CA and CPA induced by OXA, DNCB, TSCA and CLPZ in comparison to the corresponding induction controls. This marked increase did not occur, when mice were challenged with the phototoxic or irritant chemicals 8-MOP, acridine and croton oil. In contrast, IL-2 and IFN(Interferon)-γ were detectable during CA, CPA, IR and PT, whereas IL-4 and IL-10 were not found during PT and IR. In conclusion, comparison of IL-4 and IL-10 expression during induction and challenge phase treatment with test chemicals represent a reliable possibility to identify CA and CPA in a two-tiered protocol of the murine LLNA.

804 THE USE OF COMPUTER MODELLING TO DEMONSTRATE THE 3D DISTRIBUTION OF ENZYMES WITHIN THE RAT NASAL CAVITY.

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The rat nasal cavity develops site-specific lesions when exposed to toxic compounds. In some cases (eg. formaldehyde) the site-selectivity is dependent upon airflow driven dosimetry, but other factors such as regional metabolism may also be critical. The distribution of biotransformation enzymes within the nasal epithelia is generally studied using immunohistochemistry (IHC) with a small number of standard transverse histological sections, which makes relating regional metabolism to lesion distribution difficult. The aim of this study was to model the distribution of glutathione S-transferase (GST) 3-3 in 3D. A model of the nasal cavity from a six week old male Wistar rat was generated using step wax sections (1 in 100μm) and a 3D image analysis system. Imaging densitometry was then used to semi-quantify GST 3-3 within step wax sections (1 in 600μm) stained by IHC. The distribution of GST 3-3 was then displayed upon the 3D model. The anterior of the cavity expressed relatively low levels of GST 3-3, while the posterior of the cavity stained highest. Staining was most intense at the tips of the ethmoturbinates and within the nasopharyngeal duct, areas which are within the major routes of inspiratory airflow. Other enzymes will now be modelled using this approach and their distribution correlated to lesion development.

805 AN INVESTIGATION INTO THE ANTIOXIDANT STATUS OF THE RAT NASAL CAVITY.

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Oxidative stress and free radical formation occurs as a result of normal cellular metabolism, or as a by-product of xenobiotic detoxification. This work aimed to characterize the antioxidant status of the metabolically active rat nasal mucosa, and to localize the distribution of several important antioxidant enzymes. The activities of glutathione peroxidase (GP), glutathione reductase (GR), superoxide dismutase (SOD) and catalase were determined using whole homogenates of male Wistar rat (180-200g) lung, olfactory epithelium (OE) and respiratory epithelium (RE). Immunohistochemistry was then used to localize GP, catalase, SOD (Zn/Cu) and SOD (Mn) in paraffin wax nasal sections. In general the RE showed a higher level of anti-oxidant activity compared to that of the OE and lung, however, SOD activity was approximately three fold higher in the OE than the RE. GP and catalase were localized within the apical cytoplasm and cilia of the RE columnar cells, the compound tubular glands and myelinated axon bundles in the lamina propria. Within the OE the apical cytoplasm of the sustentacular cells stained positive along with the neural bundles. SOD (Mn) and SOD (Zn/Cu) both had a similar distribution to catalase and GP within the OE and RE, except the intensity of RE staining was reduced for both isoforms. Thus the nasal epithelia express different antioxidant enzyme profiles, which may reflect different protective needs for each tissue type.

806 CORRELATION OF INHALED FORMALDEHYDE FLUX PREDICTIONS WITH REGIONAL DNA-PROTEIN CROSSLINK MEASUREMENTS IN RAT NASAL PASSAGES.

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DNA-protein crosslinks (DPX) are used as a dosimeter for inhaled formaldehyde and are associated with the presence of tumors induced in rat nasal passages in chronic studies. Previous studies (E. Cohen Hubal et al., *Toxicol. Appl. Pharmacol.* **143,** 47-55, 1997) linked airflow-driven uptake with DPX formation in the anterior rat nose. A 3-D computational fluid dynamics (CFD) model of a F344 rat nasal passage developed by J. Kimbell and coworkers, was used to quantify local wall mass flux of formaldehyde. Links between regional uptake estimates throughout the rat nose are needed to provide confidence in the model predictions of formaldehyde flux. Casanova and colleagues studied regional formaldehyde dosimetry in F344 rats (M. Casanova et al., *Fundam. Appl. Toxicol.* **23,** 525-36, 1994) by experimentally measuring DPX in two sites; one corresponding to high tumor incidence and the second corresponding to low tumor incidence in chronic studies. Flux and exposure concentration (ppm) were correlated with DPX separately in the high and low-tumor sites and then the regional results were compared with each other. Correlation of ppm and DPX showed a clear difference between the two regions. When flux, rather than ppm, was correlated with DPX, regional differences decreased, although flux alone was not sufficient to completely explain these differences. Additional information such as regional tissue thickness is needed. This study showed that regional flux is an important factor in estimating DPX formation. Flux predictions using human CFD models can be used to help predict cancer risk from inhaled formaldehyde.

807 LOCATION AND EXTENT OF MAJOR EPITHELIAL TYPES IN THE NASAL PASSAGES OF THE F344 RAT.

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Many chemicals induce site-specific responses in the nasal passages of the F344 rat. Major factors affecting such responses are local tissue dose and tissue susceptibility. To help account for tissue susceptibility in nasal lesion formation, the location and extent of the major epithelial types were obtained. Serial step, histological sections through the entire nasal passages of a 315g, male F344 rat were used for digitization of airway perimeters. The locations of squamous, transitional, respiratory, and olfactory epithelial types were determined by light microscopy and mapped onto each cross section. A three-dimensional computer reconstruction was created, color-coded by epithelial type, and used for the calculation of epithelial surface areas. In general, squamous epithelium lined the nasal vestibule, extending about 6 mm into the nose from the external nares and ventrally to the incisive duct. Transitional epithelium lined extravestibular lateral walls and turbinates, interdigitated with squamous epithelium on the lateral wall, and separated respiratory from squamous epithelium on the septal walls. Olfactory epithelium lined the dorsal aspect of the dorsal medial meatus, the ethmoid recesses, and the septum adjacent to the septal olfactory organ. Surface areas of squamous, transitional, respiratory, and olfactory epithelial types were 188, 209, 707, and 764 mm2, representing 10, 11, 38, and 41% of total nasal surface area, respectively. This study provides information for incorporating airway lining characteristics into dosimetry-response models for interspecies comparisons and prediction of risks to the human respiratory tract from inhaled chemicals and air pollutants.

808 MODELING FORMALDEHYDE DOSIMETRY IN THE RESPIRATORY TRACT OF HUMANS.

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To provide estimates of lower respiratory tract doses of inhaled formaldehyde (HCHO) for use in a risk assessment, a respiratory tract (RT) dosimetry model was used to simulate the uptake of HCHO in humans. The RT dosimetry model accounted for human RT anatomy, inhalation and exhalation, convection and dispersion of HCHO in the air phase, and it's absorption at the air-liquid lining surface. An activity pattern was used to define ventilatory parameters and oronasal breathing is taken into account. Lower RT anatomy was based on the idealized symmetric model of Wiebel (1963); dimensions for human nasal passages were based on magnetic resonance image scans. The nasal passage compartment of the RT dosimetry model was calibrated by varying parameters to reproduce the same HCHO uptake that a computational fluid dynamic (CFD) model predicted for these passages during unidirectional inspiratory airflow. The calibration also ensured that the appropriate amount of HCHO was delivered to the proximal end of the lower RT during the inhalation phase of the RT dosimetry model. Model results indicate that most of the absorbed HCHO is retained during inhalation and that the CFD unidirectional simulations results are essentially equivalent to those of a complete RT simulation with explicit inhalation and exhalation. HCHO flux to airway and air space surfaces is predicted to be the highest in the nasal passages; then flux decreases distally to the trachea, increases to a local maximum (less than in the nasal passages) at the third or fourth generation, and finally decreases rapidly, resulting in very small pulmonary region surface fluxes. This information allows prediction of human RT response to inhaled HCHO as an extrapolation of rodent cancer data. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

809 COMPARISON OF MAGNETIC RESONANCE IMAGING (MRI) AND HISTOLOGY OF THE NASAL PASSAGES OF MICE.

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Alterations in the nasal passages are important indicators of the effect of irritants on the respiratory system. Classically, histology in animals has been the technique employed to visualize these changes. However, it is known that fixation of tissue causes some shrinkage and/or swelling which can alter the surface area and volume of the airspace. Recently, we have shown cell loss, mucous secretion and other structural changes in mice due to exposure to respiratory irritants by an *in vivo* magnetic resonance imaging (MRI) technique. In the present study, direct comparisons between imaging live and fixed tissue have been made. A surface coil was built to achieve a resolution below 50 μm. Mice were imaged, immediately sacrificed and the heads were fixed. The sections were then paraffin embedded and stained by traditional methods or whole heads were dehydrated with alcohol and immediately imaged to detect tissue shrinkage caused by this process. Preliminary results show dramatic differences between live and fixed heads in control mice. The greatest change, a 2.5 fold increase in area in the fixed tissue, occurs in the dorsal meatus of the turbinates. Significant changes were seen in all parts of the upper respiratory tract. The prominent differences between the images and the histology sections demonstrate the advantage of MRI in assessing respiratory morphology.

810 EFFECTS OF PRE-EXISTING RHINITIS ON OZONE-INDUCED MUCOUS CELL METAPLASIA IN RATS.

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People with inflammatory airway disease may be more susceptible to the adverse health effects of air pollutants compared to the general population. Ozone, the major oxidant pollutant in smog causes rhinitis and nasal epithelial alterations in normal healthy rats. The toxic effects of ozone on rats with pre-existing rhinitis have not been investigated. The present study was designed to determine the effect of endotoxin-induced rhinitis on ozone-induced epithelial alterations, especially mucous cell metaplasia (MCM), in the nasal transitional epithelium (NTE) of rats. Male F344/N rats were exposed to either filtered air (0 ppm) or ozone (0.5 ppm) for 3 days (8 h/day), and killed 2 h or 4 days after the end of the last exposure. Six h prior to daily inhalation exposure, rats were intranasally instilled with either saline or endotoxin (100 μ g/day). After death, the maxilloturbinate from one nasal passage was processed for light microscopy to morphometrically determine the number of epithelial cells and neutrophils, and the amount of stored mucosubstances in the NTE. The maxilloturbinate from the other nasal passage was processed for analysis of mucin-specific (rMuc-5AC) mRNA expression. At 2 h postexposure, endotoxin/ozone-exposed rats had 48 and 3 times more neutrophils in the NTE than did saline/air- and saline/ozone-exposed rats, respectively. At 4 days postexposure, the amounts of intraepithelial mucosubstances and percent mucous cells in endotoxin/ozone-exposed rats were significantly greater than those in saline/air-exposed and saline/ozone-exposed rats (5 and 2 times greater, respectively). No MCM was present in rats exposed to endotoxin and air. Ozone-exposed rats had 35 % more NTE cells than air-exposed rats. At 2 h postexposure, both saline/ozone- and endotoxin/ozone-exposed rats had 2-fold increases in mucin mRNA expression, compared to saline/air-exposed controls. The results of the present study indicated that endotoxin-induced neutrophilic inflammation augments ozone-induced MCM (Research funded by NIHHL59391.)

811 ACROLEIN-INDUCED NASAL VASODILATION IS CGRP- AND NO- MEDIATED.

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Previous studies in this laboratory have shown that inspired acrolein produces an immediate, prolonged nasal vasodilatory response without alteration in nasal air flow resistance. The current study was aimed at examining potential mechanisms of this response. Male F344 rats were anesthetized and the nasal cavity isolated by insertion of an endotracheal tube to allow for isolated upper respiratory tract exposure to acrolein vapor. Nasal acetone uptake efficiency was monitored continuously during the 50 min exposure to monitor nasal perfusion rates. As evidenced by a large increase in nasal acetone scrubbing capacity, acrolein (1-10 ppm) produced a vasodilatory response that was fully developed within minutes of the onset of exposure. This response was maintained throughout the exposure. Pretreatment of animals with capsaicin (50 mg/kg, ip) 7 days prior to exposure to defunctionalize sensory c fibers, inhibited the response (p=0.003), providing strong evidence that this response was sensory nerve mediated. The response was not altered by NK-1 receptor antagonism suggesting substance P was not involved. The CGRP antagonist hCGRP 8-37 (200 mg/kg, iv during exposure) significantly attenuated (p=0.02) the response. Similarly, the response was attenuated (p=0.0002) by the nNOS selective inhibitor 7-nitroindazole (64 mg/kg, 30 min prior to exposure). In toto these results suggest that acrolein vapor stimulates nasal sensory neurons to release NO and CGRP which mediate, at least in part, the immediate vasodilatory response associated with exposure to this irritant. (Supported by NIEHS RO1-08765.)

812 ACUTE NASAL RESPONSE TO INSPIRED ACETALDEHYDE: ROLE OF SENSORY NERVES AND METABOLISM.

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Acetaldehyde is a sensory irritant present in environmental tobacco smoke. Previous studies have shown that inspired acetaldehyde produces an immediate nasal vasodilatory response. The aims of the current study were to: 1) investigate the role of nasal sensory nerves in this response, and 2) examine the relative importance of the acetaldehyde vis-a-vis its aldehyde dehydrogenase metabolite acetic acid. Towards these ends, the upper respiratory tract of the anesthetized male F344 rat was surgically isolated by insertion of an endotracheal tube, and vapor laden air was drawn through that site for 50 min. Acetone uptake was measured continuously during the exposure period to monitor nasal perfusion rates. To examine the role of nasal sensory nerves in the response to acetaldehyde, rats were pretreated with capsaicin (50 mg/kg sc), a dose known to defunctionalize sensory nerves. This pretreatment was without effect (p>0.05) suggesting that the vasodilatory response is not mediated by sensory nerve stimulation. Exposure to acetic acid vapor (240 ug/L) produced a response identical to that of acetaldehyde, suggesting that this metabolite may play a role in the acetaldehyde-induced nasal vasodilatory response. Pretreatment with the aldehyde dehydrogenase inhibitor cyanamide (10 mg/kg sc), however, did not alter the vasodilatory response to 10, 80, or 350 ug/L acetaldehyde (p>0.05). That cyanamide neither potentiated nor protected against the vasodilatory response suggests this response may be dependent upon both the parent, acetaldehyde, and its metabolite, acetic acid. (Research supported by NIH RO1 ES03676 and 1T32 ES07163.)

813 USING STEADY-STATE CLEARANCE MODELS TO ASSESS TARGET TISSUE METABOLIZED DOSE FOR INHALED VAPORS IN NASAL TISSUES AND IN SYTEMIC ORGANS.

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Clearance concepts have been applied to the nasal airflow to determine extraction of metabolized vapors under conditions of negligible systemic (arterial) concentration. This assumption may be appropriate for vapors with metabolism in the systemic tissues and in blood. However most metabolized vapors have significant accumulation in the arterial blood. These compounds can be metabolized both in nose and in systemic tissues with the potential to produce toxicity at these sites. Risk assessments for these metabolized vapors have to be based on rates of metabolism within relevant cell types. Here we link a nasal vapor clearance model with a whole body clearance model and compute nasal and liver dose for compounds that accumulate in arterial blood. These models were used to calculate tissue dose as a function of inhaled vapor concentration for chemicals with varied partition coefficients, metabolic rates and systemic clearance in both rats and humans. Nasal tissue dose estimates differ by several fold depending on the extent of accumulation of chemical in arterial blood. In addition, for metabolic processes with similar affinity constants in nose and liver, the half maximal rate of nasal metabolism occurs at lower inhaled concentrations than does half maximal saturation in the liver. Nasal effects of metabolites are likely to be limiting for risk assessments with inhaled compounds that affect both the liver and nose.

814 EFFECTS OF METHIMAZOLE ON THE OLFACTORY NEUROEPITHELIUM IN MICE.

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Methimazole, a drug used in antithyroid therapy, has been reported to induce olfactory disturbances in humans. NMRI mice were given injections of methimazole (2x50 mg/kg; ip) or saline. Histopathology showed marked morphological changes in the olfactory mucosa; necrosis and detachment of the olfactory neuroepithelium and necrosis in the Bowman's glands were observed. Pretreatment with the cytochrome P450 inhibitor metyrapone, prevented the methimazole-induced toxicity at this site. Using an antibody towards a marker of immature neurons, growth-associated protein 43 (GAP43), the regeneration of the olfactory neuroepithelium following methimazole administration was studied. A markedly increased number of GAP43 stained cells was seen two weeks after administration. Three months after administration the number of GAP43 stained cells were similar to controls suggesting an efficient neurogenesis and repair of the neuroepithelium. Additional mice were given 3H-methimazole (5 mg/kg; iv), killed 4 or 24 hours later and subjected to autoradiography. A distinct labelling of the lamina propria, preferentially in the Bowman's glands in the olfactory mucosa occurred. Also, in solvent-extracted tissue sections, there was a labelling of the lamina propria indicating presence of tissue-bound metabolites at this site. Metyrapone pretreatment reduced the level of binding in the olfactory mucosa. These results suggest a cytochrome P450-dependent metabolic activation and toxicity of methimazole in the mouse olfactory mucosa.

Regenerating olfactory neurons were observed two weeks after administration and after three months the olfactory neuroepithelium was repaired suggesting that the basal stem cells for olfactory neurons in the neuroepithelium were not permanently damaged by methimazole.

815 EFFICACY AND SAFETY OF RECOMBINANT HUMAN INSULIN-LIKE GROWTH FACTOR (rhIGF-I) IN A CANINE MODEL OF OSTEOARTHRITIS.

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rhIGF-I is a known growth and maintenance factor for chondrocytes. To test whether this agent may have potential use in the treatment of osteoarthritis (OA), the efficacy and safety were evaluated in a canine model where the anterior cruciate ligament is transected to produce OA of the femorotibial joint. Adult mongrel dogs underwent surgery 4 weeks prior to the initiation of treatment. Animals then received intra-articular injections of 0, 1 or 5 mg rhIGF-I once a week for 13 weeks. In addition to standard toxicological evaluations, monitoring of blood glucose, pharmacokinetics, and specialized pathology of the joint tissues were conducted. Hypoglycemia occurred between 0.5 and 3 hours after the 5 mg dose, while no hypoglycemia occurred in the 1 mg group. Glucose levels corresponded with mean serum rhIGF-I levels which were 1.7 and 2.7 fold baseline values in the 1 and 5 mg groups, respectively. Transient hypoglycemia was the only adverse effect noted in the study. Histological parameters of efficacy, including assessment of cartilage by Mankin scoring of Safranin O stained sections, cartilage structural morphometry, and assessment of collagen content, all demonstrated that osteoarthritic lesions were attenuated in treated groups. The primary effect was preservation of proteoglycan staining. Chondrocytes were also maintained in rhIGF-I treated groups and some indications of chondroregeneration were observed. This study indicated that rhIGF-I treatment was well-tolerated in this disease model and may be a chondroprotective agent in osteoarthritis by acting as an anabolic agent for matrix components.

816 TELEMETRIC EVALUATION OF THE CARDIOVASCULAR EFFECTS OF TNK-tPA IN CYNOMOLGUS MONKEYS.

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TNK-tPA, a fibrinolytic being developed for myocardial infarction, is a tPA variant with site-specific amino acid substitutions that have resulted in increased fibrin specificity, PAI-1 resistance and plasma half-life. Due to its extended half-life, TNK-tPA is given bolus IV while tPA is given as a continuous infusion. A primate safety pharmacology study to assess cardiovascular changes attributed to TNK-tPA was performed. Mean arterial blood pressure (MAP), heart rate (HR) and ECG (lead II) after bolus administration of TNK-tPA were assessed in conscious cynomolgus monkeys using telemetry. TNK-tPA at doses of 0.003, 0.03, 0.3, 3.0 and 30 mg/kg were administered IV to 3 female monkeys in a dose-escalation manner. Since TNK-tPA is antigenic in most laboratory species, all animals were treated within a 10-day period. The dosing schedule is shown:

Dose (mg/kg)	0.003	0.03	0.3	3.0	30.0
Study Day	1	3	6	8	10

TNK-tPA (0.003-0.3 mg/kg) did not effect MAP, HR or ECGs. TNK-tPA at 3.0 mg/kg, approximately 6x the expected clinical dose, increased MAP (10-22%) and HR (7-13%). These effects returned to baseline levels within 30 min. No ECG changes were observed. TNK-tPA (30 mg/kg) induced an immediate reduction in MAP (60%) with an increase in HR (17-32%). Epinephrine was administered to improve the status of one animal. The ECG waveform of one animal exhibited an inverted T-wave while the amplitude of the T-waves from the remaining two animals was attenuated. These effects were evident first hour with levels returning to near normal by 6 hours post-dose. All animals were ataxic at 6 hours post-dose with complete recovery in two animals by 24 hours. Bolus IV administration of TNK-tPA up to 3.0 mg/kg was well tolerated and produced pharmacologically expected minor alterations of MAP and HR in conscious monkeys.

817 COMPARISON OF THE PHARMACOKINETIC PROFILE OF A [3H]-CHOLESTEROL-CONJUGATED AND [3H]-UNCONJUGATED P=O MOE.

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First generation phosphorothioate antisense oligodeoxynucleotides (P=S-ODN) have been shown effective as therapeutics in preclinical and clinical trials. Oligonucleotides are being developed to optimize the antisense mechanism of action while minimizing non-sequence dependent effects. The phosphodiester, 2'-methoxyethyl (P=O MOE) oligodeoxyucleotides have been designed in response to the above. However, these compounds are poorly bound to blood-proteins, resulting in rapid excretion. It is hypothesized that conjugation of a P=O MOE with a lipophilic molecule would alter its pharmacokinetic profile. Male rats were administered 3 mg/kg (1.79 uCi/mg) intravenous injections of a tritiated P=O MOE (either conjugated with cholesterol or unconjugated) via the lateral tail vein. Plasma, excreta, and tissues were collected and analyzed for radioactivity by LSC spectroscopy. The plasma elimination half-life of the cholesterol P=O MOE was significantly longer than that of the unconjugated species. In conjunction, urinary excretion of the cholesterol P=O MOE was less (<5% dose from 0-6 hours) than that of the unconjugated P= O MOE (>35% dose from 0-6 hours). Further, the tissue distribution of the cholesterol P=O MOE was significantly broader than that of the unconjugated species. Although the concentration (ug eq/g) of the unconjugated P=O MOE was greater in the kidney cortex 3 and 24 hours post dose, the concentration of the cholesterol P=O MOE was at least 2-fold greater in other tissues; including the plasma, liver, spleen and small and large intestines at 3 and 24 hours post dose. In summary, the addition of a lipophilic molecule to a P=O MOE antisense oligodeoxynucleotide was shown to improve its pharmacokinetic profile.

818 THE EFFECTS OF MODIFIED PHOSPHOROTHIOATE OLIGODEOXYNUCLEOTIDES (P=S ODN) TARGETING C-*RAF* IN THE MOUSE.

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Chemical modifications to oligonucleotides are being explored as ways to reduce toxicity and increase potency of antisense therapeutics. This study investigated the toxicity and the reduction in C-*raf* mRNA in CD-1 mice produced by 10 days of IV administration at 10, 50 or 100 mg/kg of a murine-specific 2' methoxyethyl (MOE) modified P=S ODN or a human-specific MOE modified P=S ODN. Clinical pathology indices were normal with both MOE P=S ODNs. Liver weights of mice treated with the murine MOE P=S ODN were ≈ 10% heavier than control, although no histopathology was evident. Levels of C-*raf* mRNA were unchanged from control in the heart; however, levels were decreased at all doses in the liver with the murine MOE P=S ODN. No reduction in C-*raf* mRNA levels was detected with the human-specific MOE P=S ODN. In previous studies with an unmodified P=S ODN active toward murine C-*raf*, doses <25 mg/kg were ineffective in reducing C-*raf* mRNA in mouse liver. Analysis of tissue distribution of the MOE P=S ODNs indicates concentrations are higher in spleen and lower in kidney and liver than with P=S ODNs. To further investigate the liver effect, a 28-day study at a dose of 100 mg/kg was conducted and revealed moderate hepatocellular effects that included cytoplasmic eosinophilia and correlated with an increase in serum transminase levels. These effects were less severe than observed with P=S ODNs. In addition, the MOE P=S ODN did not induce immune stimulation characteristic of the P=S ODNs. In summary, the MOE modified P=S ODNs decreased toxicity and increased potency, and thus, this modification appears promising as a new antisense oligonucleotide.

819 4-WEEK TOXICITY AND TOXICOKINETIC STUDY OF SUBCUTANEOUSLY ADMINISTERED ANAKINRA (IL-1ra) AND ORALLY ADMINISTERED METHOTREXATE IN RATS.

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Recombinant methionyl interleukin-1 receptor antagonist (IL-1ra) is a cytokine proposed to treat rheumatoid arthritis. Since IL-1ra may be used clinically in combination with Methotrexate (MTX), the objective of this study was to assess the interactive effects of IL-1ra (administered subcutaneously) and MTX (administered orally) to rats on target organ toxicity and to determine the toxicokinetic profile of each drug. One hundred fifty-three female rats (17 per group assigned to 9 groups) were treated daily with 0, 10,

or 100 mg/kg/day IL-1ra in various combinations with 0, 0.04, or 0.20 mg/kg/day MTX for 28 days. There were no test article-related effects on body weight, food intake, and ophthalmologic data throughout the study. Pale skin was observed in 15 animals treated with 0.20 mg MTX/kg alone or in combination with IL-1ra. Approximately 50% of the animals receiving 0.20 mg MTX/kg had slightly decreased (~5-10%) red blood cell counts, hemoglobin, hematocrit, and more severely decreased absolute reticulocyte counts (~42-58%). Rats treated with 0.20 MTX/kg had increased (~10-20%) platelet counts, albumin-to-globulin ratio, and potassium. Coadministration of IL-1ra with MTX had no effect on these findings. Neither IL-1ra nor MTX had any apparent effect on the systemic exposure (assessed by AUC over 24 hours) of the other when administered concomitantly. In groups treated with 0.20 mg/kg MTX (with or without IL-1ra), bone marrow hypocellularity and/or an increased incidence of light-colored femoral bone marrow was noted. An increased incidence and severity of histiocytic infiltration was present in the spleens of 5 of 5 animals given 0.20 mg MTX/kg alone. This change was not present in animals treated in combination with IL-1ra. Increased chronic-active inflammation at the injection site was seen in some animals treated with IL-1ra at 100mg/kg. There was no apparent correlation between the dose of IL-1ra and/or MTX on the antibody response to IL-1ra. In conclusion, the no-observable-effect level for MTX was 0.04 mg/kg/day. The no-observable-adverse-effect level for systemic toxicity for IL-1ra was 100 mg/kg/day. Since no evidence of any adverse drug-drug interactions were observed, clinical coadministration of IL-1ra and MTX is not expected to produce any additional or interactive toxicity.

820 CHRONIC TOXICITY ASSESSMENT OF SUBCUTANEOUSLY INJECTED PEG-rHuMGDF IN RHESUS MONKEYS.

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PEG-rHuMGDF is a modified recombinant human protein which has pronounced thrombopoietic effects through stimulation of the c-mpl receptor. The objective of this study was to assess the chronic toxicity of PEG-rHuMGDF in rhesus monkeys (3-5/sex/group) after daily subcutaneous doses of 0, 5, 25, 100 or 250 μ g/kg/day. Platelet counts were markedly increased (~5-fold baseline) in all PEG-rHuMGDF treated groups by Day 14. Mean platelet counts generally remained above baseline values for doses ≥ 25 μ g/kg/day throughout the study, although individual animals in all PEG-rHuMGDF treated groups had below normal platelet counts. Mean white blood cell counts were increased in most PEG-rHuMGDF treated animals on Day 14 and declined thereafter. Mean red blood cell counts, hemoglobin, hematocrit, mean corpuscular volume and mean cell hemoglobin were decreased in all PEG-rHuMGDF treated groups, beginning on Day 14. Reticulocytes showed only a modest increase, suggesting inhibition of RBC production as the cause of the anemia. Histologic changes to the bone marrow were characterized as megakaryocyte hyperplasia or hypoplasia, with erythroid hypoplasia and myeloid hyperplasia also observed. Increased numbers of reticulin fibers were present in the marrow of all PEG-rHuMGDF treated animals. Extramedullary hematopoiesis (EMH), predominantly erythroid, occasionally also megakaryocytic, was noted in the spleen, liver and lymph nodes. Other tissues, such as the heart, kidney, testes, and thymus also had evidence of EMH in individual animals. Antibodies to PEG-rHuMGDF developed in all animals and appeared to diminish the biological effects of (i.e., decreased platelet counts and megakaryocyte hypoplasia) and influenced the exposure to PEG-rHuMGDF in some animals, particularly in the lower dose groups. In summary, PEG-rHuMGDF was well tolerated in rhesus monkeys for up to 6 months of daily dosing, producing changes that are consistent with its pharmacologic effects.

821 SUBACUTE TOXICITY STUDY OF A RIBOZYME TARGETING VEGF RECEPTOR mRNA (ANGIOZYME™) IN FEMALE CD-1 MICE.

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Vascular endothelial growth factor (VEGF) has been implicated as a contributing growth factor to the angiogenesis of developing tumors. A ribozyme (ANGIOZYME™) has been developed against VEGF receptor subtype *Flt*-1 mRNA as a potential antiangiogenic therapeutic. In previous rodent studies, daily administration of phosphorothioate oligodeoxynucleotides has been associated with immune stimulation as well as changes in hematology and serum chemistry parameters. ANGIOZYME™ includes some phosphorothioate linkags and it is possible that repeat high-dose administration of ANGIOZYME™ could result in similar effects. To determine the toxicology and toxicokinetic profile of this ribozyme, female CD-1 mice were administered a daily sc bolus of 30, 100, or 300 mg/kg ANGIOZYME™ for 14 days. Body weight and food and water consumption were monitored throughout the 14-day study. Blood was collected for serum chemistry and hematology endpoints on days 1, 7 and 14 after initiation of dosing. In addition, the weights of major organs (kidney, liver, pancreas and spleen) were measured. On days 1 and 14, blood was collected from an additional group of animals at 30, 90 and 180 min following ANGIOZYME™ administration. ANGIOZYME™ concentrations in plasma were quantitated by high-performance liquid-chromatography. Daily sc bolus administration of 30-300 mg/kg ANGIOZYME™ for 14 days was very well tolerated in female CD-1 mice. No animals died or were euthanized prematurely during the study. There were no treatment-related changes in serum chemistry, hematology, food and water consumption, body or organ weight. Peak plasma concentrations 30 min after a 300 mg/kg sc bolus on day 1 were 154 ± 39 μg/mL. No adverse effects were observed in this study despite the appreciable systemic exposure achieved in the mice as indicated by plasma concentrations of ANGIOZYME™.

822 A 13 WEEK TOXICITY STUDY OF OSTEOPROTEGERIN (OPG) IN THE CYNOMOLGUS MONKEY.

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Osteoprotegerin (OPG) acts as an antiresorptive agent that negatively regulates osteoclast maturation which can lead to the excess accumulation of newly synthesized bone and cartilage *in vivo*. The purpose of this study was to investigate the potential toxicity of a human recombinant form of OPG in the young cynomolgus monkey during intravenous (iv) or subcutaneous (sc) injection three times per week for up to 13 weeks. Six animals/sex/group were randomized to four groups; controls received placebo iv and treated groups received OPG at 1, 5 or 15 mg/kg/occasion iv. An additional group of three animals/sex received OPG at 15 mg/kg/occasion sc. There were no deaths during the study, no clinical signs related to treatment, no effect on body weight, appetence or ophthalmology, and no toxicologically meaningful changes in routine laboratory investigations, organ weights, gross or histopathological findings. Serum ionized calcium and phosphorus were decreased at all dose levels. Evaluations were performed to monitor biochemical markers of bone resorption (N-telopeptide [NTx], deoxypyridinoline [DPD]), bone formation (skeletal alkaline phosphatase [sALP], osteocalcin [OC]), PTH and bone density of the proximal tibia and distal radius *in vivo*. Dose-related decreases in NTx and/or DPD were observed at each dose level with up to 90% decreases in NTx noted for animals treated iv or sc at 15 mg/kg/occasion. Similar decreases were observed for sALP and OC. PTH was increased for animals treated at 5 or 15 mg/kg/occasion (iv/sc). Trabecular bone density was increased for the majority of animals treated iv or sc at 15 mg/kg/occasion and males treated iv at 5 mg/kg/occasion. Microscopic examination of the sternebrae revealed corresponding increases in bone. Decreases in markers of bone turnover, and corresponding increases in bone density, were consistent with the pharmacological action of OPG as an osteoclast inhibitor. The no observable adverse effect level of OPG was 15 mg/kg/occasion.

823 A SAFETY EVALUATION OF HEMOLINK™, AN O-RAFFINOSE, CROSS-LINKED HUMAN HEMOGLOBIN-BASED OXYGEN CARRIER.

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Hemolink™ is a hemoglobin-based oxygen carrier prepared in a Ringer's Lactate solution vehicle. This material is expected to be used in such clinical indications as acute normovolemic hemodilution to enhance autologous blood donation and substitution for red cell transfusion in cases of acute blood loss. The purpose of this study was to determine the degree of tolerability by dogs of a repeated daily dosing regimen of i.v. administered Hemolink™ over a 14-day period in a daily dose at least equal to that contemplated for a single therapeutic administration. Evaluations were made of mortality, clinical observations, body weights, food consumption values, electrocardiograms, clinical pathology and hematology parameters, urinaly-

sis data, organ weight changes and gross and microscopic pathology. There were no effects of Hemolink™ at 20 and 30 ml/kg per day that resulted in any functionally significant findings, and effects were reversible (except for yellow sclera, increased liver and kidney weights, the presence of iron positive staining material was found in macrophages in some tissues and within the lumina of the proximal tubules of the kidneys). Findings at necropsy, such as organ weight or intestinal changes (thickening), were not accompanied by histopathological findings other than iron accumulation. The absence of demonstrable adverse effects in tissues with iron positive deposits suggests that there should be no long-term adverse effects as a result of Hemolink™ administration. Furthermore, as the clinical administration of this material to humans at doses comparable to 20 and 30 ml/kg would occur as a single dose, which would be cleared from the circulation relatively rapidly, the accumulation of a very large extracellular hemoglobin load as occurred in this 14-day repeat dose study would not be expected to occur under actual conditions of clinical use.

824 INHIBITION OF NF-κB IN PRIMARY HUMAN CD19+ B LYMPHOCYTES BY HYDROQUINONE.

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Hydroquinone (HQ), a reactive metabolite of benzene, is known to inhibit mitogen stimulated activation of both T and B lymphocytes. Despite extensive study, the underlying mechanism for HQ's immunotoxicity is not clear. We have previously demonstrated that 1 uM HQ inhibits TNF induced activation of NF-κB in CD4+ T cells, resulting in decreased IL-2 production. NF-κB, known to be important in T lymphocytes, also plays a critical role in normal B cell development and activation. We therefore hypothesized that alterations in NF-κB might be involved in HQ induced B cell immuno-suppression as well. In this study, we demonstrate that 1-5 uM HQ inhibits PMA/ionomycin induced activation of NF-κB in primary human CD19+ B cells. Inhibition of NF-κB is accompanied by a dose dependent decrease in PMA stimulated production of TNF with no corresponding loss in viability. HQ does not appear to alter NF-κB directly as pre-incubation of B cell nuclear extracts with HQ does not diminish activity of this protein. In contrast to our finding in T cells, inhibition of NF-κB by HQ in B cells is not reversible after 72 hours in culture suggesting a long-term functional suppression. These data support our original findings in T cells and suggests that NF-κB is particularly susceptible to inhibition by HQ. We further hypothesize that inhibition of NF-κB in lymphocytes and perhaps other cell types as well may play a significant role in HQ's toxicity.

825 INHIBITION OF MAPK (ERK) ACTIVATION BY CANNABINOL IN PMA/IO-STIMULATED MOUSE SPLENOCYTES.

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Cannabinol (CBN) is a plant derived cannabinoid compound which exhibits immunosuppressive actions with minimal CNS activity. We have previously demonstrated that the immunosuppression by CBN involves decreased AP-1 binding activity to both the proximal AP-1 site and the distal NF-AT site in the IL-2 promoter following cellular activation for 240 min. The decreased AP-1 activity was mediated, in part, by decreased nuclear expression of both c-fos and c-jun. This decrease in protein expression; however, was not due to perturbations in nuclear import, nor to inhibition of steady state mRNA expression. Thus, we investigated the effects of CBN on ERK MAP kinase activation as this is one kinase important in the post translational modification of both c-fos and c-jun. Using the MEK inhibitor D098059, we demonstrated that ERK does indeed play a role in IL-2 production and c-fos nuclear protein expression in activated mouse splenocytes. Activated (i.e., phosphorylated) ERK protein was detected with phorbol ester (PMA, 40 nM) plus calcium ionophore (Io, 0.5 mM) within 5 min, with peak activation at 20 min using Western analysis. This early ERK activation by PMA/Io was inhibited slightly in the presence of CBN. We further demonstrated that ERK activation was inhibited more robustly by CBN following a 240 minute activation with PMA/Io. Interestingly, in the absence of PMA/Io, 20 mM CBN slightly induced ERK activation, confirming earlier reports of the ability of cannabinoid agonists to activate MAP kinases. These studies provide evidence that cannabinoid compounds affect the activation of ERK MAP kinases and this may contribute to the modulation of nuclear protein expression of c-fos and c-jun, and subsequently, AP-1 activity in the IL-2 promoter. (This work was supported by NIH Grant DA070908 and E.I. Dupont DeNemours & Co.)

826 CANNABINOL INHIBITION OF INTERLEUKIN-4 EXPRESSION IN EL4.IL2 CELLS IS MEDIATED THROUGH DOWN-REGULATION OF NF-AT.

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Cannabinoid compounds are widely established as being psychoactive and immunosuppressive. One of the most sensitive immune responses inhibited by cannabinoids is the T-cell dependent IgM antibody forming cell (AFC) response which is a measure of humoral immunity and requires the function of T-cell. To elucidate the mechanisms underlying cannabinoid-mediated immunosuppression, previous experiments in our laboratory have demonstrated that cannabinol and D9-tetrahydrocannabinol inhibit the expression of interleukin-2 (IL-2) in EL4.IL2 cells, a murine T-cell lymphoma line. In addition to IL-2, IL-4 is another T-cell secreted cytokine critical for the development of humoral immunity. IL-4 is also essential in type I hypersensitivity disorders, such as asthma, by mediating immunoglobulin class switching to promote IgE production. The objective of the present study was to examine the effects of cannabinol on the IL-4 expression in EL4.IL2 cells. IL-4 expression in EL4.IL2 cells was induced by ionomycin (1 μM) alone or in combination with PMA (80 nM). It is notable that ionomycin alone induced IL-4 but not IL-2 expression, whereas ionomycin plus PMA induced both IL-4 and IL-2 expression in EL4.IL2 cells. Treatment with cannabinol (1-20 μM) 30 minutes prior to either ionomycin alone or in combination with PMA attenuated both IL-4 mRNA and protein levels in a concentration dependent manner. Decreased binding activity to the IL-4 promoter P0 site, a NF-AT motif, by cannabinol treated cells was also observed in EMSA experiments, indicating that cannabinol down-regulates IL-4 expression at transcription level by disrupting the NF-AT signaling pathway. These results suggest that the inhibition of IL-4 expression by cannabinol may contribute to its immunosuppressive effects and the marked sensitivity of the T-cell dependent AFC response to cannabinoids. (This work was supported by NIDA Grant DA09789 and DA07908.)

827 THE ROLE OF CALCIUM AND CYCLOOXYGENASE ACTIVITY ON THE INHIBITION OF IL-2 BY THE ENDOGENOUS CANNABINOID, 2-ARACHIDONYL-GLYCEROL.

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2-arachidonyl-glycerol (2-Ara-Gl), a fatty acid ethanolamide, was recently identified as a putative endogenous ligand for cannabinoid receptor types, CB1 and CB2. Follow-up evaluations demonstrated that 2-Ara-Gl possessed immunomodulatory activity including inhibition of IL-2 expression. The objective of the present studies was to investigate signaling pathways involved in 2-Ara-Gl-mediated IL-2 inhibition. Pretreatment of splenocytes from female B6C3F1 mice with 20 μM 2-Ara-Gl strongly suppressed phorbol 12-myristate 13-acetate plus ionomycin-stimulated IL-2 secretion and steady-state mRNA expression. This inhibition was partially reversed by agents which increased [Ca2+]i including the calcium ionophore ionomycin and Ca+2-ATPases inhibitors, thapsigargin or cyclopiazonic acid. Furthermore, pretreatment of splenocytes with cyclooxygenase inhibitors indomethacin or flurbiprofen completely blocked the 2-Ara-Gl-mediated IL-2 inhibition. This recovery of 2-Ara-Gl-elicited IL-2 inhibition by ionomycin and indomethacin appeared to be mediated at the transcriptional level as evidenced by a similar reversal of the inhibition of a CAT reporter gene under the control of the IL-2 promotor/enhancer region. Phenylmethylsul-fonyl fluoride, an amidase inhibitor, had marginal effects on the 2-Ara-Gl-mediated inhibition of IL-2 expression. In contrast, lipoxygenase inhibitor nordihydroguaiaretic acid failed to block this inhibition. Taken together, these data suggest that a significant component of the IL-2 inhibition by 2-Ara-Gl involves the perturbation of Ca2+ signaling and cyclooxygenase pathway.

828 DEOXYNIVALENOL (VOMITOXIN) AND TNFα ACT SYNERGISTICALLY TO INDUCE THYMOCYTE APOPTOSIS THROUGH MECHANISMS INVOLVING INTRACELLULAR Ca^{2+}, REACTIVE OXYGEN SPECIES, MAPKs, AND CASPASE-3.

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The trichothecene mycotoxins are a group of sesquiterpenoid fungal metabolites produced by several commonly occurring molds. Preliminary studies in our lab indicate that co-exposure of mice to small doses of bacterial lipopolysaccharide (LPS) and the common foodborne trichothecene deoxynivalenol (DON or vomitoxin) results in massive apoptosis of lymphoid organs and that thymocytes are a primary target. The effect of co-exposure to DON and LPS or known apoptotic mediators induced by LPS was assessed in murine thymocytes. Apoptosis was determined morphologically using DNA binding dyes. Co-exposure to DON and LPS, prostaglandin E_2 (PGE_2), dexamethasone (DEX), or anti-Fas did not significantly influence thymocyte apoptosis. Co-exposure to 250 or 500 ng/ml DON and 4 or 20 ng/ml TNFα significantly increased thymocyte apoptosis approximately 3-fold over control values ($p<0.05$). DON or TNFα alone did not significantly increase apoptosis at these concentrations. Signal transduction mechanisms were studied using several pharmacological inhibitors and chelators. Intracellular Ca^{2+} chelator BAPTA-AM, free radical scavengers N-acetyl-cysteine (NAC) and glutathione (GSH), MAPK inhibitor PD98059, SAPK inhibitor SB203580, and caspase-3 inhibitor Ac-DEVD-CHO significat\ntly reduced DON/TNFα induced apoptosis by 87.8%, 88.1%, 74.1%, 78.7%, 87.0%, and 59.9% respectively. Extracellular Ca^{2+} chelator EDTA, and caspase-1 inhibitor Ac-YAD-AOM did not significantly decrease DON-TNFα induced apoptosis. The results demonstrate that thymocyte apoptosis resulting from DON and LPS co-exposure may be mediated by TNFα and involve upregulation of secondary messengers, including intracellular Ca^{2+} and ROS, which trigger the MAP kinases and result in caspase-3 activation. (This work was supported by PHS Grants ES-03358 and T32 ESO7255.)

829 POTENTIATION AND ATTENUATION OF TRANSCRIPTION FACTOR ACTIVITY IN MURINE SPLEEN FOLLOWING ORAL EXPOSURE TO THE TRICHOTHECENE VOMITOXIN (DEOXYNIVALENOL).

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The capacity of the trichothecene mycotoxin vomitoxin (VT or deoxynivalenol) to superinduce expression of mRNAs for TNF-α, IL-6 and other cytokines is likely to involve transcriptional and /or posttranscriptional mechanisms. Using electrophoretic mobility shift assay, we assessed the *in vivo* effects of VT on several transcription factors which bind transcriptional control motifs identified in the TNF-α and IL-6 promoters. The binding of NF-IL6, AP-1, CREB, AP-2, SP-1 and NF-κB was measured at 0, 0.5, 1.5, 4 and 8 hr after oral exposure to 25 mg/kg BW of VT. NF-IL6 binding activity was elevated at 30 min and declined within 90 min with 8 hr values still bellowing control. CREB and AP-1 binding activities decreased slightly at 30 min but gradually increased, reaching a maximum at 4 hr. AP-2 and SP-1 binding activity decreased and reached a minimum at 4 hr. NF-kB was increased slightly only at 4 hr. The specificity of NF-IL6, AP-1, CREB, AP-2, SP-1 and NFκB to the relevant DNA motifs were verified by competition assays using an excess of unlabeled consensus and mutant oligonucleotides. Supershift analyses were used to identify the VT- inducible DNA binding proteins for NF-IL6 (CEBPβ), AP-1 (JunD, cJun/AP-1,cFos), CREB (ATF-2 and ATF-3) and NF-κB (p50 and cRel). Taken together, the results indicate that VT exposure can potentiate or attenuate binding activities of transcription factors specific for potential regulatory motifs in TNF-α and IL-6 promoter region. These altered activities may contribute, in part, to VT-induced gene expression of the cytokines. (Supported by PHS Grant ES 03358.)

830 DOWN-REGULATION OF A ENDOPLASMIC RETICULUM CHAPERONE, GRP78/BiP, BY VOMITOXIN (DEOXYNIVALENOL) IN MURINE EL-4 THYMOMA CELLS.

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The mechanisms by which vomitoxin (VT or deoxynivalenol) causes cytokine superinduction and apoptotic cell death are not fully understood. In the present study, we have applied mRNA differential display analysis to evaluate changes in gene expression induced by VT in murine EL-4 thymoma cells stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin (ION). These conditions have been previously shown to promote cytokine superinduction. We found that VT decreased the expression of GRP78/BiP, a stress-response gene induced by agents or conditions that adversely affect endoplasmic reticulum function. Competitive RT-PCR analysis revealed that 250 ng/ml VT decreased GRP78/BiP mRNA expression in both unstimulated and PMA/ION-stimulated EL-4 cells at 6 hr and 24 hr after VT treatment. In accordance with the RT-PCR analysis, Western blot analysis confirmed that GRP/BiP protein level was significantly diminished in a dose response fashion by VT (50 to 1000 ng/ml) in PMA/ION-stimulated cells. GRP78/BiP has been shown to play a role in regulation of protein folding and secretion, and to protect a cell from apoptosis. In PMA/ION-stimulated cells, 200 bp DNA laddering, a hallmark of apoptosis, was shown in a dose-dependent fashion from 50 to 1000 ng/ml VT at 24 hr. In contrast, when the cells were treated with 50 to 1000 ng/ml VT only for up to 48 hr, the 200 bp DNA laddering was not found. These results suggest that the capacity of VT to induce cytokine superinduction or apoptotic cell death may be due, in part, to a reduction of the GRP78/BiP stress response.

831 INHIBITION OF HUMAN CYTOKINE PRODUCTION BY PHENOLIC COMPONENTS OF CIGARETTE TAR.

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Cigarette smoking has been shown to induce profound, but localized, suppression of T cell responses in the lungs and lung-associated lymph nodes. We previously demonstrated that both HQ and catechol, but not phenol, cause an immediate cessation in DNA synthesis in human lymphocytes. In the current project, we studied the effects of HQ, catechol and phenol on human cytokine production *in vitro*. Human peripheral blood mononuclear cells were treated with 50μ M HQ, catechol or phenol for three hours, then stimulated with 1μ g/ml ConA and 3nM PMA. Supernatants were collected at 16 hrs (IL-1β and TNFα) and 24 hours (IL-2 and IFNγ) and assayed for cytokines levels by ELISA. HQ, catechol and phenol inhibited production of IL-1β , IL-2 and IFN-γ by >75-95% without loss of cell viability, but TNFα production was relatively resistant to these phenolic compounds. Unlike the effects on DNA synthesis, which were immediate and reversible, the inhibitory effects of HQ and catechol on IL-2 and IFNγ production required a three-hour incubation with the cells and could not be reversed by washing. These experiments suggest that HQ and catechol have distinct effects on early and late events in T cell activation, and that phenol also has immunosuppressive properties. (Supported by NIH grant HL60538.)

832 DOSE RELATED SUPERINDUCTION AND SUPPRESSION OF CYTOKINES BY MACROCYCLIC TRICHOTHECENES IN MURINE CLONAL T-CELL AND MACROPHAGE MODELS.

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Foodborne type A and B trichothecenes including vomitoxin (deoxynivalenol, DON) have been previously assessed for their cytotoxic and immunotoxic effects. Several macrocyclic (group D) trichothecenes, notably the satratoxins, have been associated with cases of sick building syndrome. The effect of macrocyclic trichothecenes on cytokine secretion were assessed in murine EL-4 cells as well as RAW 264.7 cells. The EL-4 cells were stimulated with phorbol 2-myristate 13-acetate (5 ng/ml) and ionomycin (100 ng/ml) for 72 hrs to a various concentrations of satratoxin G (ST-G), satratoxin H (ST-H), isosatratoxin F (iST-F), roridin A (ROR-A), and verrucarin A (VER-A). After 72 hrs incubation, IL-2 secretion was increased by the concentrations of 0.5, 1.0, 1.0, 0.1, and 0.25 ng/ml for ST-G, ST-H, iST-F, ROR-A, and VER-A, respectively. At higher mycotoxin concentrations, cytokine production and proliferation were markedly impaired. RAW 264.7 murine macrophage cells were unstimulated or stimulated with lipopolysaccharide (LPS) and exposed to various concentrations of the macrocyclic trichothecenes mentioned above for 72 hrs. With LPS stimulation (50 ng/ml), the secretion of TNF-α was increased 2-3 fold compared to controls after 48 or 72 hrs incubation in the presence of 1.0, 2.5, 2.5, 1.0, and 1.0 ng/ml of ST-G, ST-H, iST-F, ROR-A, and VER-A, respectively. TNF-α

production was suppressed at higher mycotoxin concentrations. IL-6 secretion was not superinduced even with LPS stimulation. Without LPS stimulation, neither TNF-α nor IL-6 was induced. These results suggest that the macrocyclic trichothecenes can superinduce secretion of IL-2 in EL-4 cells and secretion of TNF-α in RAW 264.7 cells.

833 POTENTIATION OF THE INHIBITORY EFFECT OF HYDROQUINONE ON HUMAN TNFα PRODUCTION BY OXIDATIVE STRESS.

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Hydroquinone is a major constituent of cigarette tar that may play an important role in the suppression of T cell immunity in the lungs of smokers. We have found that hydroquinone (HQ), at concentrations found in smokers lungs, is a potent inhibitor of human IL-1β , IL-2, and IFNγ production, but is less inhibitory against TNFα production. TNFα is an inflammatory cytokine that is induced by oxidative stress. However, since oxidants can also oxidize HQ into the more reactive quinone, we decided to investigate the combined effects of HQ and H_2O_2 on human TNFα production *in vitro*. Human peripheral blood lymphocytes were treated with HQ and H_2O_2 in RPMI1640 + 10% FBS for three hours, then stimulated with 1μ g/ml ConA and 3nM PMA. Supernatants were collected 16 hrs later and assayed for IL-1β and TNFα levels by ELISA. 20μ M HQ, in the absence of H_2O_2, inhibited IL-1β by >70%, but had no effect on TNFα production. Concentrations of H_2O_2 up to 50μ M, in the absence of HQ, enhanced TNFα production. However, the simultaneous addition of 5μ M H_2O_2 and 20μ M HQ resulted in >80% suppression of TNFα and >99% suppression of IL-1β production. HPLC analysis of the culture supernatants demonstrated that H_2O_2 enhanced the disappearance of HQ from the media. (Supported by NIH grant HL60538.)

834 SUPPRESSION OF IL-2 GENE EXPRESSION IN MURINE SPLENOCYTES AND THYMOCYTES BY MICROCYSTINS AND NODULARIN.

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Microcystins and nodularin are cyclic peptide toxins produced by cyanobacteria. Both toxins are known to affect hepatocytes and macrophages. In the present study, we investigated effects of microcystins and nodularin on T-cell function. Treatment of microcystins and nodularin significantly inhibited ConA-induced T-cell proliferation in B6C3F1 mouse splenocytes. Microcystins and nodularin also inhibited phorbol 12-myristate 13-acetate (PMA) and ionomycin (Io)-induced IL-2 mRNA expression in murine splenocytes and thymocytes as determined by quantitative RT-PCR. Electrophoretic mobility shift assays indicated that treatment of nodularin showed an inhibition of NF-AT binding in PMA/Io-stumulated murine splenocytes. AP-1 DNA binding was also slightly inhibited, but no effect was observed in NF-kB and Oct DNA binding. These results suggested that microcystins and nodularin inhibit IL-2 gene expression through the down regulation of NF-AT activation. (Supported by the grant from the Ministry of Science and Technology, Korea, #97-N1-05-03-A-01.)

835 FETAL THYMIC ATROPHY FOLLOWING LOW-LEVEL MATERNAL TCDD EXPOSURE IS CHARACTERIZED BY THYMOCYTE HYPOCELLULARITY WITHOUT ALTERED CD4 AND CD8 MARKER EXPRESSION.

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We previously reported altered fetal thymocyte surface marker expression and fetal thymic hypocellularity in gestational day (gd) 18 B6C3F1 mice following subacute maternal exposure to 1.5 μg/kg/day TCDD from gd 6-14. In continued experiments, both marker expression and thymic hypocellularity remained significantly changed at doses of TCDD down to 0.3 μg/kg/day. However, at these lower doses it was noted that the magnitude of the hypocellularity effect was greater than that of the inhibited differentiation (i.e., marker) effect. These data suggested that these two effects of TCDD (i.e., induction of thymic hypocellularity and inhibited thymocyte differentiation) may be mechanistically independent and thus may be separable by a further reduction in the subacute dosing with TCDD. Continued experiments in outbred ICR mice verified this hypothesis. Specifically, fetuses of timed-pregnant ICR mice dosed with 0.3-3.0 μg/kg/day TCDD displayed significant thymic hypocellularity without altered marker expression. Given that no TCDD-related reduction of $CD4^+8^+$ (DP) thymocytes was observed in the presence of significant reduction in thymocyte number, these results suggest that TCDD-induced apoptosis of DP cells did not contribute to the fetal thymic atrophy caused by low-level subacute chemical exposure. Taken together with reports by other authors, these data also indicate that the T-lymphocyte progenitor cell (prothymocyte) may represent a more sensitive target of TCDD exposure than does the thymocyte. (Supported by NIH ES 07612-01 SRC.)

836 ACUTE ADMINISTRATION OF ETHANOL IN VIVO DECREASES THE ACTIVATION OF NK CELLS BY POLY I:C IN VIVO OR IN VITRO: AN IN VITRO SYSTEM FOR MECHANISTIC STUDIES.

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Acute administration of ethanol (EtOH) in a mouse model for binge drinking suppresses both basal and polyinosinic-polycytidylic acid (poly I:C)-enhanced natural killer (NK) cell activity. An in vitro system was developed to allow investigation of mechanistic aspects of EtOH-induced inhibition of NK cell activation by poly I:C. Initial experiments indicated that mouse splenocytes cultured at high density (108/ml) can be effectively activated by poly I:C with regard to NK cell lytic function (as measured by a standard 51Cr-release assay using YAC-1 target cells). As previously shown in vivo, addition of corticosterone in vitro suppresses NK cell activation by poly I:C in a concentration-dependent manner. Splenocytes from mice treated in vivo with EtOH respond significantly less effectively to poly I:C in vitro than do splenocytes from control mice treated only with the vehicle for EtOH (water). This suggests that the effects of EtOH in vivo are relatively long-lasting and are evident during similar time frames during subsequent exposure to poly I:C in vivo or in vitro. In addition, administration of RU 486 (a glucocorticoid antagonist) in vivo significantly decreased the EtOH-induced suppression of NK cell activation in response to poly I:C in vitro. The addition of anti-interferon-alpha antibodies to spleen cell cultures significantly decreased poly I:C-induced activation, and addition of exogenous Interferon-alpha partially overcame the suppressed NK cell activation noted when splenocytes were obtained from EtOH-treated mice. Finally, Western blot analysis indicated that upregulation of perforin and granzyme B were induced by poly I:C, and EtOH inhibited this upregulation. These results indicate that EtOH may function by preventing interferon-alpha production or responsiveness and subsequently upregulation of perforin and granzyme B. Glucocorticoids are partially responsible for effects of EtOH in vivo, which persist during NK cell activation in vitro. (This work was supported by NIH grants AA09505 and ES09158, and S.B.P. is supported by an NIAAA Research Scientist Development Award.)

837 TRIBUTYLTIN OXIDE (TBTO) EXPOSURE *IN VIVO* BLOCKS THYMOCYTE MATURATION AT THE $CD25^-CD44^+$ STAGE.

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In a previous study we have shown that *in vivo* bis(tri-*n*-butyltin)oxide (TBTO) exposure of rats resulted in reduced CD25 expression on thymocytes. $CD3^-CD4^-CD8^-$ (triple negative, TN) thymocytes maturate in mice through several stages: $CD25^-CD44^+ \rightarrow CD25^+CD44^+ \rightarrow CD25^+CD44^- \rightarrow CD25^-CD44^-$. This prompted us to establish whether TBTO exposure blocks maturation of TN thymocytes at a particular stage. Thymi from rats exposed for 2 and 6 weeks to TBTO at a dose that did not cause thymus atrophy, and from unexposed controls, were double immunostained for CD25/CD44 and analyzed using two-color Confocal Laser Scan Microscopy. Cells staining for CD25 showed a reticular appearance in the medulla, and a thymocyte-like appearance in the cortex and cortico-medullary region. Both a reticular appearance and a thymocyte-like appearance was found for cells staining for CD44. $CD25^-CD44^+$ thymocyte-like cells were confined to the cortex. TBTO exposure resulted in a decreased intensity and number of $CD25^+$ cells, with at the same time an increased intensity of CD44 staining. These effects were more profound after 6 weeks than after 2 weeks. These results indicate a block in thymocyte maturation at the $CD25^-CD44^+$ stage. Most likely these thymocytes are TN. As these thymocytes have the potential to develop a.o.

into T-cells and NK cells, a reduced development of these cell types may result from this block, in line with previous *in vivo* function studies. Exposure of mice to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin has been shown to block TN thymocyte maturation at this stage. Possible mechanisms that result in this block include reduced interaction with thymic and bone marrow stromal cells, and reduced transcription factor expression.

838 A STRUCTURE ACTIVITY RELATIONSHIP OF VARIOUS POLYCHLORINATED DIBENZO-p-DIOXIN CONGENERS: INHIBITION OF IMMUNOGLOBULIN EXPRESSION VERSUS AhR-MEDIATED ENZYME INDUCTION.

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The immune system has been identified as a sensitive target for the toxic effects produced by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Furthermore, the B-cell has been identified as a sensitive cellular target of TCDD by previous cell-type fractionation studies from this laboratory. The actual molecular mechanism responsible for the immunotoxic effects produced by TCDD is unclear; however, many of the biological effects produced by TCDD are thought to be mediated by the aryl hydrocarbon receptor (AhR). Our previous results describing the CH12.LX and BCL-1 B-cell lines, which differ markedly in their expression of the AhR and in their sensitivity to TCDD, have implicated a role for the AhR in TCDD-induced inhibition of B-cell function. The purpose of the present studies was to further investigate this role of the AhR through a characterization of the structure activity relationship between inhibition of immunoglobulin (Ig) secretion and of Ig heavy chain transcription and AhR-mediated enzyme induction in CH12.LX cells. Results demonstrated an inhibition of IgM secretion with the following rank order potency, TCDD > 1,2,3,4,7,8-hexachlorodibenzo-p-dioxin (HxCDD) > 2,3,7-trichlorodibenzo-p-dioxin (TriCDD) > > 1-monochlorodibenzo-p-dioxin (MCDD). The rank order potency for induction of *Cyp1a1* expression in the CH12.LX cells for these congeners is TCDD > HxCDD > TriCDD >> MCDD. This correlates well with the previously reported induction of aryl hydrocarbon hydroxylase (AHH) activity and with the sensitivity to inhibition of IgM secretion from CH12.LX cells. In addition, TCDD, HxCDD, TriCDD and MCDD also had no effect on IgM secretion from the AhR-deficient BCL-1 cells. Furthermore, preliminary results demonstrate a concentration-dependent inhibition of Ig heavy chain gene transcription by TCDD and HxCDD that closely resembles the concentration-dependent induction of Cyp1a1 gene expression suggesting a potential DRE-mediated regulation of the Ig heavy chain gene. The results from these studies further support an AhR-dependent mechanism for the inhibition of IgM secretion by dibenzo-p-dioxin congeners. (These studies were supported by the NIH grant ES02520.)

839 IMMUNOMODULATION OF MACROPHAGE RESPONSE BY 2,4-DICHLOROPHENOXYACETIC ACID: TIME- AND TISSUE-SPECIFIC DIFFERENCES IN MUMMICHOG (FUNDULUS HETEROCLITUS).

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The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is a known peroxisome proliferating agent (PPA) with potential immunomodulating abilities. PPA exposure induces hepatic fatty acid metabolizing cytochromes P450 (CYP2K1 and CYP2M1 in fish) and may modulate liver and extrahepatic tissue macrophage activities. Hallmarks of macrophage activation are induction of nitric oxide synthase (iNOS) and generation of reactive oxygen species (ROS). The aim of this study was to evaluate the immunomodulatory effects of 2,4-D through measurement of hepatic iNOS expression and ROS production by anterior kidney macrophages. 2,4-D exposure was evaluated by immunodetection of induced CYP2K1-like proteins. Female fish were exposed to 2,4-D (0.1 or 1.0 ppm) or vehicle control for 1, 7, 14 or 21 days. At each time point, livers and anterior kidneys were collected. Pooled livers (n=3 pools/ treatment) were processed to retrieve microsomal and cytosolic fractions. Proteins were separated by SDS-PAGE and transferred to PVDF membrane. Immunodetection of induced microsomal CYP2K1 and cytosolic iNOS was performed using rabbit anti-trout CYP2K1 (D.R. Buhler) and rabbit anti-mouse iNOS (Transduction Labs). Macrophages from the anterior kidneys of individual fish (n=4 individuals/treatment) were tested for ROS production as measured by luminol-augmented chemiluminescence, using zymosan as an immunostimulant. Preliminary results indicate that 2,4-D increased hepatic CYP2K1 by day 7, but decreased levels to near control by day 21, whereas iNOS expression was increased at day 1, suppressed at day 7, and near control levels at other time points. Immunostimulated ROS production is slightly increased at day 14, while baseline (non-stimulated) ROS production is suppressed at day 21. These results suggest that 2,4-D is capable of modulating macrophage function, however the response varies over time, and differs by macrophage tissue source.

840 INHIBITION OF THE LYSOSOMAL PROTEASE CATHEPSIN L BY TRIVALENT ARSENIC.

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Gallium arsenide (GaAs), a semiconductor with useful electronics applications, is classified as an immunotoxicant. Previous work by our laboratory has shown that intraperitoneal injection of GaAs into mice suppresses antigen processing by splenic macrophages. Additionally, GaAs exposure diminishes cathepsin L (cathL) activity in splenic macrophages. CathL is a widely expressed lysosomal cysteine protease that plays an important role in antigen processing through the major histocompatibility class II pathway. Previous studies have demonstrated that GaAs dissociates *in vivo* into its respective components and the arsenic component is primarily responsible for immunosuppression. To identify the mechanism by which GaAs inhibits cathL, we characterized arsenic inhibition of cathL *in vitro*. We utilized the trivalent form, arsenite, because it is a well-characterized inhibitor of enzymes containing active site sulfhydryl groups, such as cathL. Lysates from the murine B cell line, TA3, as well as cathL purified from human liver were sources of cathL for this study. Enzymatic activity was measured by fluorometric assay using a synthetic peptide substrate and specific inhibitor. Arsenite caused a dose-dependent inhibition of cathL in TA3 lysates and completely ablated cathL activity at higher doses. Arsenite also decreased activity of purified cathL in a dose-dependent manner, although not completely. Reversal experiments, using an equimolar concentration of dithiothreitol, demonstrated that arsenite inhibition is reversible. Dixon and Lineweaver-Burk analyses suggest that arsenite is a mixed inhibitor of TA3-derived cathL; however, arsenite inhibits the purified enzyme differently. These findings offer insight into one mechanism by which GaAs causes immunosuppression. (This work was supported by National Institutes of Health grant ES07199. M.T.H. was supported by training grant T32ES07087.)

841 IMMUNE ALTERATIONS IN SPRAGUE DAWLEY RATS EXPOSED TO GENISTEIN FOR 77 DAYS.

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Genistein, an endocrine disruptor, is found occurring naturally as a phytoestrogen in several plants including the soya plant which is used increasingly in food products. These studies were conducted in Sprague Dawley rats utilizing the F0 females and F1 generation males and females to assess the potential of immunogical alterations. Rats were exposed to genistein both *in utero* and in feed for up to 77 days. NIH-31C diet was the control with genistein doses of 25, 250 and 1250 PPM. The exposures took place at the National Center for Toxicology Research. On the day of sacrifice the spleen and bone marrow samples were shipped to Richmond for assay assessment the following day. A significant decrease in body weight was observed at 1250 PPM (9 to 12%). A decrease in relative spleen weight was seen in the F1 male and female rats. In the bone marrow evaluation, data from the F1 males showed a significant decrease in colony forming units (CFU) for granulocytes-monocyte (GM) cells and CFU-M cells up to 32% as compared to the control. In the immune parameters, no alteration was observed in the T-dependent antibody forming cells response to sheep red blood cells with exception of the F1 female rats with a significant increase at 1250 PPM. A significant dose-dependent increase (75%) was observed with the F0 female rats at all effector-to- target ratios in the natural killer cell response at 250 and 1250 PPM. Using the anti-CD3 antibody to assess T cell receptor-mediated proliferation, splenocytes from both F1 male and females demonstrated significant dose-dependent increased proliferation up to 41% and 59% respectively. No effect was seen with the absolute number of splenic surface markers in the F0 rats, however, a significant increase in splenic surface markers was seen with T cell and T cell subsets for both the F1 male and female rats. In the F1 generation rats exposed to genistein for 77 days, alterations were observed in both T cell number and T cell function. (These studies were conducted under NIEHS Contract ES 55387.)

842 GENDER-BASED DIFFERENTIAL HUMORAL RESPONSES TO A FOREIGN VERSUS SELF ANTIGEN AFTER IN OVO EXPOSURE TO LEAD.

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To examine the potential role of gender in the nature of immunotoxic responses to lead, five-day old avian embryos were injected once with lead acetate (5 or 10 ug) or sodium acetate as control. At four weeks post-hatch, animals were immunized with a foreign antigen (BSA) and serum collected two weeks after. At six weeks and eight weeks of age, a self-antigen, thyroglobulin (Tg) injected as thyroid extract was used as a sensitizing antigen. At 10 weeks of age, various immune parameters were examined. In females, absolute thyroid weights and anti-thyroglobulin antibody levels were significantly decreased following lead treatment. In comparison, males showed no differences in response to thyroglobulin between treatment groups but immunomodulatory effects were observed after exposure to a foreign antigen, BSA. Males differed in anti-BSA antibody levels and total leukocyte counts between treatment groups, whereas females did not. A significant difference in ex vivo interferon-gamma production was also observed in males. No differences were observed in males or females in the delayed-type hypersensitivity response or in spleen, thymus or bursa weights. These results suggest that there is a persistent differential immunotoxic effect after in ovo exposure to lead based on gender. While both genders exhibited certain changes in antibody production following lead exposure, females were more sensitive to dose-response permutations in response to thyroglobulin, a self-antigen, whereas male humoral response changes were detected only with BSA, a foreign antigen. (Supported by NIEHS grant #ES05950.)

843 ENDOGENOUS METALLOTHIONEIN ALTERS IMMUNE RESPONSES TO EXTERNAL STIMULATION.

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Metallothionein (MT) has been characterized as a stress-induced protein capable of modulating a cell's exposure to heavy metals and reactive intermediates. Toxicants, such as metals cations, organic compounds and acute phase proteins, can induce the expression of MT; therefore, it has been proposed that one of MT's function is to reduce the toxic effects of these compounds. However, MT also plays an important role in normal cellular functions. MT can act as a reservoir for essental trace metals, regulate the activity of Zn-dependent transcription factors, and maintain a reduced cytoplasmic environment. These various roles are clearly essential to normal cellular homeostasis. One of the critical systems effected by toxicant exposure is the immune system. The central premise of this work is that MT can function as an intermediate in toxicant-induced immunomodulation. Previous work has shown that exogenous MT can influence a developing immune response, reducing the levels of humoral antibodies produced after immunization. The negative regulatory functions of endogenous pools of MT during a humoral immune response is demonstrated in the experiments described here. MT-1 mRNA expression decreases in immune tissues during a normal immune response, as shown by RT-PCR. These observations suggest that MT expression during a normal immune response is tightly regulated, decreasing during the height of an immune response. The absence of MT-1 and MT-2 in 129/Sv-*Mt1tm1BriMt2tm1Bri* (MT-null) mice correlates with a two-fold increase in antibody titer to a T-dependent antigen challenge when compared to wild-type control animals (129/SvJ). Assays of lymphocyte function show that MT-null mice are hyper-responsive to external mitogen and antigen stimulation, as demonstrated in proliferation and ELISPOT assays. Naive MT-null mice have increased total numbers of plasma cells and splenic weights, although markers of autoimmune disease are not upregulated. Our experiments suggest that MT can be a potent modifier of humoral immune responses, and that manipulation of MT levels may serve as a novel therapeutic treatment to autoimmune disease or inflammation.

844 TOPICAL PERMETHRIN EXPOSURE ALTERS SKIN IMMUNE RESPONSES AND PRODUCES SYSTEMIC IMMUNE EFFECTS.

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Inhibited immune responses have been observed following occupational, inadvertent, or therapeutic exposure to chemically-diverse xenobiotics. In the present studies, preliminary data were generated showing limited but significant systemic and regional immunotoxicity following low-level topical exposure to the pyrethroid insecticide, permethrin (formerly not considered an immunotoxicant). Permethrin was applied to the shaved dorsal interscapular region of C57Bl/6N mice at doses of 0.5, 1.5, or 5.0 μl/day. The highest of these doses is approximately equal to 215 μg/kg/day, which is about seven times the estimated daily human exposure in individuals wearing permethrin treated clothing for insect protection. Mice were exposed to permethrin daily for 10 or 30 consecutive days, or every other day for 7 or 14 exposures. Body weight was not affected by the treatment. However, thymic weight was decreased and splenic weight increased 2 days after termination of the topical exposure. Splenic macrophages showed significantly depressed chemilumeniscent responses up to 10 days following termination of exposure. The contact hypersensitivity response, measured by quantitating the ear swelling response to chemical exposure, was also significantly depressed. Taken together, these data indicate that low-level topical permethrin exposure may produce both local and systemic immunotoxicity.

845 EFFECTS OF THE NON-STEROIDAL ANTI-INFLAMMATORY DRUGS NAPROXEN AND INDOMETHACIN ON CANINE LEUKOCYTE CHEMOTAXIS *IN VITRO*.

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Experiments were conducted to determine whether dog leukocyte chemotaxis *in vitro* is affected by treatment with the non-steroidal anti-inflammatory drugs (NSAIDs) naproxen and indomethacin. Peripheral blood leukocytes from male beagle dogs were isolated using a Ficoll gradient procedure. Isolated leukocytes were pre-incubated for 40 minutes with 0, 1, or 10 μ M naproxen or indomethacin. Chemotaxis of 100,000 cells was assessed in 96-well chemotaxis chambers in the presence or absence of 0.5% zymosan-activated dog serum. Treatment of leukocytes with 1 or 10 μ M naproxen or indomethacin resulted in similar, concentration-dependent increases in chemokinetic activity in the absence of chemoattractant. In the presence of chemoattractant, naproxen and indomethacin treatment resulted in concentration-dependent decreases in the mean number of cells migrating through the filter. Similar results were obtained in replicate experiments done with peripheral blood from seven different dogs. These results show that *in vitro* NSAID treatment at therapeutically relevant concentrations can inhibit canine leukocyte chemotaxis.

846 MODULATION OF THE IMMUNE RESPONSE BY THE DRINKING WATER DISINFECTION BY-PRODUCT DIBROMOACETIC ACID.

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Dibromoacetic acid (DBA) is a disinfection by-product contaminant found in drinking water. DBA is formed as a result of the bromination process used to purify water to acceptable drinking water standards. The purpose of this study was to determine the potential effects of DBA on the immune system when administered in the drinking water. Female B6C3F1 mice were exposed daily, *ad libitum*, to DBA in the drinking water at dose levels of 125, 250, 500, 1000, and 2000 mg/l for 28 days. While body weight was not affected, a decrease (40%) in body weight gain was observed in the high dose (2000 mg/l) animals. While the high dose produced a decrease in absolute thymus weight (36%), increases were observed in spleen (25%), liver (46%) and kidney (17%) weights. All dose levels produced significant increases in absolute liver weight and the four highest dose levels produced elevated kidney weights. Hematological parameters were not affected with the exception of reticulocytes which were elevated (30%) at the three highest dose levels. Increases in splenic B cell and T cells appeared to be related to an increase in spleen cell number following exposure to DBA. A dose related increase in splenic macrophages ($Mac1^+$ cells) was observed. DBA produced a dose dependent decrease (50%) in the antibody forming cell response to sRBC conversely an increase in natural killer cell activity was observed. No effect was observed on the mixed leukocyte response (MLR). These studies demonstrate that the immune system can be a target for disinfection by-products and that both immune suppression and enhancement can result following exposure to dibromoacetic acid in the drinking water. (Supported by NIEHS Contract ES 55387.)

847 EFFECT OF *N, N*-DIETHYLANILINE ON SISTER CHROMATID EXCHANGE OF LYMPHOCYTE, NATURAL KILLER ACTIVITY, CYTOTOXIC T LYMPHOCYTE ACTIVITY AND MITOGENS-STIMULATED LYMPHOCYTE PROLIFERATION.

Q Li, Y Hirata, S Piao and M Minami. *Nippon Medical School, Tokyo, Japan.* Sponsor: T Yoshida.

N,N-Diethylaniline (DEA) is a reagent used in organic synthesis and is an important intermediate in the manufacturing of dyes. We found that DEA induced high frequency sister chromatid exchange (SCE) of lymphocytes *in vitro* that was about 5 times greater than the control, and was as great as positive controls of SCE such as cyclophosphamide. To determine whether or not DEA affects other functions of lymphocytes, we evaluated the immunotoxicity of DEA using CBA/N mice. Mice were divided into 4 groups and received 0, 100, 200, or 400mg/kg body weight of DEA by a single subcutaneous injection. The following items were investigated on days 3 and 7 after the injection: weight of spleen, number of splenocytes, natural killer (NK) activity, cytotoxic T lymphocyte (CTL) activity, and concanavalin-A (Con-A) and lipopolysaccharide (LPS)-stimulated lymphocyte proliferation using splenocytes. NK and CTL activity in exposed groups significantly decreased compared to controls in a dose-dependent manner. Spontaneous proliferation of lymphocytes, the relative weight of the spleen and number of splenocytes in exposed groups were significantly higher than in the control. On the other hand, DEA did not affect the Con-A or LPS-stimulated lymphocyte proliferation. The results from day 3 were similar to those from day 7. These findings indicate that DEA induces SCE and selectively affects NK and CTL activity, but not mitogens-stimulated lymphocyte proliferation. This study was supported by a grant from the Ministry of Education, Science and Culture of Japan.

848 ALTERATION OF T-2 TOXIN INDUCED IMMUNOTOXICITY BY A SELENIUM ENRICHED DIET IN BALB/C MICE.

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The objective of this project is to determine whether a selenium (Se) enriched diet will reduce immunotoxicity induced by T-2 toxin. Preliminary results from our laboratory, using mice orally exposed to T-2 toxin for five days at 1.5 mg/kg, revealed that: (1) Se supplementation has significant protection on reduction of spleen weight, thymus cellularity and weight, contact hypersensitivity response, and cytotoxic T-lymphocyte activity. (2) Se supplementation did not alter suppression of humoral immunity. The present work investigates Se protection on immunotoxicity induced by T-2 toxin *via* topical exposure, a main route for occupational exposure. Female BALB/c mice were fed a defined diet containing either 0.75, 1.5 or 2.5 ppm Se for 4 weeks. After topical exposure to T-2 toxin at 1.0 mg/kg for four days, several immunotoxic endpoints were determined. In mice topically treated with T-2 toxin, total thymus cellularity reduced to 25% of the control, and thymus weight was reduced to 58% of the control. However, dietary Se supplementation did not alter these suppressive effects induced by T-2 toxin. As compared to control group, no significant changes were observed in spleen weight and cellularity, cytotoxic T-lymphocyte activity in T-2 treated control. On the other hand, IgM plaque forming responses were significantly decreased in T-2 treated control and 0.75 ppm Se groups. This suppressive effect was not observed in T-2 treated and Se-supplemented (1.5 and 2.5 ppm) groups. Expression of major histocompatiblity class II (MHC II), a surface marker on epidermal Langerhans cell, was also investigated. Oral exposure to T-2 toxin at 1.5 mg/kg did not alter MHC II expression. However *in vitro* T-2 toxin treatment at a concentration of 10-7M significantly inhibited MHC II expression, this inhibition was not protected by Se supplementation. These results suggested that protective effects of Se against to T-2 toxin depend on the route of exposure to T-2 toxin.

849 QUANTITATIVE MODELING OF STRESS-INDUCED IMMUNOSUPPRESSION.

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A number of chemicals induce neuroendocrine stress responses, some of which can be immunosuppressive. Although the impact of stress-related immunosuppression in humans and animal models has been well documented, it is not yet possible to estimate the degree of immunosuppression by simple measurements of the concentrations of neuroendocrine mediators. The present study was conducted to obtain such data and to ascertain the relative importance of glucocorticoids as opposed to other stress-induced mediators in suppression of selected immune parameters. The area under the corticosterone concentration vs. time curve (AUC) was measured following subcutaneous administration of various dosages of corticosterone and during the course of restraint stress (1-8 hr) in female B6C3F1 mice. The percentage of bound vs. free corticosterone was not substantially affected by exogenous corticosterone or restraint, indicating that measurement of total corticosterone is appropriate. Twelve hours after the initiation of stress (or corticosterone administration) spleens were obtained. Natural killer (NK) cell activity was measured using a standard 4-hr 51Cr release assay, and expression of major histocompatibility class (MHC) II proteins on splenocytes was evaluated by flow cytometry. Th1 and Th2-dependent antibody responses (IgG2a and IgG1, respectively) to keyhole limpet hemocyanin as well as host resistance to B16F10 cells were also evaluated. The results demonstrate that corticosterone AUC correlates well with the suppression of each of these immunological parameters, but there were marked variations in sensitivity to corticosterone (MHC II > B16F10 > NK > antibody response). The linear regression models for MHC II expression were not significantly different for exogenous corticosterone and restraint. This was also true for NK cell activity. The results indicate that corticosterone AUC can be used to predict at least the minimal immunosuppression expected following exposure to an acute stressor. Because glucocorticoid measurements can easily be done for human subjects, these models could provide valuable information for cross-species extrapolation. (This work was supported by NIEHS grant ES09158.)

850 FLOW CYTOMETRY IMMUNOPHENOTYPING FOR THE MEASUREMENT OF LEUKOCYTE SUB-POPULATIONS IN THE RAT.

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Immunophenotyping by flow cytometry is the method proposed by the Center for Drug Evaluation and Research (Rockville, MD) to evaluate potential immunosuppression by drugs (SOT '98, Seattle, WA). In toxicology, the rat is a standard rodent species for use in preclinical studies. The objective of this study was to validate a flow cytometry immunophenotyping method to quantitate five leukocyte sub-populations in the Fisher rat. EDTA blood samples were collected from non-treated animals and were analyzed with anti-rat antibodies to determine the percentage of five leukocyte sub-populations using a flow cytometry method according to ClinTrials BioResearch's Standard Operating Procedures. The following parameters were assessed: intra- and inter-assay precision, physiological range and 24-hour stability at 2 - 8°C of samples fixed with Formalin (0.5% v/v). Intra- and inter-assay precision were considered acceptable at 1.06% and 1.28% for thymocytes and T lymphocytes (CD5-positive cells), 5.92% and 4.47% for B lymphocytes (CD45RA-positive cells), 1.46% and 1.78% for T helper lymphocytes (CD4-positive cells), 2.74% and 2.91% for T suppressor/cytotoxic lymphocytes (CD8-positive cells) and 15.96% and 19.4% for monocytes/macrophages (ED1-positive cells). Physiological distribution was determined with 5 males and 5 females for each of the five respective markers. Samples fixed with Formalin 0.5% v/v kept for 24 hours at 2 - 8°C were shown to be stable for all markers, except for thymocytes and T lymphocytes in the females. A higher percent difference for monocytes/macrophages was obtained due to the small absolute values for this cell type. The experimental procedures used during the course of this validation were considered appropriate since acceptable precision was obtained and physiological range of the five sub-populations was as expected.

851 IMMUNOTOXICOLOGY GUIDELINE STUDIES: SOME PRACTICAL CONSIDERATIONS.

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The new EPA immunotox guidelines emphasize the antibody (Ab) response to SRBC, which can be measured as either a plaque assay or an ELISA. While the ELISA may afford greater technical flexibility, the database which was used to support the selection of the Ab response is based on the plaque assay, and there is much more limited experience with the ELISA. One of our goals was to determine if the technical flexibility of the plaque assay

could be increased. We observed good agreement when various parts of the spleen were recorded as percent of wet weight when compared to the same parts recorded as percent of total cell number. We also observed good agreement between the Ab response of the whole spleen when compared to the response by various parts of the spleen. These results indicate that it should be feasible to use half of the spleen for the Ab response (i.e., plaque assay) and the other half of the spleen for other purposes (i.e., histopathology or phenotypic analysis). The EPA guidelines also indicate that an NK assay may be required when the Ab response is negative. A second goal was to determine the feasibility of measuring these endpoints in the same animals (female B6C3F1 mice). Dexamethasome (DEX) was administered (daily for 14 days) as a known immunosuppressant. NK cytotoxicity was reduced due to immunization in both control and immunosuppressed mice - the reduction due to DEX was comparable to that associated with SRBC, and the two were additive. These results indicate that a one-mouse model is not suitable for guideline studies in immunotoxicology.

852 INTEGRATION OF PUPS AND WEANLINGS INTO STUDIES TO ADDRESS THE POTENTIAL FOR DEVELOPMENTAL IMMUNOTOXICOLOGY.

P K Anderson, C M Wiescinski and M P Holsapple. *HERL, Dow Chemical Co., Midland, MI.*

There is increased interest in determining whether the developing immune system demonstrates a unique sensitivity to chemical perturbation. The overall goal of these studies is to integrate both pups (i.e., 10 days old) and weanlings (i.e., 21 days old) into immunotox studies. The antibody (Ab) response to SRBC is the focus of current guideline studies in young adults, and will be the primary endpoint in these studies. Our initial results have indicated that the conditions to elicit an Ab response in weanlings were comparable to the optimal conditions used in young adults. To further address the feasibility of using weanlings, we determined the effects of maternal food restriction (FR) during *in utero* and neonatal life. Groups of time-mated Sprague-Dawley rats were fed standard diets at 100%, 90%, 70% or 50% of the historical feed consumption quantities, which resulted in treatment-related body weight gain decreases in the dams and their pups. Necropsies conducted on pups 24 hours after they were weaned showed that relative thymus and spleen weights were significantly decreased. Parallel studies indicated that the Ab response was not significantly affected. Therefore, changes in organ weights caused by FR were not associated with marked functional impairment. Our results to-date with 10-day old pups have been disappointing. We have had no success with i.v. injections in pups until about 16 days of age. Immunization by the i.p. route in pups has not elicited an acceptable Ab response (i.e., either "plaque" or ELISA). Taken together, these results indicate that weanlings; but not pups can be integrated into developmental immunotox studies, which include a functional parameter.

853 EVALUATION OF CYCLOPHOSPHAMIDE IMMUNOTOXICITY IN RATS: THE USE OF FUNCTIONAL ASSAYS AND FLOW CYTOMETRY.

D F Lanham, S M McHugh, S R Wilkins and P F Uphill. *Huntingdon Life Sciences Limited, Huntingdon, Cambs, England.* Sponsor: R J Harling.

In order to comply with recent EPA and OPPTS guidelines we have evaluated three specific tests to assess immunomodulation in rats, with the aim of incorporating them into standard sub-chronic toxicity studies. These tests were: the plaque forming cell (PFC) assay for the detection and enumeration of antigen specific B cells, induced by sheep red blood cells (SRBC) a T cell dependent antigen. A ^{51}Cr release natural killer (NK) assay, to assess changes in innate immunity, and thirdly flow cytometric analysis of T, B and NK cell populations in both blood and spleen. Using Cyclophosphamide (CY) a known immunosuppressive agent we treated five groups of female CD rats (10/group) i.p.; three groups received CY for a total of 8 days at 1, 3 or 10 mg/kg/day, one group received a single dose of 50 mg/kg 2 days before sacrifice and one group received saline. In addition half the animals in each group were challenged i.v. with SRBC four days before sacrifice, to allow measurement of the primary humoral response in the PFC assay. We demonstrated 1) dose-dependent reductions in the lymphocyte populations of both spleen and blood, 2) a dose dependent reduction in the PFC response from over 28,000 plaques/spleen in untreated animals to less than 50 in the 10 mg/kg/day group, also reflected by an almost complete loss of the CD45R+ (B cell) population in spleen and blood, 3) an enhancement of NK activity, associated with a relative enrichment in NKR+ cells amongst the declining lymphocyte populations, this was most notable in the spleen, also 4) a reduction in the CD4:CD8 T cell ratios of both spleen and blood, indicating the preferential depletion of CD4+ cells from the T cell population and survival of CD8+ cells. These observations show that it is viable to use the above parameters for assessment of immunomodulation in rats, but emphasise the importance of combining flow cytometry, functional assays and pathology data when attempting to assess immunotoxicity.

854 COMBINED EFFECTS OF CHEMICAL AND NATURAL STRESSORS ON NONSPECIFIC IMMUNITY IN RED VERSUS BLACK ABALONE.

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The chemotactic and phagocytic efficiency as well as gross morphological characteristics of hemocytes withdrawn from red (Haliotis rufescens) and black abalone (H. cracherodii) after in vivo exposure to 25, 35, or 45 0/00 seawater salinity plus 1.2 mg/l pentachlorophenol (PCP) was determined. Red and black abalone exposure durations of 3.5 and 6.5 h, respectively, were based on species specific metabolic endpoints (MEPs) derived from previous NMR data. Extracted hemocytes were incubated with FITC conjugated Saccharomyces cerevisiae and the percent phagocytosis (%P: number of cells that had phagocytosed yeast) and phagocytic index (PI: number of yeast within each phagocytic cell) were quantified using epifluorescence microscopy. Chemotactic activity was quantified in Boyden chambers using a live marine bacterium as the chemotactic stimulus. Hemocyte chemotaxis was found to be augmented by low salinity in red but not black abalone while high salinity seemed to reduce chemotactic ability in both species. PCP did not potentiate the effects demonstrated by salinity variations alone in red abalone although chemotaxis was further compromised in black abalone exposed to both high salinity stress combined with PCP. Although chemotactic ability was similar among both species both %P and PI were greater among black abalone. Generally, low salinity seems to offset the effects of PCP in red but not black abalone. Whereas red abalone demonstrate a reduction in phagocytic ability upon exposure to high salinity alone, black abalone appear more resilient to high salinity stress alone. Furthermore, whereas the effect of high salinity plus PCP is subadditive among red abalone, high salinity seems to potentiate the effect of PCP on black abalone. Finally hemocytes exposed to acute salinity variations plus PCP show varying morphological characteristics when compared to controls. When allowed to settle on glass, over 30% of the hemocytes of exposed abalone remain rounded and generally unattached with only a few pseudopod extensions. Furthermore, they do not clump. Hemocyte preparations incubated in rhodamine-conjugated phalloidin were observed with fluorescence microscopy and reveal an altered actin filament pattern. While PCP is a potent inhibitor of ATP synthesis, use of varying water salinities simulated an additional stress imposed on intertidal animals by transient or seasonal environmental changes.

855 EFFECTS OF SUB-ACUTE *IN VIVO* EXPOSURE OF FORMALDEHYDE (HCHO) ON FISH.

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Formaldehyde (HCHO), an inhalational toxicant, dermal contact irritant, mutagen and carcinogen has been shown to exert its effect in both laboratory animals and humans based on concentration, exposure route and duration of exposure. A 1998 report from the U.S. EPA indicates that HCHO was detected at levels greater than the cancer benchmark concentration in approximately 90% of the 60,000 census tracts measured in the United States. Since HCHO mixes with water extremely well and is a common aquatic contaminant, it is reasonable to believe that aquatic life is also exposed. Further, HCHO is used therapeutically in aquaculture facilities to remove bacterial pathogens from gills of fish. A study was undertaken to evaluate the effects of sub-acute exposure to HCHO on a variety of immune endpoints in tilapia (*Oreochromis niloticus).* The fish were exposed to 0, 50,100, 150 and 250 ppm of HCHO. Immune parameters examined included blood hematology, spleen and pronephros body weight ratios, spleen and pronephros total cellularity, leukocyte blastogenesis, mixed lymphocyte reaction and natural killer cell activity. Organ/body weight ratios and total cellularity were not significantly different from controls. Similarly, mitogen response and mixed lymphocyte reactions were not significantly altered. However, natural killer cell activity was enhanced. These findings agree with a previous study in mice in which no significant immunosuppression

was observed with similar endpoints, suggesting fish may respond immunologically in a manner similar to mammals following exposure to HCHO. (Supported by NIH ES 07612-01 SRC.)

856 CHEMICAL EXPOSED TILAPIA SHOW SIMILAR IMMUNE EFFECTS AS RODENTS.

S D Holladay, D A Smith, S A Smith, and G G Schurig. *Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, USA.*

The ability to produce specific antibody following challenge with antigen has been found to be a powerful predictor of immunosuppression in chemical-exposed rodents. Similarly, ability of cytotoxic immune cells to target and lyse foreign cells has been widely used as a predictor of immunosuppression in rodents. The efficacy of these assays for predicting immunosuppression in non-rodent species remains unknown, however. In the present report, tilapia (*Oreochromis niloticus*) were exposed to 9 chemical agents known to inhibit humoral and/or cytolytic immune responses in mice (benzo[a]pyrene (B[a]P), 7,12-dimethylbenzanthracene (DMBA), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), dimethylnitrosamine (DMN), cadmium chloride ($CdCl_2$), azathioprine (AZA), hexachlorocyclohexane (lindane, HCH-g), T2 mycotoxin (T2 toxin), and toluene) and 5 chemical agents not classified as rodent immunotoxicants (oxymethalone, acetonitrile, diethylstilbesterol (DES), *t*-butylhydroquinone (TBHQ), and formaldehyde). Eight of nine humoral immunotoxicants caused decreased antibody production in fish. All five of the compounds with negative humoral effects in rodents were also negative in fish. In like manner, eight of nine agents which inhibit rodent cytolytic responses also caused decreased cytolytic activity in fish. Thus, in each case (i.e., when a humoral and when a cytolytic endpoint was evaluated) 13 of the 14 chemical agents tested gave similar results in tilapia as reported in rodents, indicating a comparable pattern of immunosuppression in chemical-exposed tilapia. (Supported by NIH ES 07612-01 SRC).

857 ROLE OF METABOLISM IN THIOACETAMIDE-INDUCED SUPPRESSION OF ANTIBODY RESPONSE IN MALE BALB/c MICE.

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Effects of thioacetamide, a potent hepatotoxicant, on T-dependent antibody response to sheep red blood cells(SRBCs) were investigated in male BALB/c mice. When mice were treated intraperitoneally with thioacetamide once 30 min prior to the immunization with SRBCs, the number of antibody-forming cells (AFCs) enumerated 4 days after was significantly reduced at the dose of 200 mg/kg in accompanying with the elevation of SGPT and SGOT activities. When mice were treated with 10, 20, or 50 mg/kg of thioacetamide for 7 consecutive days, the number of AFCs was reduced at the dose of 50 mg/kg. To determine the possible role of metabolic activation by cytochrome P450 (P450) in thioacetamide-induced suppression of the antibody response, mice were pre-treated with 80 mg/kg of phenobarbital intraperitoneally for 3 days, followed by the intraperitoneal administration of 100 mg/kg of thioacetamide for 3 days. Four days after the immunization with SRBCs, the AFCs were enumerated. The spleen and thymus weights were significantly reduced when mice were pre-treated with phenobarbital prior to thioacetamide administration. The elevated SGOT and SGPT activities by thioacetamide were significantly potentiated by the pre-treatment with phenobarbital. In liver microsomes, the activities of P450 2B-specific enzymes induced by phenobarbital recovered to normal level a week after the pre-treatment. Meanwhile, the suppression of antibody response by thioacetamide was significantly potentiated by the pre-treatment with phenobarbial. Our present results indicate that thioacetamide may require metabolic activation by P450 2B to its immunosuppressive form(s). (Supported by a grant from the MOST, Korea.)

858 ETHANOL DECREASES HOST RESISTANCE TO PULMONARY METASTASES IN A MOUSE MODEL: ROLE OF NATURAL KILLER CELLS AND THE ETHANOL-INDUCED STRESS RESPONSE.

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Ethanol (EtOH) consumption activates the hypothalamic-pituitary-adrenal (HPA) axis. EtOH consumption, even at moderate levels, is also associated with an increased incidence of cancer at several anatomical locations in humans, and these cancers are often associated with subnormal natural killer (NK) cell function. Mediators induced by activation of the HPA axis such as glucocorticoids and catecholamines can suppress the activity of NK cells, which are important in resistance to some cancers. The work described here was done to evaluate cause-effect relationships between the EtOH-induced stress response, NK cell activity, and resistance to experimental metastases of B16F10 melanoma cells in female B6C3F1 mice. Increased numbers of metastatic nodules in the lungs were noted 2 weeks after challenge in mice treated with dosages of EtOH at 5 or 6 g/kg (dosages that produce peak blood levels of ~0.25-0.4%). Increased numbers of nodules were observed when EtOH (6 g/kg) was administered from 1 h before to 10 h after intravenous administration of tumor cells. Depletion of NK cells using a monoclonal antibody substantially increased the number of B16F10 nodules in the lungs, confirming a key role for NK cells. Treatment of NK cell-depleted mice with EtOH did not further decrease resistance to B16F10 cells, indicating that suppression of NK cell activity is the major mechanism by which EtOH suppresses resistance to B16F10 metastases. Adrenalectomy or a glucocorticoid antagonist was used to prevent the production or effects of glucocorticoids and this partially prevented EtOH-induced increases in the number of B16F10 nodules in the lungs. Administration of exogenous corticosterone increased the number of B16F10 nodules to a similar extent as EtOH. These results indicate an important role for the EtOH-induced stress response in decreasing resistance to B16F10 metastases. Along with similar findings in rats in another laboratory, these results suggest that this is a generalized phenomenon, which could occur in humans. (This work was supported by NIH grants AA09505 and ES09158. S.B.P. is supported by a Research Career Development Award from NIAAA.)

859 MORPHOLOGICAL EVALUATION OF PROMOTION AND PROTECTION IN ORGANOPHOSPHORUS ESTER-INDUCED DELAYED NEUROPATHY (OPIDN) IN HENS USING PERIPHERAL NERVE TEASED MYELINATED FIBERS.

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The serine/cysteine protease inhibitor phenylmethylsulfonyl fluoride (PMSF) has been used to promote or to protect against neuropathic events of OPIDN in hens (Lotti et al., Toxicol. Appl. Pharmacol. 108:234). This is the first study evaluating peripheral nerve teased myelinated fibers to demonstrate lesion intensity in protection and promotion of OPIDN. Single phenylsaligenin phosphate (PSP) dosages of 0.5 and 2.5 mg/kg were administered to adult hens. PMSF (90 mg/kg) was given 4 hours after 0.5 mg/kg PSP (promoted group) or 12 hours prior to 2.5 mg/kg PSP (protected group). PMSF and negative (vehicle) controls were also used. Scores of clinical signs (weakness, ataxia, 0-8 scale) and teased fiber lesions (axonal/fiber degeneration, 0-4 scale) were determined. At 15 days post-dosing, the following teased fiber lesion scores were observed: PSP 2.5 mg/kg alone 2.3 ± 0.6; promoted group (PSP 0.5 mg/kg) 2.0 ± 0.3 (n=2-4). No lesions were noted in the following groups of hens: 0.5 mg/kg PSP, the protected group, PMSF alone, and negative controls (n=4-8). At the time of sacrifice, there was a significant correlation (r=0.91) between the terminal clinical and the neuropathology scores ($p<0.0001$). This study demonstrates that PMSF-induced protection against OPIDN of 2.5 mg/kg PSP and promotion of 0.5 mg/kg PSP are associated with significant changes in the degree of peripheral nerve lesions ($p<0.0001$). In addition, the nerve teased fiber technique provides a powerful method to quantitate peripheral nerve lesions observed in toxic neuropathies.

860 COMPARATIVE EFFECTIVENESS OF ORGANOPHOSPHORUS (OP) PROTOXICANT ACTIVATING SYSTEMS IN NEUROBLASTOMA CELLS AND BRAIN HOMOGENATES.

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Oxidation in the presence of bromine and incubation with rat liver microsomes (RLM) can convert the OP protoxicants chlorpyrifos and parathion to inhibitors of human neuroblastoma cell acetylcholinesterase (AChE) [Barber et al., *Toxicological Sciences*, in press]. Products formed and effectiveness of AChE inhibition differed between the activating systems. To determine if this was compound and/or species specific, additional OP protoxicants (TOTP, EPN, leptophos, fenitrothion, malathion) were examined for their capability to inhibit AChE and neuropathy target esterase (NTE) in human neuroblastoma cells and brain homogenates from the hen, which is the animal model for OP neurotoxicity. Only preincubation with RLM activated

TOTP; inhibition of AChE was less in hen brain (31% ± 14) than in neuroblastoma cells (73% ± 1) at 10^{-6} M (mean ± SE, n>3); inhibition of NTE was more in hen brain (89% ± 1) than in the cells (72% ± 4). For EPN and leptophos, bromine activation resulted in greater esterase inhibition than RLM and human cell esterases were more sensitive to inhibition than hen brain esterases. Bromine activation also resulted in greater AChE inhibition than RLM with fentitrothion (>65% compared to <50%) and malathion (>90% compared to <60%) at 10^{-6} M. These results suggest that bromine activation, although effective for many OP protoxicants, is not effective for all, and that differences exist in the esterase inhibition determined in human neuroblastoma cells and hen brain homogenates following OP activation. (Supported by EPA grant R825356.)

861 INTERACTIONS OF ACETYLCHOLINESTERASE (ACHE) WITH ORGANOPHOSPHATES (OPS).

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The interactions of acetylcholinesterase (AChE) with organophosphates such as paraoxon (*O,O,* diethyl-*O-p*-nitrophenyl phosphate)(PO) and methyl paraoxon (*O,O*-dimethyl *O-p*-nitrophenyl phosphate)(MPO) have been characterized previously. However studies from this lab designed to model mouse brain AChE inhibition by PO MPO in vitro suggest that the current view of how these organophosphates interact with AChE must be modified. The computer model developed included the bimolecular rate constant K_i to quantify the inhibitory power of PO and MPO toward AChE, as well as a first order rate constant describing reactivation of phosphorylated AChE. These studies revealed that the $appK_i$ for inhibition of mouse brain AChE by PO and MPO decreased as the organophosphate concentration increased. For example 0.5nM MPO yielded an $appK_i$ of 0.54 $nM^{-1}h^{-1}$, while 5 nM MPO gave an $appK_i$ of 0.10$nM^{-1}h^{-1}$. Furthermore, studies utilizing mouse brain recombinant AChE showed similar findings. For example a PO concentration of 0.25nM yielded an $appK_i$ of 0.023$nM^{-1}h^{-1}$, while 10nM yielded an $appK_i$ of 0.0048$nM^{-1}h^{-1}$. These results together with the current understanding of the structure of AChE, suggest that attenuation of AChE phosphorylation by MPO and PO might occur through their binding to a peripheral anionic site known to exist on AChE.

862 MORPHOLOGIC STUDY OF NON-LETHAL CYTOPATHIC EFFECTS OF PARATHION AND PAROXON IN SH-SY5Y HUMAN NEUROBLASTOMA CELLS.

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The organophosphate pesticide parathion and its oxygen analog, paraoxon, produce toxicity associated with inhibition of acetylcholinesterase (AChE) in exposed animals, but other actions could contribute to their toxicities *in vitro*. Retinoic acid-differentiated SH-SY5Y human neuroblastoma cells were treated with 10^{-5} M parathion or paraoxon for 24 hours. Acute cytopathic effects of parathion and paraoxon on these cultures were evaluated in morphologically viable cells using light and electron microscopy. A striking feature in treated cells was the presence of regions of cytoplasmic clearing, primarily related to lysis and granular degeneration of cytosol and cytoskeleton. While similar small lytic areas were occasionally observed in controls, the effect was markedly enhanced in toxicant-treated cells. The lesions suggest that the activity of intracellular proteolytic enzymes may increase after treatment with parathion or paraoxon. Parathion does not exert anticholinesterase effects without being converted to paraoxon and little conversion occurs in SH-SY5Y cultures (Barber et al., Toxicological Sciences, in press). As similar effects were caused by treatment with parathion or paraoxon, the results suggest that anticholinesterase mechanisms were not a primary cytotoxic factor. (Supported by USEPA grant R825356.)

863 CHANGES IN ACTIVATION AND DETOXIFICATION ENZYMES RESPONSIBLE FOR ORGANOPHOSPHATE (OP) METABOLISM DURING PREGNANCY.

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Fetal susceptibility to the toxic effects of OP insecticides may be determined, in part, by a balance among pathways of maternal and fetal metabolic activation and detoxification. It is hypothesized that OP activation/detoxification by cytochrome P450 (CYP450) and detoxification by carboxyl- (CaE) and A-esterase (A-est) enzymes is modified during pregnancy in response to normal hormonal modulation of these enzymes. Changes in enzyme expression can quantitatively and qualitatively impact OP metabolism and thus the dose of OP and its toxic metabolite, OP-oxon delivered to the fetus. The present study examines the expression and marker substrate activities of these critical enzymes at 4 gestational stages. Plasma and liver subcellular fractions were prepared from age-matched nulliparous control female or pregnant Sprague-Dawley rats at gestation days 8, 13 and 20 (GD8, GD13 & GD20, respectively). Western blots of CYP450 isoforms implicated in activation/ detoxification of OPs, indicated that isoform activities did fluctuate during gestation. Specifically, CYP2B3 was increased (~2.5 – 2.7 fold) from GD8 to GD13, while CYP1A1 steadily increased to a peak (3 fold) at GD20. CYP3A exhibited considerable fluctuation, peaking at GD13 (~1.5 fold), but decreasing markedly to below control activity by GD20. In contrast, CYP2B2 was suppressed (50%) throughout pregnancy, while CYP1A2 and female-specific CYP2C12 were unchanged. Plasma A-est activity was suppressed at GD8 and GD20, whereas hepatic CaE and A-est were unchanged from control levels. In conclusion, these data suggest that alterations in the expression and activity of enzymes involved in OP metabolism could result in changes in maternal activation and detoxification of OPs.

864 ORGANOPHOSPHORUS COMPOUND-INDUCED MODIFICATION OF SH-SY5Y HUMAN NEUROBLASTOMA CELL MITOCHONDRIAL TRANSMEMBRANE POTENTIAL.

K Carlson and M Ehrich. *Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA, USA.*

Decreases in mitochondrial respiration and ATP generation, inhibition of mitochondrial enzymes, and structural changes such as swelling occur following organophosphorus (OP) compound exposure and implicate mitochondria as primary subcellular targets for *in vitro* cytotoxicity. The health and function of cellular mitochondria following OP compound exposure was further assessed by evaluating the mitochondrial transmembrane potential ($\Delta\Psi$), a loss of which can be induced through the opening of a permeability transition pore (PTP). To assess $\Delta\Psi$, SH-SY5Y cells were incubated with rhodamine 123 (5μ g/ml), a mitochondrial-specific cationic fluorochrome, and OP test compounds (tri-ortho-tolyl phosphate - TOTP, phenyl saligenin phosphate - PSP, parathion, and paraoxon) for 7.5 to 960 minutes. Following incubation, the plates were read on a Cytofluor II multiwell fluorescence spectrometer (ex530nm em590nm). Cytotoxic doses of OP compounds (10^{-5} to 10^{-3}M) induced significant dose-dependent mitochondrial hyperpolarization with monomodal or bimodal peaks at 60 and 120 minutes. Following this, the mitochondrial membranes gradually depolarized. Thirty hour pretreatment with the PTP inhibitor cyclosporin A (5μ M) and the phospholipase A_2 inhibitor aristolochic acid (500nM) decreased the hyperpolarization induced by 10^{-3}M paraoxon (20%), parathion (50%), TOTP (70%), and PSP (120%), indicating potential modulation through a PTP. In contrast, 30 hour pretreatment with the muscarinic agonist carbachol (1mM) increased the amount of mitochondrial hyperpolarization induced by TOTP by approximately 90% (10^{-4}M) and 73% (10^{-3} M) and also delayed subsequent depolarization. This indicated that second messenger systems were also involved in the generation of mitochondrial hyperpolarization. Hyperpolarization of the mitochondrial membrane 1 to 24 hours prior to depolarization, a loss of substrate adhesion, and an increase in DNA fragmentation indicated that mitochondria were a primary target in OP compound-intiated cytotoxicity. (Supported in part by a Novartis predoctoral fellowship from the Society of Toxicology.)

865 EVIDENCE OF AZINPHOS-METHYL HEMOGLOBIN ADDUCT.

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Azinphos-methyl (AZM) is an organophosphate insecticide commonly used by U.S. growers on a variety of crops. Agricultural laborers who apply AZM or work in AZM treated fields are exposed to AZM. Acute, high-level AZM exposure causes cholinesterase inhibition leading to short-term neurological dysfunction. The effects of chronic, low-level AZM exposure, typical of most worker scenarios, are unknown. Methods for monitoring occupational AZM exposure have limitations. Urinary metabolites can only be detected within 48 hours of exposure. Cholinesterase monitoring requires pre-exposure testing because of high individual variation in cholinesterase activity. Our work has focused on using hemoglobin adducts as a biomarker of internal dose for AZM. Hemoglobin adducts are useful biomarkers of exposure to xenobiotics because of sampling ease (a 5-10 mL blood sample contains about 1 g of hemoglobin) and the long biological lifespan of hemoglobin (120 days in humans), which allows for retrospective exposure monitoring.

We hypothesize that an AZM metabolite forms an adduct to hemoglobin present in red blood cells. Previously, we reported a dose dependent association of radioactivity with hemoglobin collected from [^{14}C]AZM treated rats. Recent experiments provide more evidence to support our hypothesis. We exhaustively washed hemoglobin from [^{14}C]AZM treated rats with organic solvents. After washing, 30% of the radioactivity remained. HPLC analysis of washed hemoglobin resulted in a single radioactive peak with an elution time consistent with the hemoglobin alpha chain. Washed hemoglobin was hydrolyzed under mild acid conditions. We found ~50% of the radioactivity in the organic phase extract of the hydrolysate. These results indicate that the radioactive moiety is covalently bound to hemoglobin, supporting our hypothesis of an AZM hemoglobin adduct.

866 THE USE OF OPH ENZYME FOR THE DETOXIFICATION OF METHYL PARATHION.

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Methyl parathion (MP) is a pesticide widely distributed in the environment and chemically similar to nerve agents used in biological warfare. Bioremediation of this class of compounds is a critical environmental concern. MP toxicity is primarily associated with the inhibition of cholinesterase activity and resulting effects on the nervous system. MP can induce a genotoxic effect in bacterial and mammalian cells. However, the major metabolite of MP degradation, p-nitrophenol, is generally considered less toxic. This study reports on the evaluation of the genotoxic effect of MP, p-nitrophenol (PNP), and a 1:1 MP:PNP mixture. While MP induced a positive genotoxic response in *S. typhimurium* TA100 at a dose of 1000 μ g per plate, PNP and a 1:1 mixture of PNP and MP did not induce a positive response. The cytotoxic effect of MP was also evaluated in a human neuroblastoma cell, SH-SY5Y. At the highest dose of MP tested (50 μ M), significant decreases of both cell proliferation and viability were observed. Studies were also conducted to evaluate the utility of an organophosphate hydrolase (OPH) enzyme for degradation of MP. MP dissolved in LB broth containing 5 % methanol was treated with intact *E. coli* cells containing the OPH enzyme. Significant rates of MP biodegradation were observed in the liquid culture. Within 50 hours, approximately 85 % of the MP was converted to PNP as measured spectrophotometrically. After the samples were extracted by liquid/liquid extraction with methylene chloride, PNP and MP were analyzed by capillary gas chromatography with NP detector. With this extraction procedure, the average recovery of PNP and MP was 66% and 89%, respectively. The genotoxic potential of extracts of OPH treated and untreated MP amended media was measured with *S. typhimurium* strain TA100. The untreated media induced about 2.3 times the number of spontaneous revertants at a dose equivalent to 54 ml of medium (MP 0.025 mg/ml) per plate, while the OPH treated media failed to induce a positive genotoxic response. Chemical analysis indicated that about 90% of MP was removed from the media by treatment with OPH enzyme. These studies indicate that the OPH enzyme is capable of degrading MP and eliminating its genotoxic potential in liquid culture.

867 INHIBITION OF BRAIN SYNAPTOSOMAL Ca-ATPase BY ORGANOPHOSPHOROUS (OP) COMPOUNDS.

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Exposure to neuropathy-inducing OP compounds can result in elevated intracellular calcium (Ca_i). We hypothesize that OP exposure could contribute to changes in Ca_i by inhibition of Ca-ATPases in susceptible animals. To examine this, synaptosomes were prepared from the brains of adult hens, which are susceptible to OP-induced delayed neuropathy (OPIDN) and adult rats, which are not. Synaptosomes were incubated with OP compounds which can produce neuropathy [chlorpyrifos (CPF), chlorpyrifos-oxon (CPO), tri-*o*-tolyl phosphate (TOTP), phenyl saligenin phosphate (PSP), diisopropyl phosphate (DFP)] and with parathion (PTN) and its active metabolite paraoxon (PXN), which do not induce OPIDN. In hen brain synaptosomes, incubation with CPF and PTN for 30 minutes produced significant inhibition of Mg/Ca-ATPase activity (35% and 25%, respectively). In rat brain synaptosomes, no compound produced significant inhibition of Mg/Ca-ATPase activity. Ca-ATPase activity in brain synaptosomes prepared from hens treated with DFP (0.5 x LD50, 1 mg/kg) was decreased by 50% 24 hours after dosing. Ca-ATPase was not inhibited in hens treated with CPF (0.5 x LD50, 25 mg/kg). The results suggest that inhibition of Ca-ATPase occurs rapidly after exposure to OP insecticides and this may contribute to their neurotoxicity. (Supported by EPA grant R825356.)

868 INCREASED IN VITRO ANTICHOLINESTERASE ACTION OF THE INSECTICIDE CHLORPYRIFOS WHEN TESTED IN MIXTURES CONTAINING SEVERAL POLYCYCLIC AROMATIC HYDROCARBONS.

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Research on complex mixtures is of high of priority to EPA, NIH, and other agencies because most environmental agents that affect human health are found in mixtures. The study reported here tested the hypothesis that several polycyclic aromatic hydrocarbons (PAHs) found in house dust modify the ability of the insecticide chlorpyrifos (CPF) to inhibit acetylcholinesterase (AChE) in vitro. AChE (EC 3.1.1.7; 0.125 Units) purified from electric eel was incubated at 25°C for 4 min in buffer containing increasing concentrations of CPF-oxon dissolved in ethanol (0.01-800 nM). AChE activity was then determined by standard colorimetric analysis using acetylthiocholine iodide as substrate. CPF-oxon inhibition curves were generated in the presence or absence of different concentrations of the PAHs pyrene, benzo[a]pyrene, anthracene, and fluoranthene (0.1-10 ppm dissolved in ethanol). AChE inhibition curves were also established for these four PAHs alone, relative to ethanol-only controls. Results indicate that all four PAHs inhibited AChE activity potently with IC50 values in the range of 3-7 ppm, in close agreement with another recent study demonstrating this effect [Kang and Fang, *Biochem Biophysic Res Comm* 238, 367-369 (1997)]. When AChE was incubated with CPF-oxon together with the PAHs, the efficacy of CPF-oxon was dramatically increased. This was exemplified by large (~70%) and significant ($p<0.001$) decreases in AChE activity at nanomolar concentrations of CPF-oxon, and similar decreases in the Vmax estimates from kinetic studies. The effects of the PAHs followed a dose-response relationship in all cases, and kinetic studies indicated that the PAHs inhibited AChE in a non-competitive manner. These findings may have implications on the hazard assessment of indoor exposure to CPF, especially since these PAHs increased markedly the anticholinesterase activity of low nanomolar concentrations of this insecticide. (Supported by JHU Professional Development Award to DAJ.)

869 cAMP-DEPENDENT PROTEIN KINASE (PKA) ACTIVITY IN THE BRAINS OF RATS AFTER ACUTE EXPOSURE TO CHLORPYRIFOS.

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We have previously shown that anticholinesterases such as paraoxon cause a decrease in intra-neuronal levels of the second messenger cAMP, either by direct interaction with G-protein linked neurotransmitter receptors, or by some indirect mechanism [e.g. Jett et al., *Pest Biochem Physiol* 39:149-157 (1991)]. Others have found similar results with other organophosphate insecticides [e.g. Ward and Mundy, *Brain Res Bull* 39:49-55 (1996)], and it is generally believed that these compounds preferentially affect cAMP signaling when compared to other signal transduction pathways. Also it was recently demonstrated that adenylyl cyclase is a primary target for the developmental toxicity of chlorpyrifos [Song et al., *Toxicol Appl Pharmacol* 145:158-174 (1997)]. These findings are significant because cAMP signaling is known to be extremely important in learning and memory in both vertebrates and invertebrates. Surprisingly, nothing is known about the significance of OP-induced changes in cAMP signaling downstream of adenylyl cyclase activity. We report here on the first of several experiments on the effects of chlorpyrifos on cytoplasmic and nuclear cAMP signaling. Subadult Long-Evans rats (~150 g) were injected subcutaneously with 125 mg/kg chlorpyrifos dissolved in peanut oil; control rats were given oil alone. Rats were euthanized 48 hr after dosing, and the cerebral cortex was dissected on ice and immediately frozen and stored at −70°C. In this study, cAMP-dependent protein kinase (PKA) levels were determined by a colorimetric assay using the fluorescent dye-labeled Kemptide substrate (LRRASLG) and SpinZyme affinity columns, both from Pierce Chem. Co. Results indicated that rats exposed acutely to chlorpyrifos had ~30% decrease in PKA activity as judged by the amount of phosphorylated substrate in the assay. These data suggest that the documented decrease in cAMP levels by OPs may result in changes in PKA activity in vivo. Research is underway to examine the effects of chlorpyrifos on transcriptional events and gene expression associated with this pathway. (Supported by JHU NIEHS Center in Urban Health Pilot Project to DAJ.)

870 ALTERATIONS OF RAT BRAIN DNA AND NEUROTROPHIN LEVELS BY PRENATAL EXPOSURE TO CHLORPYRIFOS.

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From embryogenesis to senescence, neurotrophic factors are intimately involved in proliferation, differentiation, plasticity, and survival of neurons and glia in the central nervous system. In the present study, the effects of organophosphate pesticide chlorpyrifos (CPF) on the regional and temporal distribution of neurotrophic factors (NGF, BDNF and NT-3), and DNA and protein levels were examined in the developing brain. Pregnant Long-Evans rats were dosed daily (*p.o.*) with CPF in corn oil (0, 3, 5, 7, and 10 mg/kg) on gestational days 14-18. Results show that CPF (5 h after last dose) decreased the level of BDNF in a dose-dependent manner (5-40%), increased the level of NGF (20-40%), and increased the content of DNA (3%-15%) but had no effect on NT-3 in fetal whole brain. The rest of the rats (0, 5, 7, and 10, CPF mg/kg) were allowed to give birth and the pups were sacrificed on postnatal day (PND) 1, 4, 7, 21, or 114. CPF did not alter pregnancy outcomes appreciably at any dose. The content of DNA, protein, NGF, BDNF and NT-3 were quantitated in the cerebral cortex (CTX), hippocampus (HPC), cerebellum (CB) and brainstem (BS). These regions were chosen based on differences in cholinergic innervation and differential maturational profiles. In the CTX and HPC, CPF decreased the level of BDNF (5-60%) and NT-3 (10-55%) in a dose-dependent manner across all the ages except at PND-1, but had no effect on regional NGF levels. CPF decreased the level of NGF, BDNF and NT-3 in CB only at PND-1 and in the BS at PND-114 as compared to controls, suggesting that CPF had differential effects on neurotrophins during development in different regions of the brain. No alterations were noted on regional DNA and protein content.The postnatal effects on regional neurotrophin levels were much more robust in regions (CTX and HPC) developing primarily during the perinatal period. The effects of gestational CPF exposure were long-term and persistent on regional levels of BDNF and NT-3, suggesting that subtle alterations might be manifested in plasticity and maintenance of neural function within the adult nervous system.

871 AN EVALUATION OF THE SIGNIFICANCE OF DICHLORVOS (DDVP) INDUCED ALTERATIONS OF CHOLINERSTERASE (ChE) LEVELS IN BIOLOGICAL SYSTEMS: FINAL REPORT OF THE EXPERT PANEL.

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A group of 11 internationally-known scientists were members of an Expert Panel (the Panel) which evaluated the biological significance of DDVP induced ChE inhibition. The Panel's major conclusions included: - The primary source of information for estimating human health risks is the presence or absence of clinically observable signs of toxicity. - Brain ChE activity (not plasma or RBC) is predictive of toxicity only after a significant threshold has been surpassed. - Significant RBC AChE inhibition (< 50%) occurs in the absence of clinical signs or symptoms of toxicity. - DDVP has extensive human safety data available. - The human acute dietary NOEL of DDVP is 1.0 mg/kg based upon human data. - The human chronic dietary NOAEL and NOEL are 0.3 and 0.1 mg/kg/day, respectively. - The chronic dietary NOAEL and NOEL in dogs are 1.0 and 0.05 mg/kg/day, respectively. - Extensive human data demonstrate the safety of maintaining humans at continuously reduced RBC cholinesterase levels (50 - 75%) for periods in excess of six years.

872 FUR RESIDUE AND CHOLINESTERASE INHIBITION OF DOGS DIPPED WITH CHLORPYRIFOS OR PHOSMET.

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Dogs were dipped with a commercial chlorpyrifos (Dursban[R]) or phosmet (Imidan[R]) flea dip using the recommended guidelines for four consecutive treatments in order to determine the residues available for transfer to humans from the fur of the dogs. The inhibition of plasma cholinesterase in the dogs was also determined over the study as a biomarker. Twelve dogs were used for each of the chlorpyrifos experiments and the fur was sampled by rubbing with cotton gloves for 5 min. in a 10 x 4 in. area along the upper back before dipping and at times 4 hrs, 7, 14, and 21 days after dipping. Over the 4 dippings the 4 hr samples averaged 1229 ± 158 μg, the 7, 14, and 21 day samples averaged 332 ± 68 μg, 215 ± 61 μg, and 139 ± 43 μg, respectively. The highest sample was 7000 μg at 4 hrs. Chlorpyrifos (CP) was quantified using gas chromatography with electron capture detection. For phosmet 24 dogs were used and were sampled at 4 hours and at days 1, 3, 7, and 14. Over the 4 dippings the 4 hr samples averaged 3723 ± 426 μg, the 1, 3, 7, and 14 day samples averaged 1431 ± 205 μg, 728 ± 131 μg, 231 ± 59 μg, and 62 ± 18 μg, respectively. The highest sample was 13817 μg at 4 hrs. The gloves were extracted using an accelerated solvent extractor. Plasma cholinesterase levels were measured as butyryl cholinesterase (BChE) using butyrylthiocholine (BTCh) and as acetylcholinesterase (AChE) using acetylthiocholine (ATCh). The average specific activities (nmole min^{-1} mg $protein^{-1}$) at zero time were: 54 ± 3 for BChE, and 10 ± 1 for AChE. The average percent inhibition ± standard error of BChE for the four dippings of CP were: 4 hours - 60 ± 4; 7 days - 76 ± 2; 14 days - 66 ± 3; and 21 days - 54 ± 3. The average percent inhibition ± standard error of AChE for the four dippings of CP were: 4 hours - 13 ± 2; 7 days - 18 ± 3; 14 days - 15 ± 3; and 21 days - 11 ± 2. Phosmet did not significantly decrease the levels of plasma BChE or AChE during the study, with inhibition ranging from 0 to 15%. (Supported by EPA R 825170)

873 POSSIBLE INFLUENCE OF VITELLOGENIN SYNTHESIS ON THE TOXICITY OF CHLORPYRIFOS IN CHANNEL CATFISH.

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Vitellogenin, the egg yolk precursor, is secreted by the liver of oviparous animals in response to estrogen during vitellogenesis. Vitellogenin is rich in serine hydroxyl residues, the same residues present in the esteratic site of acetylcholinesterase (AChE), which is the target enzyme for organophosphorus insecticides. The oxon-metabolite of the phosphorothionate insecticide chlorpyrifos (C=S) inhibits AChE by phosphorylating these serine residues. The effect of vitellogenin synthesis on AChE inhibition by C=S was investigated in channel catfish (*Ictalarus punctatus*) fingerlings. Fish were injected i.p. with 2 mg/100g 17β-estradiol to induce vitellogenesis, and exposed to 0.125 mg/L C=S at 24, 36, and 48 hours post-injection in a static environment. Fish were sacrificed 24 hours after termination of the C=S exposure, and brain and liver AChE activities were determined. The presence or absence of vitellogenin in the serum was confirmed by native gel-electrophoresis. Brain AChE inhibition for estrogen treated fish at 24, 36, and 48 hours was about 76%, 75%, and 71% respectively, as compared to 86%, 87%, and 88% for non-induced fish . Liver AChE inhibition for all groups was about 90%. These results suggest that vitellogenin may provide a means of partial protection against brain AChE inhibition following organophosphorus insecticide exposure, possibly by providing alternate phosphorylation sites for the oxon-metabolite of chlorpyrifos.

874 COMPARATIVE CHOLINERGIC NEUROTOXICITY OF ORAL CHLORPYRIFOS EXPOSURES IN NEONATAL AND ADULT RATS.

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Chlorpyrifos (CPF) is an organophosphorus acetylcholinesterase (AChE) inhibitor and commonly-used insecticide. Previous studies have demonstrated that neonatal (7 days of age) rats are more sensitive than adults to the acute lethality of high subcutaneous dosages of CPF. The present study examined age-related differences in neurochemical and functional signs of

neurotoxicity following oral CPF exposures. Lethality studies determined substantial differences in acute sensitivity following high dosages (LD_{10}: adult = 136 mg/kg; neonate = 15 mg/kg). Neonatal and adult rats (n=3-6/group) were given CPF (0, 0.15, 0.45, 0.75, 1.5, 4.5 or 7.5 mg/kg/d) for fourteen days and sacrificed 4 hrs after either the 1st or 14th dose for neurochemical measurements (AChE in plasma, RBC, frontal cortex and diaphragm, and muscarinic receptor ([^{3}H]QNB) binding and nicotinic receptor ([^{3}H]epibatidine) binding in frontal cortex). No overt signs of cholinergic toxicity were noted in either age group 4 hrs after the 1st dose. After acute exposure, AChE in neonates was inhibited similarly in all tissues ($ED_{50\approx}$ 0.5-2.5 mg/kg). In contrast, only RBC and plasma AChE were inhibited 4 hrs after the 1st exposure in adults ($ED_{50\approx}$ 3 mg/kg). Four hrs after the 14th dose, levels of AChE inhibition in neonates were again relatively similar in all tissues ($ED_{50\approx}$ 1-2 mg/kg). In contrast, there was a wide range of sensitivity to inhibition in adult tissues (ED_{50}: RBC = 0.5 mg/kg; plasma = 1 mg/kg; cortex = 2.3 mg/kg; diaphragm = 6.6 mg/kg). Interestingly, dose-related inhibition of cortical AChE was essentially the same (ED_{50} = 1.9-2.4 mg/kg) and similar reductions in both cortical QNB and epibatidine binding were noted between the age groups after repeated dosing. We conclude that while neonates are more sensitive to acute effects of oral CPF, very similar cholinergic neurochemical changes are noted in frontal cortex in both age groups following repeated exposures. (Supported by U.S. EPA R 825811-01-0.)

875 RESULTS OF A 2-MONTH CHLORPYRIFOS FEEDING STUDY IN ADULT, MALE RATS.

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Chlorpyrifos [CPF; (*O,O'*-diethyl *O*-3,5,6-trichloro-2-pyridyl) phosphorothionate] is a commonly used organophosphorus pesticide. The widely accepted mechanism of toxicity for CPF is cholinesterase (ChE) inhibition. The present study was devised to assess the toxicodynamic and toxicokinetic responses of animals exposed to a relatively long-term, low-dose level of CPF in the feed. Long-Evans, male rats were given CPF in their feed at a level designed to deliver a dosage of 0, 0.8 or 4.0 mg/kg/day. After 2 months of feeding, half the animals in each feeding group were challenged with a single, oral dose of CPF (60 mg/kg in corn oil). This experimental design results in six groups of animals (n=5/group): the unchallenged group at the three feeding levels and the challenged group at the three feeding levels. Twenty-four hours after the challenge dose, the animals were killed, and blood, brain, and liver were collected and frozen. In the unchallenged animals, the 0.8 mg/kg dose produced only ChE inhibition in the blood with no inhibition in the brain, whereas the 4.0 mg/kg dose produce inhibition in both blood and brain (~ 50%). The animals challenged with CPF showed maximal blood and marked (~ 70%) brain ChE inhibition which did not differ according to the amount of CPF in the feed. There were no changes in the muscarinic receptor density in any of the dosage groups. Levels of CPF, CPF-oxon, and trichloropyridinol (TCP) were measured by HPLC in both brain and liver. No CPF or metabolites were detected in brain in any of the dosage groups, whereas both CPF and TCP were detected in liver of the challenged animals. In the unchallenged animals, only the 4.0 mg/kg/day group showed measurable liver TCP levels. It appears, therefore, that previous, low-level exposure to CPF does not alter the biochemical responses to a challenge dose of higher level of CPF.

876 TIME OF ADMINISTRATION AFFECTS ORGANOPHOSPHATE PESTICIDE-INDUCED ALTERATIONS IN BODY TEMPERATURE.

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Chlorpyrifos (Cpf) is an organophosphate pesticide (OP) and is among the most common and widely used commercial insecticides. Cpf, as well as other OPs, exerts its toxic effects through inhibition of acetylcholinesterase, resulting in increased cholinergic activity. Human intoxication results in a typical set of responses, which include an immediate and long lasting hyperthermic response. Rodent exposure, however, results in a biphasic thermal response: short-term hypothermia followed by subtle hyperthermia several days after administration. The current study was designed to determine whether exposure to Cpf at different times of the day would influence this biphasic response. Male Sprague-Dawley rats (200-250 gm) were exposed to Cpf via oral gavage at one of four time points (0600, 1200, 1800, 2400). Core body temperature was recorded every six hours from two days prior to administration to three days after administration. The results of this study show that the timing and magnitude of the initial (hypothermic) response (6 hours post-dosing) was independent of time of exposure, while the delayed (hyperthermic) response (48-72 hours post-dosing) was related to light/dark phase. Regardless of time of administration, at six hours after treatment, animals exposed to Cpf demonstrated a significant decrease in body temperature as compared to controls. However, the delayed hyperthermic effect differed with time of administration. Light phase administration resulted in increased temperature only during the light phase, while dark phase administration resulted in sustained temperature elevation. Identifying these mechanisms and the underlying neurochemistry is necessary in order to evaluate protective limits for exposure to compounds such as Cpf.

877 ERYTHROCYTE ACETYLCHOLINESTERASE ACTIVITY IN A KOREAN POPULATION.

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To assess the degree of organophosphate poisoning, acetylcholinesterase (AChE) activity is measured. While the plasma AChE activity is easy to determine, it is labile and is not specifically correlated with the enzyme activity in neuronal tissues. AChE activity in red blood cells (RBC) is not labile and is more closely related to the enzyme activity in neuronal tissues. However, there were no reference range of AChE activities in RBC for Korean population. Thus, RBC AChE activities were measured using modified Ellman's method in 109 healthy Korean adults (age: 19 - 58 yr). Blood sample (3 ml) was collected in heparinized tube and RBC was separated from plasma within 1 - 2 hr. Interference by butyrylcholinesterase was eliminated by quinidine sulfate, a specific inhibitor. RBC AChE activities in Korean subjects were unimodally distributed with inter-individual variation of 15% (coefficient of variation) similar to the values obtained in other ethinic groups. The RBC AChE activity ranged from 8,132 to 23,562 U/L packed RBC. Comparing the RBC AChE activity of 76 male and 33 female subjects, they were not significantly different (Mann-Whitney U test, $p = 0.675$). Median activities for male were 13,526 (range: 10,402 - 21,027) and for female were 13,408 (range: 8,132 - 23,562). However, there was a positive correlation for smoking intensity (Spearman's correlation coefficient = 0.253, $p = 0.048$, smoking index = daily cigarette packs × smoking years) but a negative correlation for age (Spearman's correlation coefficient = -0.184, $p = 0.055$). (Supported by an intramural research grant from Inha University in 1998.)

878 REVERSIBLE INHIBITION OF CHOLINESTERASE BY SPIN TRAPPING AGENTS.

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The spin trapping agent phenyl-N *tert*-butylnitrone (PBN) prevented anticholinesterase induced fasciculations and seizures, withouth affecting kainic acid or pilocarpine produced seizures. Consequently this study was performed to quantitate and identify the probable mechanism of action. The inhibitory potency of PBN and α-(4-pyridyl 1-oxide)-N-*tert*-butylnitrone (POBN) on rat brain and diaphragm, purified mouse brain and electric eel acetylcholinesterase (AChE; EC 3.1.1.7) were studied in vitro using spectrophotometric kinetic assay with acetylthiocholine (ASCh) as substrate. The inhibition was in a reversible and concentration-dependent manner. Double reciprocal plots of the reaction velocity against varying ASCh concentration were linear and generally indicated mixed competitive/non-competitive inhibition. PBN was the most potent inhibitor of mouse brain AChE with Ki and Ki' of 0,58 mM and 2.99 mM respectively. PBN was less effective for rat brain (Ki=0.69 mM, Ki'=3.87 mM) and diaphragm (Ki=1.33 mM, Ki'=2.40 mM) and was the weakest inhibitor of electric eel AChE in contrast to POBN which showed the highest affinity for this enzyme, with Ki and Ki' values of 1.065 mM and 3.15 mM respectively. These data support the suggestion that preinhibition with these agents prevents critical irreversible inhibition of AChE in brain and muscle and consequently seizures and fasciculations. (Supported by NIH Grant, ES04597.)

879 PIPERONYL BUTOXIDE POTENTIATES THE SYNAPTOSOME ATPase INHIBITING EFFECT OF PYRETHRIN.

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Pyrethrins are widely used insecticides both in agriculture and households. In many commercial formulations piperonyl butoxide (POB) is used with pyrethrins. POB is a known synergist of pyrethrins, used to intensify their effect. The cellular target of pyrethrins is the sodium channel in the membrane. In the present study the activity of membrane-bound integral protein ATPase was studied as a biomarker for the membrane effect of pyrethrin and POB. Male Sprague-Dawley rats were used for cerebral synaptosome preparation. The isolation of synaptosomes was performed with the Percoll gradient method. Both total ATPase and Mg^{2+} activated ATPase were studied by determining inorganic phosphate. Exposure to 0.1, 1, 10, 100 and 1000 μ M of pyrethrin and to 0,4, 4, 40 and 400 μ M of POB decreased ATPase activity dose-dependently. Most efficient was the mixture of pyrethrin and POB (1:4). The activity of total ATPase decreased 10 % in concentrations of 1-10 μ M pyrethrin alone and the 50 % decrease was found at 100 μ M pyrethrin. The mixture of pyrethrin and POB caused 30-60 % decrease in the total ATPase activity at 1-10 μ M pyrethrin and 4-40 μM POB. 85 % decrease was found after exposure to the mixture of 100 μM pyrethrin and 400 μM POB. POB alone had no effect at 1-10 μM concentrations, but a marked effect was seen at over 40 μ M concentrations. The results illustrate that POB is an effective synergist of pyrethrin and that it is very toxic in high concentrations. The results also confirm that sodium homeostasis is a target of the neurotoxic effect of pyrethroid compounds.

880 BIOCHEMICAL AND MICROSCOPIC CHANGES IN DIAPHRAGM MUSCLE BY ACUTE CARBAMATE TOXICITY.

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The objective of this study was to investigate the biochemical and microscopic changes in diaphragm muscle of rats acutely intoxicated with aldicarb (0.4 mg/Kg, ip) or carbofuran (1.5 mg/Kg, sc). With either carbamate, rats developed signs of muscle fasciculations within 30-60 min. During this time, diaphragm AChE was maximally reduced (75-80%). Biomarker enzymes, such as CK (isoenzyme CK-MM3) and LDH (isoenzymes LDH-1→ LDH-5) showed characteristic changesin muscle. These enzymes/isoenzymes leaked into circulation due to significant depletion of high-energy phosphates (ATP, 20% and PCr, 30%) in muscle. Declines in the corresponding ratios of ATP/ADP (26%), ATP/AMP (39%) and PCr/Cr (20%) were suggestive of a greater utilization of ATP and PCr in response to their increased demand for carbamate-induced sustained high-frequency muscle fasciculations. Lesions in muscle were greater with aldicarb than with carbofuran, causing cell swelling characterized by loss of myofibrillar structure, cytoplasmic clearing and necrosis. Electron microscopic changes included mid focal loss of myofibrillar structure, inter-myofibrillar clearing (fluid accumulation)m and swelling of mitochondria. It can be concluded from these findings that carbamate-induced muscle hyperactivity caused muscle lesions due to a depletion of high-energy phosphates, which are required to maintain mitochondrial/cellular membrane permeability and integrity. (Partially supported by NIEHS Grant 5RO1ES05193-03).

881 RELATIVE CONTRIBUTION OF TRANSMITTER RELEASE AND GABA ANTAGONISM IN THE ACTIONS OF CYCLODIENES ON THE MURINE NIGROSTRIATUM.

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Neurochemical changes were investigated in C57BL6 mice given 3 intraperitoneal doses of the cyclodiene insecticide, heptachlor, over a 2 week period. Heptachlor increased [^{3}H]dopamine uptake in *ex vivo* striatal synaptosomes, which we attribute to dopamine transporter up-regulation in response to increased synaptic levels of dopamine. Neurotransmitter release studies with striatal synaptosome preparations revealed that heptachlor and heptachlor epoxide stimulated release of pre-loaded dopamine, serotonin, glutamate and GABA. The potency for release of dopamine was at least 7-fold greater than that of the other transmitters. Picrotoxinin and bicuculline were inactive in this assay, which precludes an interaction with the GABAa receptor as a mechanism for release of transmitter. Accordingly, mice treated with sublethal doses of picrotoxinin failed to show any upregulation of dopamine transport in *ex vivo* synaptosomes. In electrophysiological studies of murine striatal brain slices, the cyclodiene dieldrin mimicked the inhibitory action of applied dopamine, an effect consistent with induced release of dopamine, but not GABA antagonism. These studies suggest that cyclodiene-dependent release of transmitter from nerve terminals, and not GABA antagonism, induces upregulation of dopamine transport and may serve as a predisposing factor in environmental Parkinsonism.

882 DEET BLOOD LEVEL STUDIES IN RATS FOLLOWING A SINGLE ORAL ADMINISTRATION, AND SINGLE AND REPEATED DERMAL APPLICATIONS.

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Studies were conducted in rats to determine diethyl-*m*-toluamide (DEET) blood levels following oral and dermal administration of DEET using the same dosing procedures and dose levels used previously in an acute oral neurotoxicity study in rats and a 90-day dermal toxicity study in rats. The purpose of the present studies was to develop information regarding the blood profile and maximum DEET blood levels likely to have occurred at the highest no observable effect levels in the previous studies. In the acute oral study, DEET plasma levels were determined following bolus administration of 200 mg/kg undiluted DEET to rats. Three rats/sex were anesthetized and exsanguinated 15, 30, 45, 60, 90, 120, 180 and 240 min. after dosing. DEET was detected in plasma within 15 min. and reached an apparent mean plateau of 9.6 and 13.6 μg/ml for males and females, respectively, between 45 to 240 min. In the dermal study, DEET plasma levels were determined following application of 1000 mg/kg undiluted DEET to rats on a single occasion or once per day for five consecutive days. Four rats/sex were anesthetized and exsanguinated 2, 4, 8, 12, and 24 hours after the first or final applications. DEET plasma levels in males reached a mean plateau of 4.5 μg/ml between 2 and 12 hours and decreased thereafter. In females, mean plasma levels peaked during the 4 to 8 hour period at 17 to 24 μg/ml and decreased thereafter. There was no evidence of DEET accumulation with repeated application. These data were compared to analogous blood level data generated recently for humans following dermal application of DEET under a worst-case exposure scenario. These comparisons, along with the toxicity data from previous studies, indicate margins of exposure of at least 10-fold between the no-effect blood levels in animals and peak blood levels in humans. (Sponsored by the DEET Joint Venture/CSMA, Washington, DC.)

883 PARADOXICAL ALTERATION OF DIBROMOETHANE (EDB)-INDUCED NEPHROTOXICITY BY ADRENERGIC AGENTS.

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Stress, adrenergic agonists, and antagonists can influence chemical and drug-induced hepatotoxicity. Previous studies have shown that adrenergic agonists such as phenylephrine (PE) can enhance the cytotoxicity produced by selected hepatotoxicants and by bromohydroquinone, a known nephrotoxicant. The purpose of this study was to extend these observations to evaluate the effect of adrenergic agents on the pesticide dibromoethane (EDB), a nephrotoxicant. Male ICR mice, 23-26gms were treated with EDB and subsequently with either an adrenergic agonist, antagonist, or a combination of both. Each treatment group contained 8-10 mice. All animals were maintained in a controlled environment and provided food and water ad libitum. EDB treatment resulted in kidney cytotoxicity as measured by increases in urinary gamma glutamyl transpeptidase (GGTP). Severe to moderate cell damage was detected with this first treatment regimen. Concomitant with the increase in urinary GGTP, a significant decrease in kidney tissue glutathione was detected. Phenylephrine (PE) (50mg/Kg ip) potentiated the cytotoxic effects of EDB resulting in a significant increase in urinary GGTP. Several pathological changes were also observed in kidney tissue, consistent with the observation of hematuria. The enhancement of the pesticide-induced nephrotoxicity was antagonized by an adrenergic antagonist, phentolamine. The results of this study indicate that adrenergic agonists and antagonists can significantly alter the toxicity of the pesticide EDB. The mechanism by which

adrenergic modulation alters the nephrotoxicity of this pesticide remains to be elucidated. However, the adrenergic modulation of cytotoxicity may be a useful tool for evaluating drug and chemical-induced organ changes. (Supported in part by NIOSH Grant T42/CCT 412874.)

884 COMPARISON OF KINETIC PARAMETERS FOR *IN VITRO* METABOLISM OF ALACHLOR AND 2-CHLORO-*N*-(2,6-DIETHYLPHENYL)ACETAMIDE (CDEPA) IN MOUSE, RAT, AND HUMAN.

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Alachlor (2-chloro-*N*-methoxymethyl-*N*-(2,6-diethylphenyl)acetamide) is a widely used pre-emergent herbicide which has been shown to be carcinogenic in rats. The mechanism for carcinogenesis has not been elucidated, however it has been proposed that alachlor forms a DNA reactive metabolite, 2,6-diethylbenzoquinone imine (DEBQI). A key step in the formation of DEBQI is the metabolism of 2-chloro-*N*-(2,6-diethylphenyl)acetamide (CDEPA) to 2,6-diethylaniline (DEA) through an aryl amidase reaction. It has been suggested that humans lack the aryl amidase necessary to metabolize CDEPA to DEA. We have shown that humans are able to metabolize alachlor to CDEPA and that this is catalyzed by CYP 3A4. We have also shown, for the first time, that CDEPA is metabolized to DEA in humans at a rate which is greater then that of rats and mice. We have determined the K_m and V_{max} of the formation of DEA from CDEPA using liver microsomes from male rats (231.9 μM, 1.218 μM/min/mg), male mice (257.0 μM, 1.541 μM/min/mg), and male humans (9.0 μM, 7.246 μM/min/mg). Further investigation of the kinetic parameters of CDEPA formation from alachlor and DEA metabolism are being pursued using microsomes from rats, mice and humans.

885 A COMPARATIVE ANALYSIS OF DNA ADDUCT FORMATION BY ALACHLOR METABOLITES *IN VITRO* AND *IN VIVO* IN RAT LIVER AND OLFACTORY TISSUES BY ^{32}P-POSTLABELING.

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Alachlor (2-chloro-2'6'-diethyl-N-(methoxymethyl) acetanilide) is a restricted use herbicide associated with nasal carcinogenicity and hepatotoxicity in rats; however, the mechanism responsible for its biological effects remains unclear. The complex metabolism of alachlor to reactive metabolites capable of binding DNA has been implicated in its carcinogenicity. This study was undertaken to investigate the formation of DNA adducts upon bioactivation of alachlor and 2,6-diethylaniline (DEA), a major metabolite of alachlor, *in vitro* with calf thymus DNA in the presence of liver and olfactory microsomal activation systems from male Long-Evans rats. Results were compared with adducts formed by alachlor *in vivo* in DNA from liver tissues of rats exposed ip to 126 mg/kg/day of alachlor for 1, 4, or 28 days. DNA adduct analysis was performed using ^{32}P-postlabeling with adduct enrichment by either nuclease P1 or butanol extraction and adduct resolution by thin layer chromatography. Comparison of adduct migration patterns resulting from alachlor bioactivation in olfactory and hepatic *in vitro* activation systems identified a metabolism dependent adduct specific for each tissue. Metabolism of DEA using the same activation systems revealed comigration of three adducts in addition to one adduct unique to liver metabolism. *In vivo* adduct formation in DNA from liver tissues revealed several adducts for all time points. One adduct common to all *in vivo* treatments comigrated with the adduct formed following *in vitro* liver metabolism. These data indicate that bioactivation of both alachlor and DEA result in formation of reactive metabolites capable of covalent binding to DNA as evidenced by the formation of multiple DNA adducts in both *in vitro* systems and *in vivo* treatments. Finally, evidence is provided indicating bioactivation of alachlor to tissue specific adduct-forming metabolites consistent with different target tissue effects.

886 THE RATIONAL SELECTION OF HPPD INHIBITORS FOR AGROCHEMICAL AND PHARMACEUTICAL USE.

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Zeneca discovered a series of selective herbicides with a novel mode of action - the inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD) in plants. Investigative toxicology studies at CTL showed that these chemicals also inhibited hepatic HPPD in rats and mice. Collaborative studies with Dr Sven Lindstedt revealed that the purified human liver enzyme was also inhibited. This finding led to the concept of using of one of these chemicals for the treatment of tyrosinaemia type 1, a hereditary disorder of tyrosine catabolism, by blocking the formation of the toxic metabolites of tyrosine which are produced in this disease. The detailed understanding of the biochemical toxicology associated with inhibition of HPPD in mammals led to the rational selection of one of this series of chemicals, namely NTBC, for the treatment of tyrosinaemia type I in children and another, namely ZA1296, as a selective herbicide. For the treatment of tyrosinaemia type I, where prevention of toxic metabolites of tyrosine catabolism is required, a chemical which produces a rapid and prolonged inhibition of HPPD is most desirable. Therefore chemicals, such as NTBC, which exhibit low enzyme-inhibitor dissociation rates are favoured for the therapeutic use of HPPD inhibitors. In contrast, in the selection of an agrochemical, although exposure to the human population will be negligible, if the inhibition of HPPD and hence interference with tyrosine catabolism is slight, the toxicological consequences of exposure are minimised. Therefore, chemicals such as ZA1296, which exhibit a fast enzyme-inhibitor dissociation rate, complimented by rapid urinary clearance are preferred agrochemical products.

887 BLOOD PROTEINS AND DNA ADDUCTS OF ATRAZINE IN *PEROMYSCUS LEUCOPUS* AS BIOLOGICAL INDICATORS OF TRIAZINE HERBICIDE EXPOSURES IN THE ENVIRONMENT.

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Atrazine is being used extensively in agriculture around the globe as an herbicide for the control of broadleaf and grass weed growth. Atrazine has been detected in ground water, streams, rivers, and lakes in several areas where agricultural crops are predominant. We have investigated the potential of atrazine to form adducts with blood proteins and DNA, and use of these adducts as biological indicators for exposure assessment. *Peromyscus leucopus* were given varied concentrations of [^{14}C]-atrazine (1-280 mg/kg, sp. act. 109-5.7 mCi/m mol) as a single oral gavage and sacrificed at different intervals (0.33-70 days). Our results indicate that atrazine forms stable adducts with the major blood proteins globin and albumin in a dose dependent manner. The adduct levels were maximum at 24 (albumin), and 48 hours (globin) after treatment. Albumin adduct levels were six times higher than globin adducts; however, albumin adduct levels decreased faster than did those of globin. Seven days after treatment, the radioactivity retained in globin and albumin were 80% and 20%, respectively. Atrazine also formed stable adducts with DNA in liver and testis. The binding levels in testis DNA (45 pico mol/mg DNA) were 8 fold higher than in the liver DNA. The peak adduct levels were detected at 24 h. These results demonstrate that both blood proteins and DNA adducts may be used as indicators for assessing atrazine and other triazine herbicide exposures in the environment.

888 QUANTITATION OF ATRAZINE IN HAIR.

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Atrazine, a s-triazine herbicide used extensively in commercial agriculture, has been implicated in endocrine disruption and certain cancers. Exposure to atrazine is highest among applicators and agricultural workers; residues are also found in foodstuffs and in groundwater. The use of hair as a biomatrix to measure pesticide exposure offers the ability to monitor passive exposure (acute or chronic) with minimally invasive sampling and possible temporal correlation to the time of exposure. The rodent model used to test the incorporation of atrazine into hair was the Long Evans (LE) rat, an animal that has both pigmented and nonpigmented hair. Atrazine was administered to male LE rats for five consecutive days at 90, 120 or 150 mg/kg (n=6/dose) via intraperitoneal injection. An area of the dorsal region was shaved prior to dosing and again 14 days after the initiation of dosing. Using a previously developed assay, atrazine was measured in the pigmented and nonpigmented hair. The concentrations (mean±SEM) at the low, mid and high doses were 0.16±0.02, 0.97±0.4 and 0.93±0.09 ng/mg in pigmented hair, and 0.48±0.07, 1.72±0.3 and 1.49±0.24 in nonpigmented hair. Since the potential for surface contamination of hair is of concern with respect to the interpretation of atrazine hair concentrations, decontamination (wash) studies

were performed. First, pesticide-free pigmented and nonpigmented LE rat hair was fortified with atrazine at concentrations similar to those observed in the hair of dosed animals. Fortified hair was then washed with one of five conditions: water, 0.1 % SDS, 1% SDS, Tris buffer (pH 6) or Tris buffer (pH 10). The experiment was then repeated using hair from animals that had been dosed with atrazine. After washing with solutions of either 0.1 or 1% SDS, all atrazine was removed from the fortified hair. The atrazine remaining in the dosed hair samples ranged from 47.03 to 73.18% (0.1% SDS) and from 37.89 to 70.38% (1% SDS). Results of the other wash treatments were more variable; generally, more atrazine was removed from fortified hair than from the dosed hair.

889 AGGREGATE EXPOSURE MODEL FOR PESTICIDE DRIFT.

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The Food Quality Protection Act (FQPA) directs the United States Environmental Protection Agency (USEPA) to perform an aggregate exposure assessment (which considers exposure from all sources and all routes) when setting tolerances for pesticide residues in food. We have used this approach to determine risks associated with exposure to methidathion in a community exposed to pesticide drift from a nearby farm. A field study was performed to determine air concentrations and surface deposition of pesticide residues in the community. Data from the field study were supplemented with air modeling to provide estimates of pesticide concentrations throughout the community. Based on a comparison of age-specific inhalation rate to body weight (BW) and skin surface area to BW ratios, the most exposed population was determined to be the 2-3 year old child. Relevant sources/routes of exposure were direct dermal contact, ingestion from hand to mouth activity and homegrown vegetables, and inhalation of airborne pesticide. Hazard indices were determined by comparing route-specific toxicity factors to pesticide levels for inhalation, dermal, and ingestion routes of exposure. The total hazard for the 2-3 year old from all of these sources combined exceeded a Hazard Index of 1.0 (a typical risk management target). Of this total risk, dermal exposure accounted for 76%, ingestion of homegrown vegetables accounted for 13%, ingestion from hand to mouth activity accounted for 10%, and inhalation accounted for 0.1%. Thus, a more traditional "non-aggregate" approach would have missed about 23% of the total exposure. Other sources of pesticide exposure such as well water consumption, soil ingestion, and laundry hanging outdoors were qualitatively evaluated and determined to be relatively insignificant.

890 DEVELOPMENT OF AN IMMUNOASSAY FOR THE DETERMINATION OF HERBICIDE IN SEMEN.

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Exposure studies on the uptake and distribution of chemical contaminants that involve human subjects require non-invasive methods of analysis to profile the distribution of chemicals throughout the body. In those studies involving males where the chemical agent may be a reproductive toxicant, analysis of semen is often the most expedient way to determine the extent of exposure. However, small sample volumes combined with a non-homogeneous matrix present complex problems for chemical analysis using traditional chromatographic methods. We have recently developed a method for the analysis of herbicides in semen using ELISA. The primary advantage of this method is the minimum sample preparation in comparison with that required using chromatographic methods. The analytical resolution of the method, LOD and LOQ of 5 and 25 ppb, respectively, was sufficiently sensitive to determine the presence of a herbicide. The detection limits were verified using samples spiked with radiolabeled herbicide. Moreover, the small quantities required for the ELISA left enough sample to conduct more traditional determinations for sperm count and sperm morphology. Method development for the detection of a toxicant is important from a regulatory perspective. The ease of development of this method indicates that other chemical agents, with an existing ELISA, can be detected with relative ease in semen as well as other complex matrices.

891 ROLE OF CALCIUM CHANNELS IN THE SEXUAL DIMORPHISM OF CADMIUM-INDUCED HEPATOTOXICITY.

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Acute cadmium (Cd) toxicity has been evaluated in many *in vivo* and *in vitro* toxicological studies. *In vivo* rat studies assessing the effect of Cd have demonstrated sexual dimorphism, with the female species being more susceptible to Cd uptake, accumulation, and toxicity. The mechanism of female sensitivity to acute cadmium exposure has not been determined. However, it has been reported that as much as 51% of Cd entering the cytoplasm of hepatocytes does so via the receptor mediated Ca^{2+} channels. The effects of progesterone on Ca^{2+} channels has been implicated in the mechanism of acute toxicity. Given these findings, the hypothesis that progesterone is acting as a Ca^{2+} agonist, thus facilitating increased uptake, accumulation, and cytotoxicity of Cd through receptor mediated Ca^{2+} channels was examined. Male rat hepatocytes were used to evaluate the role of Ca^{2+} channels in the sexual dimorphism of acute cadmium toxicity. Twenty four hour cultures of primary rat hepatocytes were exposed to Cd, progesterone, and/or verapamil for 4 hours and cytolethality was measured. Using isolated hepatocytes it was shown that pretreatment with the Ca^{2+} channel blocker verapamil increased the Cd concentration producing 50% cytolethality (LC_{50}) from 8.1 μ M to 19.8 μ M. In contrast, pretreatment with progesterone enhanced the Cd LC_{50} from 8.1 μ M to 4.0 μ M. The pretreatment combination of progesterone followed by verapamil inhibited the progesterone enhanced Cd cytolethality yielding an LC_{50} of 7.3 μ M (Cd alone LC_{50} = 8.1 μ M). Verapamil and/or progesterone in the absence of Cd did not affect hepatocyte viability. Overall, the results of this study demonstrate that inhibition of progesterone-induced Ca^{2+} influx with the Ca^{2+} channel blocker verapamil, decreases Cd cytolethality in primary isolated rat hepatocytes. These findings suggest that progesterone activation of receptor-mediated Ca^{2+} channels is involved in the sexual dimorphism seen following acute Cd exposure.

892 INVOLVEMENT OF PHASE II ENZYMES IN HEPATOTOXICITY BY E2011.

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Sponsor: T Satoh.

(R)-3-[2-(S)-3-Cyano-1-hydroxypropyl)banzothiazol-6-yl]-5-methoxymethyl-2-oxazolidinone (E2011), a benzothiazole derivative, is a selective inhibitor of monoamine oxidase type A. A subchronic toxicity study of E2011 in rats after 13 weeks of p.o. administration revealed hepatotoxicity including GST-P positive liver foci and karyomegaly in hepatocytes (female > male). The present study was intended to clarify the role of phase II enzymes in the hepatotoxicity by repeated administration of E2011. Female SD rats were orally treated with E2011 daily for 2 weeks at a dose of 150 mg/kg. Forty-five minutes before the E2011 administration, the animals were pretreated with drug metabolizing enzyme inhibitors, i.e., 2,6-dichloro-4-nitrophenol (DCNP) (10 mg/kg, i.p.; inhibitor of sulfotransferase (ST)), pentachlorophenol (PCP) (5 mg/kg, i.p.; inhibitor of both ST and acetyltransferase (AT)) or ranitidine (RA) (100 mg/kg, p.o.; inhibitor of UDP-glucuronosyltransferase (UDP-GT)). Treatments with E2011 in combination with DCNP caused more dramatic karyomegaly in hepatocytes than treatments with E2011 alone. On the other hand, the karyomegaly was clearly diminished by pretreatment with PCP. No additional effect was found in animals pretreated with RA. The differences were attributed to inhibitory effects of AT in addition to ST by PCP. Ames tests of E2011 and its related compounds using Salmonella typhimurium strains TA98 and YG1024 suggested that E2011 exhibits mutagenicity after decarbonation and sequential metabolic activation by cytochrome P450 and AT. In conclusion, AT rather than ST or UDP-GT plays an important role in E2011-induced karyomegaly in hepatocytes. A further studies to clarify the mechanisms of E2011-induced GST-P positive foci are now in progress in our laboratory.

893 HEPATOPROTECTION BY DIMETHYL SULFOXIDE AND AMINOBENZOTRIAZOLE: COMPARISON OF EFFECTS ON HEPATIC MICROCIRCULATION WHEN ADMINISTERED 24 HOURS AFTER CHLOROFORM.

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Both dimethyl sulfoxide (DMSO) and aminobenzotriazole (ABT) protect against developing $CHCl_3$-induced centrilobular necrosis when given 24 hr after the toxicant. Although both DMSO and ABT are CYP2E1 inhibitors, recent studies have shown that ABT, but not DMSO, decreases the bioactivation and covalent binding of $CHCl_3$ at this late time. Since DMSO is also known to enhance organ blood flow following ischemia and to have anti-inflammatory effects, these potential mechanisms of protection were compared between the two antidotes. Groups (N=6-9) of male Sprague Dawley rats (250-300 g) received 0.75 ml/kg $CHCl_3$, 20% in corn oil, po, followed 24 hr later by either 2.0 ml/kg DMSO, 30 mg/kg ABT, or saline vehicle, ip. In vivo microscopy was then utilized to examine events occurring within the hepatic sinusoids 4 hr after antidotal treatment. At 28 hr after $CHCl_3$ dosing, the number of sinusoids containing blood flow was decreased by 40%. DMSO, but not ABT, caused a restoration of blood flow to 85% of normal. Only DMSO moderated the 3-fold increase in centrilobular Kupffer cell phagocytic activity caused by $CHCl_3$. $CHCl_3$ intoxication also increased the numbers of leukocytes adhering to sinusoidal walls in the centrilobular region by 10-fold and increased numbers of swollen (i.e., activated) periportal endothelial cells by 4-fold. Both antidotes equally reduced these parameters by 57% and 78%, respectively. The shared effects by DMSO and ABT on these indicators imply that attenuation of developing liver injury was responsible for the observed decreases in the inflammatory response. That DMSO helped maintain sinusoidal blood flow, while ABT did not, indicates this as a potential mechanism of DMSO's protective action. (ES 08414)

894 HEPATOPROTECTION BY DIMETHYL SULFOXIDE AND AMINOBENZOTRIAZOLE WHEN ADMINISTERED 24 HR AFTER CHLOROFORM: ROLE OF INHIBITION OF ONGOING BIOACTIVATION.

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Dimethyl sulfoxide (DMSO) rapidly attenuates developing $CHCl_3$-induced liver injury when given as late as 24 hr after the toxicant. This protection has been duplicated with aminobenzotriazole (ABT). Since both DMSO and ABT inhibit CYP2E1 which is responsible for bioactivating $CHCl_3$ to a reactive intermediate, they were evaluated for their effect on the degree of $CHCl_3$ binding to hepatic protein following their late administration. Male Sprague Dawley rats (300-350 g) received 0.75 ml/kg $CHCl_3$ (30 μCi/kg $^{14}CHCl_3$), 20% in corn oil, po, followed 24 hr later by either 2.0 ml/kg DMSO, 30 mg/kg ABT, or saline vehicle, ip. Groups (N=4) were then killed at 24, 30 and 34 hr after $CHCl_3$ dosing. ^{14}C concentrations in liver, blood, and fat, as well as ^{14}C covalently bound to hepatic protein, were measured. There were no differences in total ^{14}C content of the liver between DMSO and ABT treated animals. Covalent binding to hepatic protein (6.5 nmol/mg protein) remained unchanged from 24 to 34 hr after $CHCl_3$ dosing in the animals that received saline. While DMSO had no effect on this binding, ABT caused a 35% reduction by 34 hr. The normal loss of ^{14}C from fat (1520 to 270 nmol/g fat) that occurred during this time was not significantly affected by DMSO but was inhibited 60% by ABT. Blood ^{14}C concentrations (160 nmol/ml) did not change from 24 to 34 hr and were not significantly affected by DMSO. However, ABT caused a 50% decrease by 34 hr. These studies indicate that at 24 hr after its administration, there was ongoing biotransformation of $CHCl_3$ and covalent binding of reactive intermediates in the liver that may well contribute to lesion development. However, the lack of effect by DMSO on the disposition of $CHCl_3$ and covalent binding in the liver indicates that other beneficial effects may be responsible for its protective action. (ES08414)

895 ROLE OF MDR2 P-GLYCOPROTEIN IN THE CHOLESTASIS MEDIATED BY ESTRADIOL-17β(β-D-GLUCURONIDE) ($E_2$17G) IN ISOLATED PERFUSED MOUSE LIVER.

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MDR1 substrates/modulators protect against $E_2$17G-induced cholestasis and $E_2$17G is transported by MDR1. mdr2/MDR3, localized in the canalicular membrane of hepatocytes, translocates phospholipids into bile. Recent studies show that MDR3 contributes to the transport of several MDR1 substrates. The present studies were designed to determine the role of mdr2 in cholestasis mediated by $E_2$17G and its biliary excretion. **Methods**: The single-pass isolated perfused liver from FVB/NJ and mdr2(-/-) mice (5-7 weeks old) was used as a model to monitor bile flow (BF, μl/min/g liver) and biliary concentration of $E_2$17G equivalents following administration of a bolus dose of ^3H $E_2$17G. Bile duct was cannulated and gallbladder was ligated. Values are mean±SE, n=3. **Results**: Liver weights of female control (FC), male control (MC), female mdr2(-/-) (FO) and male mdr2(-/-) (MO) mice were ~ 0.8, 1.1, 1.8, and 1.8 g, respectively. In FC, $E_2$17G (0.6 μmol) decreased BF maximally by 51.6%±5.4% with the peak biliary concentration of $E_2$17G of 5.1±0.3 mM. In FO, $E_2$17G (2 μmol) inhibited BF maximally by only 21.4 %±2.8% ($p<0.05$, vs FC) with the peak $E_2$17G concentration in bile of 1.5±0.02 mM; BF recovered to initial level in 20 min. In MO, $E_2$17G (2 μmol) inhibited BF by 49.7%±7.0% and BF did not recover to initial level. Maximal biliary $E_2$17G concentration (1.7±0.06 mM) in MO was similar to that in FO. BF in MC was inhibited 68.0% ±2.5% by 0.6 μmol $E_2$17G with the peak concentration of 5.5±1.1 mM. Following a noncholestatic dose of $E_2$17G, cummulative excretion over 80 min was 94.7%±1.7%, 68.5%±5.9%, 55.3%±0.9% in MC (0.1 μmol), MO (0.1 μmol), MO (0.2 μmol) with peak $E_2$17G concentrations of 3.1±0.4, 1.1±0.1, 1.7±0.1 mM, respectively. **Conclusions**: FO are resistant to $E_2$17G cholestasis. Maximal concentrations of $E_2$17G achieved in bile are significantly lower ($p<0.05$) in FO and MO than control. These data indicate that mdr2 contributes to $E_2$17G cholestasis and in concentrating $E_2$17G into bile.

896 HEPATOBILIARY DISPOSITION OF ACETAMINOPHEN AND ITS METABOLITES IN MALE CD-1 MICE PRETREATED WITH CLOFIBRATE.

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Mice pretreated with the peroxisome proliferator clofibrate (CFB) are protected against acetaminophen (APAP) toxicity. CFB is also known to induce P-glycoprotein in canalicular membrane of hepatocytes. In this study, we investigated the effect of CFB pretreatment on the biliary excretion of APAP and its metabolites. Male CD-1 mice received 500 mg CFB/kg, daily for 10 days. Controls were given corn oil. After overnight fasting, the common bile duct was cannulated and 1mmol APAP/kg was administered *i.v.* Bile was collected for 180 min, and then analyzed by HPLC. CFB had no effect on bile flow rate, nor did it alter the excretion of APAP or its metabolites. To better understand the mechanism underlying the biliary excretion of APAP and its metabolites, indocyanine green (ICG), a non-metabolizable organic anion that is exclusively excreted into the bile via an ATP-dependent process, was administered *i.v.* to bile duct cannulated mice immediately prior to APAP. ICG (30 μmol/kg) significantly reduced the biliary excretion of APAP-GSH. In conclusion, our studies indicate that CFB dosing does not alter the biliary excretion of APAP or its metabolites, and that biliary excretion of APAP-GSH might be mediated through a canalicular transport protein for organic anions. Further studies are needed to fully elucidate the role of ATP-dependant transport proteins in the biliary disposition of APAP (Supported by a grant from Zeneca Pharmaceuticals.)

897 HEPATOCYTE PLASMA MEMBRANE ATP-DEPENDENT TRANSPORT PROTEIN EXPRESSION FOLLOWING INDUCTION OF HEPATOCELLULAR PROLIFERATION *IN VIVO.*

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Chemically-induced mitogenic and compensatory hepatocellular proliferation can result in increased protection to hepatotoxicants. Enhanced expression of liver plasma membrane transport proteins may contribute to this protection. To investigate this, mitogenic proliferation was induced in male CD-1 mice receiving 500 mg clofibrate (CFB)/kg, *i.p.*, for five or ten days; while compensatory proliferation was induced with 350 mg acetaminophen (APAP)/kg, *i.p.* Control mice received vehicle only. Portions of livers were formalin-fixed for immunohistochemical analysis of proliferating cell nuclear antigen (PCNA). Liver plasma membrane fractions were generated by sucrose and percoll density gradients for western blot analysis of the fol-

lowing ATP-dependent transporters: p-glycoprotein (P-gp), multidrug resistant protein-1 (Mrp1) and multidrug resistant protein-2 (Mrp2). Enhanced PCNA staining was evident in both CFB and APAP treatment groups. P-gp expression was increased in CFB treated mice, but not in the APAP group; while Mrp1 expression did not change following either treatment. Interestingly, Mrp2 expression was elevated in both treatment groups. In conclusion, hepatocellular proliferation increases the expression of one or more plasma membrane transporters, which could potentially increase resistance to hepatotoxicants (Supported by grants from Zeneca Pharmaceuticals and PhRMA Foundation.)

898 INVERSE ROLES OF TUMOR NECROSIS FACTOR-α AND NITRIC OXIDE IN ACUTE LIVER INJURY INDUCED BY CARBON TETRACHLORIDE.

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Our laboratory has been investigating the role of macrophages in chemically-induced hepatotoxicity. In a number of different experimental models, hepatotoxicity is prevented or reduced by pretreatment of animals with the macrophage inhibitor, gadolinium chloride. These findings demonstrate that macrophages play a critical role in the pathogenesis of liver injury. Macrophages are known to release a number of different inflammatory mediators with cytotoxic potential. In the present studies we analyzed the role of two macrophage-derived mediators, tumor necrosis factor-α (TNF-α) and nitric oxide (NO), in liver injury induced by carbon tetrachloride (CCl_4). Treatment of mice with CCl_4 (0.3 ml/kg, i.p.) resulted in a time-dependent induction of centrilobular hepatic necrosis. This was correlated with a dramatic increase in serum transaminase levels. CCl_4 induced hepatotoxicity was associated with increased serum and hepatic TNF-α as well as inducible nitric oxide synthase (NOS II) in the liver. To study the role of TNF-α and NO in hepatotoxicity, we used knockout mice lacking the gene for the 55 kDa TNF-α receptor, TNF-α cytokine, or NOS II. We found that CCl_4 was significantly less effective in inducing hepatotoxicity in mice lacking the p55 TNF-α receptor or the TNF-α cytokine. These mice also expressed less hepatic NOS II. In contrast, CCl_4-induced liver injury was markedly increased in knockout mice lacking the gene for NOS II. This was associated with a dramatic increase in serum TNF-α levels. These data suggest that NO is hepatoprotective in this model system. Moreover, the hepatotoxic effects of TNF-α in CCl_4 induced injury may be mediated in part by the inhibition of NO. (Supported by NIH GM34310, ES06897, and the Burroughs Wellcome Fund.)

899 TOXICITY OF CONCANAVALIN-A AND CADMIUM IN IL-6 GENE KNOCK-OUT MICE.

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IL-6 is a well-known inducer of hepatic acute phase protein synthesis. Recently we found that the induction of hepatic metallothionein (MT) after partial hepatectomy was considerably decreased in IL-6 gene knock-out (GKO) mice. MT plays important role in protection of liver and kidneys against various chemicals. In the present study toxicity of concanavalin (Con) A and cadmium (Cd) were compared in IL-6 GKO and B6J129Sv (wild-type control) female mice. Con A was injected ip at a dose of 25 mg/kg; Cd was injected sc at a dose of 2 mg/kg, 2 times with a 24 h interval. At 24 h after injection liver, kidneys and blood were collected. MT level in the tissues was measured by MT RIA. Liver and kidney damage was estimated by activities of GPT, GOT, LDH, ALP, and levels of creatinine, BUN in the serum. We found that Con A induced liver injury in both kinds of mice. Nevertheless, GPT, GOT and LDH activities were increased more in the IL-6 GKO mice than in the wild-type mice. The induced hepatic MT level in IL-6 GKO mice was one-half of that in wild-type mice. On the other hand, Cd induced a similar level of MT in the liver and kidney in the both kinds of mice. The present study shows that IL-6 GKO mice are more susceptible to Con A due to decreased MT synthesis.

900 HIGHER EFFICIENCY OF THE PHOSPHORYLATIVE SYSTEM OF DIABETIC GOTO-KAKIZAKI (GK) RATS.

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Liver mitochondrial bioenergetics of Goto-Kakizaki (GK) rats (a model of non-insulin dependent diabetes mellitus - NIDDM), reveals a mitochondrial membrane potential upon energization with succinate that is significantly increased relative to control animals. The repolarization rate following ADP phosphorylation is also significantly increased in GK mitochondria in parallel with increased ATPase activity. The increase in the repolarization rate and ATPase activity is presumably related to an improved efficiency of F0F1-ATPase, either from a better phosphorylative energy coupling or as consequence of a greater number of catalytic units. Titrations with oligomycin indicate that diabetic GK mitochondria require excess oligomycin pulses to completely abolish phosphorylation relative to control mitochondria. This uggests that mitochondria of diabetic GK rats are provided with extra catalytic units relative to mitochondria of non-diabetic rats.

901 MECHANISM OF INHIBITION OF RAT LIVER CLASS 2 ALDH BY 4-HYDROXYNONENAL.

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4-hydroxy-2-trans-nonenal (4-HNE) is an aldehydic product of lipid peroxidation which produces a diversity of adverse cellular effects. Previous studies from our laboratory have established that 4-HNE is a potent competitive inhibitor (K_i = 0.48 ?M) and, to a lesser extent, a mixed-type inhibitor of acetaldehyde oxidation of the high-affinity form of Class 2 rat liver aldehyde dehydrogenase (ALDH). Because of this complex pattern of enzyme inhibition, experiments were designed to further elucidate the mechanism of 4-HNE inhibition using purified Class 2 rat liver ALDH incubated with or without NAD^+ in the presence of 0 to 250 μM 4-HNE. At all concentrations of 4-HNE evaluated, the activity of ALDH declined most rapidly in the absence of NAD^+, suggesting that the inhibition was not mechanism-based. To assess the reversibility of ALDH inhibition by 4-HNE, experiments were performed with varying concentrations of ALDH and 4-HNE, in the presence or absence of NAD^+. Plots of the data revealed that after 5 minutes of incubation, the inhibition was reversible, however, irreversible inhibition was observed after 120 minutes as evidenced by an increasing *x*-intercept with increasing 4-HNE concentrations. The irreversible nature of ALDH inactivation by 4-HNE adduction was verified to be concentration-dependent through ELISA procedures using antisera produced against 4-HNE adducted proteins. Samples were also processed for quantitation of thiol groups obtained after 2 hours of incubation with 250 μM 4-HNE demonstrating adduction by the loss of over 50% of the sulfhydryl groups. Additionally, increased ALDH-bound carbonyl groups were detected following derivitization with 2,4-dinitrophenylhydrazine (DNPH) and subsequent detection by Western blot analysis using anti-DNPH antisera. The immunoblots demonstrated a concentration-dependent increase in carbonyl formation with 125 μM HNE exhibiting the strongest signal. These data imply that, during the time course studied, a number of nucleophilic amino acid residues in ALDH2 can be alkylated leading to irreversible inactivation. (Supported by AA09300 and AA05536.)

902 TUMOR NECROSIS FACTOR RECEPTOR (TNFR) SIGNALING ON CELLS OF HEMOPOIETIC ORIGIN CONTRIBUTES TO DIMETHYLNITROSAMINE (DMN)-INDUCED HEPATOTOXICITY.

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Repeated exposures to low doses of DMN in humans and mice causes a centrilobular necrosis with inflammatory cell infiltration. Studies in this laboratory involving TNFR knockout mice have demonstrated a role for TNF in this pathology. The purpose of this study was to test whether TNFR on cells of hemopoietic origin were contributing to this event. Irradiated wild type mice (TNFR intact) were rescued with 10^7 white blood cells (WBC) from bone marrow from wild type or from TNFR knockout mice lacking type 1

and type 2 receptors (DKO). After 10 weeks the mice receiving DKO bone marrow cells had greater than 90% of the circulating blood cells of DKO genotype. At this time mice were injected i.p. with 5 mg/kg DMN daily for 7 days. Mice were sacrificed on days 1, 3, 4, 5, and 7 six hours after the DMN injection. Counts of WBC were determined and serum ALT, AST, and SDH activities were measured. No significant differences in circulating WBC were observed between these two treatment groups. Serum enzyme activities were greatest on day 4, with decreasing amounts on days 5 and 7. Significant decreases in the maximum serum enzyme activity were measured on day 4 from irradiated mice having DKO donors when compared to TNFR intact mice (in units/L., 195±44 vs. 533±62 for ALT; 214±22 vs. 330±47 for AST; 214±30 vs. 315±37 for SDH). These data suggest that signaling through TNFR on hemopoietic cells contributes to DMN-induced hepatotoxicity. (Supported by NIH grants 5F32 ES05745-02 and ES04348.)

903 EFFECT OF DICLOFENAC ON CELL FUNCTION AND PROTEIN BINDING IN PRECISION-CUT WISTAR RAT, CYNOMOLGOUS MONKEY AND HUMAN LIVER SLICES.

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Diclofenac, a non-steroidal anti-inflammatory agent, can alter liver cell function which is associated with the binding of diclofenac metabolites to cellular proteins. This study examines the biochemical changes and metabolism of diclofenac in precision-cut liver slices (8 mm diameter, ~250 μm thick) derived from rat, monkey and man. Liver slice viability, as assessed by slice ATP, GSH and potassium content, and LDH leakage, was decreased significantly compared to control slices only after a 48 hr exposure to 350 μM diclofenac. The metabolism and binding of [^{14}C]-diclofenac (30-60 μM) products to cellular proteins was investigated at 2, 24 and 48 hr of culture. Both the rat and monkey liver slices showed a time and dose dependent increase in protein binding of radiolabeled diclofenac products, as measured by non-extractable radioactivity. [^{14}C]-Diclofenac metabolite formation correspondingly increased with culture time, indicating that metabolite formation was necessary for binding. Additionally, dead slices lacked metabolite formation and non-extractable binding of diclofenac products. Separation of both liver slice homogenate and microsomal proteins by 10% polyacrylamide gel electrophoresis followed by imaging on a phosphor screen revealed several distinct radiolabeled protein bands, which increased in intensity with slice culture time. A majority of the proteins binding radiolabeled diclofenac products were in the microsomal fraction. This study has confirmed the association of diclofenac metabolism and cellular protein binding in conjunction with altered cell viability as shown previously with rat hepatocyte cultures. The species similarities and differences of rat, monkey and man will be presented.

904 ROLE OF P4502E1 IN RETINOL'S ATTENUATION OF CARBON TETRACHLORIDE HEPATOTOXICITY IN THE MOUSE.

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Retinol has been shown to attenuate carbon tetrachloride (CCl4)-induced hepatotoxicity in the mouse. To determine the mechanism of this attenuation, we have investigated cytochrome P4502E1 activity. Swiss Webster mice were administered retinol (75 mg/kg/d) or vehicle for 3 days prior to a dose of CCl4 (30 μl/kg i.p.) or corn oil. Hepatotoxicity produced by CCl4 was assessed 24 hours after the CCl4 dose by plasma alanine aminotransferase (ALT) activity (1391 ± 430 vs. 274 ± 92 IU/L for vehicle + CCl4 and retinol + CCl4 treatments respectively, $p < 0.05$). Hepatic microsomes were prepared to assess P4502E1 activity. Aniline hydroxylase activity was significantly reduced in vehicle + CCl4 (5.70 ± 0.75) vs retinol + CCl4 (9.02 ± 1.02, $p < 0.05$) treatments. Results from western blotting demonstrated that P4502E1 immunoreactive protein was 67% lower in vehicle + CCl4 vs retinol + CCl4 treatment groups. Retinol's ability to attenuate CCl4 hepatotoxicity potentiated by acetone (4.8 mg/kg) was also determined. Protection against potentiated hepatotoxicity was also observed in the acetone-nduced group (ALT activity of 2741 ± 1066 vs 247 ± 77 IU/L for vehicle and retinol treatments respectively, $p < 0.05$). In acetone-induced mice, a significant decrease in vehicle + CCl4 vs retinol + CCl4 for aniline hydroxylation was also observed. These results demonstrate that retinol itself does not alter cytochrome P4502E1 activity or polypeptide levels. However, in combination with CCl4, retinol does reduce the amount of CCl4 bioactivated to its toxic metabolite. We conclude that retinol's attenuation of CCl4-induced hepatotoxicity is associated with a decrease in CCl4 bioactivation but not a decrease in P4502E1 activity or polypeptide levels. (Supported by grants from the University of Otago Medical School.)

905 INDOCYANINE GREEN PRETREATMENT DOES NOT ALTER SUSCEPTIBILITY TO ACETAMINOPHEN HEPATOTOXICITY IN MALE CD-1 MICE.

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Recent studies showed that the administration of the non-metabolizable organic anion indocyanine green (ICG) significantly reduced the biliary disposition of APAP-GSH in mice, suggesting that this APAP metabolite may compete with other organic anions for excretion into the bile via a canalicular organic anion transporter. This study was conducted to determine if changes in biliary disposition of APAP induced by ICG could lead to alterations in susceptibility to APAP toxicity. To investigate this, groups of overnight fasted male CD-1 mice received 30 μmol ICG/kg, *i.v.*, immediately prior to APAP dosing (600 mg/kg, *i.p.*). Controls were given vehicle. Mice were killed at 4 or 24 hr after APAP dosing for immunochemical detection of APAP protein binding and assessment of liver toxicity, respectively. Hepatocellular necrosis was evident at 24 hr in control mice receiving APAP as determined by sorbitol dehydrogenase activity and histopathology. Pretreatment with ICG did not alter the incidence or severity of hepatocellular necrosis induced by APAP. Furthermore, ICG did not alter APAP-induced glutathione depletion nor selective covalent binding to cytosolic target proteins at 4 hr after dosing. These data indicate that ICG does not change the susceptibility to liver injury produced by high doses of APAP (Supported by a grant from PhRMA Foundation.)

906 EFFECTS OF WEEKLY DAPM EXPOSURES ON LIVER AND LUNG IN FEMALE RATS.

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Background: Humans acutely exposed to methylene dianiline (4,4'-diaminodiphenylmethane, DAPM), a chemical used in the production of polyurethanes and epoxy resins, develop jaundice, toxic hepatitis and cholangitis. But DAPM exposures in the workplace are likely chronic, intermittant and very low. Recent acute studies suggest females are more susceptible to DAPM than males. ***Objective:*** Evaluate once-a-week exposures to a low dose of DAPM in female rats. ***Methods:*** Female Sprague Dawley rats (~250 g) were gavaged with 25 mg/kg of DAPM in 35% ethanol or vehicle once a week for 17 weeks. Animals were sacrificed at 8, 12, and 17 weeks. Tissues and sera were collected for histology and assessment of hepatotoxicity and liver function. Morphometric analysis of blood vessel architecture in lung and liver was performed using Optimas® software. ***Results:*** *8 WEEKS:* DAPM caused prominent eosinophilia in liver and lung with mild fibrosis around bile ducts and proliferation of biliary epithelial cells (BEC). *12 WEEKS:* Liver weights and serum bilirubin were elevated. Bile duct fibrosis and BEC proliferation were more marked, and inflammation increased in lung. *17 WEEKS:* Serum bilirubin and liver weights remained elevated. Increased fibrosis occurred around smaller bile ducts with concentric fibrosis around larger bile ducts. Nests of proliferated BEC extended from larger portal tracts. Minor lung fibrosis was observed. The thickness of arterial walls in both liver and lung were increased. ***Conclusions:*** Female rats exposed to a "non-hepatotoxic" dose of DAPM on a weekly basis show chronic BEC injury and proliferation with mild to moderate lung injury, and a proliferative effect on arterial vasculature. The pathological effects of intermittant, low-dose exposure to DAPM are more severe and involve more tissues in female than male rats. (Supported by ES 06348 and T32 ES07254.)

907 INCREASES IN ENTEROCYTE GLUTATHIONE AND ALKALINE PHOSPHATASE AS POTENITAL ADAPTIVE RESPONSES TO DICLOFENAC-INDUCED ENTEROPATHY.

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Background: Our laboratory is currently investigating the mechanism of small intestinal (SI) ulcerogenesis following administration of the non-steroidal anti-inflammatory drug diclofenac (Voltaren®). In a rat model of diclofenac-induced enteropathy, we have found that the treatment interval affects the enteropathy of 10 mg/kg, the recommended dose for rodents.

Specifically, two-thirds fewer SI ulcers are observed when the drug is given at 24 hr versus 12 hr intervals. **Objective:** Determine if potentially adaptive biochemical changes can be detected in enterocytes at 24 hr versus 12 hr following diclofenac treatment. **Experimental Design:** Fed adult male Sprague-Dawley rats were treated with either 0 or 50 mg/kg diclofenac via oral gavage. At 12 and 24 hours after diclofenac treatment, enterocytes were isolated from each quintile of the SI and assayed for glutathione (GSH) content and alkaline phosphatase (ALP) activity. **Results:** Consistent with the known heterogeneity of the SI, both GSH content and ALP activity decreased progressively from the 1st to 5th quintiles of the SI with levels in the 5th quintile being 15% and 1% of the 1st quintile, respectively. Diclofenac treatment produced time-dependent increases in both GSH content and ALP activity. Most notably at 24 hr, ALP activity rose by 67% in the 1st quintile, while GSH levels were markedly elevated by 600% in the 3rd and 4th quintiles, which are most vulnerable to ulceration. **Conclusions:** Our observations of GSH and ALP increases in the SI by 24 hr after diclofenac treatment are consistent with an adaptive response in this target tissue. This response may explain why diclofenac is better tolerated at 24 hr versus 12 hr intervals in our rat model. Increased brush border ALP activity could exert a beneficial effect through its ability to detoxify bacterial endotoxin to a biologically inactive form. Higher enterocyte levels of the nucleophile GSH could facilitate the detoxification of electrophilic metabolites of diclofenac or enhance protection against oxidative insults mediated by an inflammatory response to either diclofenac or permeating endotoxin. (Supported by NIH DK 34806, NIH T32E507254, and Brown Family Foundation.)

908 CLONING AND EXPRESSION OF THREE HUMAN INTESTINAL UDP-GLUCURONOSYLTRANSFERASES (UGTs):1A5, 1A8 AND 1A10.

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Although the liver has been considered as the most important organ involved in glucuronidation, recent studies have shown that the gastrointestinal tract plays a much greater role than previously thought in the metabolism of xenobiotics. We have identified three UGT isoforms of human intestinal mucosa which are absent in liver using reverse transcriptase-polymerase chain reaction (RT-PCR) technique. mRNAs of UGT1A8 and 1A10 were found in both small intestine and colon, whereas the transcript of UGT1A5 was identified only in jejunum and ileum but not in 25 other human tissues screened, including colon. Full-length cDNAs for UGT1A5, 1A8 and 1A10 were amplified from ileal RNA by RT-PCR and inserted into the mammalian expression vector pcDNA3. Membrane preparations of HK293 cells stably expressing UGT1A5, UGT1A8 or UGT1A10 were obtained and the expression of each isoform was observed by western blot analysis. Stably expressed UGT1A8 and 1A10 catalyzed glucuronidation of catechol estrogens, coumarins, flavonoids, anthraquinones and phenolic compounds. Unlike UGT1A10, UGT1A8 also catalyzed the glucuronidation of certain opioids, bile acids, fatty acids and drugs, such as ciprofibrate, clofibrate, valproic acid, furosemide and diflunisal. However, glucuronidation activity has not been detected for stably expressed UGT1A5. These studies show that human intestinal UGTs may play an important first step in the detoxification of xenobiotic compounds and, in some cases, limit the bioavailability of therapeutic agents. (This research was supported by NIH Grant GM26221 (TRT) and DK49715 (AP-R).)

909 DO DICLOFENAC PROTEIN ADDUCTS PLAY A CAUSAL ROLE IN SMALL INTESTINAL ULCEROGENESIS?

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Background: Diclofenac, a NSAID used worldwide for the treatment of arthritis, produces liver injury occasionally and more frequently GI tract damage. Mechanisms responsible for the toxicity of this drug are not well understood and could involve the bioactivation of diclofenac to a reactive acyl glucuronide which forms potentially injurious diclofenac protein adducts (Dcf-pA). **Objective**: Explore the hypothesis that Dcf-pA have a causal relationship to intestinal ulceration by determining if Dcf-pA precede morphological and biochemical characteristics of small intestinal ulceration. **Experimental Design**: Male fed Sprague-Dawley rats were administered diclofenac by a physiological oral gavage route at doses of 0 or 50 mg/kg. Blood, liver and other tissues were collected at 0.5, 1, 3, 6, 12, 24 or 48 hr. GI tracts were grossly inspected for number of ulcers; area of ulceration was measured by image analysis; intestinal leakage was estimated by serum protein levels. Immunohistochemistry was performed using polyclonal antisera against diclofenac adducted proteins. **Results**: Ulcers were evident in the small intestine at 6 hr and were chiefly located in the quintiles 2 to 4. No ulcers were evident in the stomach or colon. Area of intestinal ulceration increased in a time dependent manner from 6 to 24 hr concurrent with progressive declines in serum protein levels. Dcf-pA were first observed in the cytoplasm of small intestinal enterocytes at 1 hour; by 6 hr adduct localization shifted to enterocyte brush borders. Sites of intestinal ulceration were associated with intense Dcf-pA staining. Semiquantitative immunohistochemistry for Dcf-pA in intestinal rings at 3 hr showed greatest intensity in quintiles with ulcers. **Conclusions**: Key observations consistent with a causal role for Dcf-pA in ulceration were: first, that Dcf-pA formation within enterocytes preceded the formation of intestinal ulcers and second, that Dcf-pA were most intense I the ulcer-prone intestinal quintiles. (Supported by NIH DK 34806, Brown Family Foundation, US Army, T35-ESO7294.)

910 EFFECT OF INTERMITTENT EXPOSURES OF AFLATOXIN B_1 ON HEPATIC AND TESTICULAR GLUTATHIONE S-TRANSFERASE IN RATS.

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Glutathione S-transferase (GST) plays a major role in the detoxification of the potent hepatocarcinogen aflatoxin B_1 (AFB_1). Since AFB_1 is an inducer of hepatic GST, the quantitation of this enzyme is an important biomarker in AFB_1 activation. The induction of GST activity has been studied in animals treated with a single exposure or continuous exposure of AFB_1; however, the induction of hepatic GST activity by intermittent exposure of AFB_1 has not yet been investigated. Also little is known about the effects of AFB_1 on testicular GST activity. Male Fischer 344 rats were fed diets containing AFB_1 (0, 0.01, 0.04, 0.4 and 1.6 ppm) inter- mittently at 4-week intervals up to 20 weeks. The control animals were fed an AFB_1-free NIH-31 diet. Rats consuming diets with low AFB_1 concentrations did not show the induction of hepatic or testicular GST activity. Intermittent exposures at high concentrations of AFB_1 significantly increased the GST activi- ties. The increase of the enzyme activity was proportional to the dose and length of AFB_1 exposure.

911 SODIUM ARSENITE INDUCES CYTOKERATIN EXPRESSION IN WRL-68 CELL LINE.

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Intermediate filaments (IF) constitute an important superfamily of genes that form part of the cytoskeleton. Among the type I and type II IF proteins are the keratins, which are broadly expressed in epithelial cells. It is now clear that a major function of skin keratins is to impart mechanical integrity to cells, but there are still many questions to be answered with regards to the role cytokeratins play in other epithelial tissues. Altered expression of these proteins have been observed in liver after exposures to carcinogens like aflatoxin B1 and in some disorders like hepatic cirrhosis. Liver cancer and cirrhosis have been associated to chronic exposure to arsenic by drinking water. We investigated the effects of sodium arsenite on cytokeratins expression in WRL-68 cells, a human hepatic cell line of epithelial origin, that maintains enzymatic activities of liver cells. Non-proliferating cells were treated during 16 hs with different concentrations of sodium arsenite (10^{-5} to 10^{-9} M). Fluorescent immunostaining using Pancytokeratin polyclonal and cytokeratin 18 monoclonal antibodies showed heavier staining in cytoplasm and in the nuclear envelope of treated cells that obscured the delicate net-like pattern observed in controls. Also, increased amounts of protein were detected in total protein westernblotts. This increased expression could represent a mechanism to compensate impairments in the polymeric association of cytokeratins because of the presence of the metalloid and could help to explain the keratin changes observed in skin epithelial cells by arsenic poisoning. (This work was partially supported by PAPIIT IN207196.)

912 CADMIUM PRETREATMENT PROTECTS METALLOTHIONEIN-NULL MICE AGAINST TESTICULAR TOXICITY BUT NOT HEPATOTOXICITY.

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Low dose cadmium (Cd) pretreatment has been shown to protect animals against subsequent toxic and lethal doses of Cd. This protection is thought to be due to induction of intracellular metallothionein (MT), but Cd pretreatment alters a host of other defense mechanisms in animals. To define conclusively the role of MT in Cd-induced self-tolerance, control and MT-I/II null mice were pretreated with 2 or 5 μmol Cd/kg sc daily for two days, followed by 10, 20, or 30 μmol Cd/kg ip 24 hrs later. Cd toxicity was determined 24 hrs later. Cd-induced lethality was 0%, 11% and 44% after 10, 20 and 30 μmol Cd/kg, respectively, in control mice. In MT-null mice lethality was 0%, 40% and 100% after 10, 20 and 30 μmol Cd/kg, respectively. Cd pretreatment eliminated lethality after these doses of Cd in both control and MT-null mice. Pretreatment with Cd protected against hepatotoxicity in control mice. In contrast, Cd pretreatment enhanced hepatotoxicity in MT-null mice. For example, Cd pretreatment increased ALT from 600 to 1650 U/L after 15 μmol Cd/kg. Pretreatment provided protection in control mice against Cd-induced testicular injury, and surprisingly, it also provided similar protection in MT-null mice. This study demonstrates that (i) MT plays a major role in Cd-induced tolerance to Cd-induced hepatotoxicity, and (ii) MT is not responsible for Cd-induced protection against Cd-induced testicular injury. (Supported by NIH grants ES-01142 and ES-07079.)

913 INHIBITION OF CONJUGATION AS A TARGET FOR ACETAMINOPHEN (APAP) TOXICITY IN CULTURED HUMAN HEPATOCYTES.

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Simultaneous exposure to phenobarbital (PB) and acetaminophen (APAP) caused toxicity in cultured human hepatocytes prepared from different donors, as measured by inhibition of protein synthesis and reduction of MTT. A 6 hr exposure to the combination of APAP and PB was associated with complete disappearance of glycogen, enlargement of endoplasmic reticulum and blebbing of mitochondria membranes, as demonstrated by electron microscopy. Pretreatment with 2 mM PB (48 h) did not increase APAP toxicity unless PB was readded with APAP. PB decreased APAP-glucuronide and APAP-sulfate formation by 85% and 50%, respectively. Salicylamide and 7-hydroxycoumarin, substrates of sulfation and glucuronidation, also increased APAP toxicity when added with APAP. There was no correlation between toxicity and binding of radiolabeled APAP to cellular proteins. The amount of APAP bound to protein was similar following treatment with APAP alone or in combinations with PB or salicilamide. PB and salicylamide each delayed the metabolism of 7-hydroxycoumarin, suggesting an interference with conjugation. These data suggest that compromising conjugation of APAP, particularly glucuronidation, by simultaneous administration of APAP and other drugs may result in hepatotoxicity. This work was supported in part by the Dept. of Veterans Affairs and the Anatomical Gift Foundation.

914 SUSPENSIONS OF FRESHLY ISOLATED RAT HEPATOCYTES GENERATE HIGH LEVELS OF NITRIC OXIDE.

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Nitric oxide (NO) is a reactive radical and intracellular/intercellular messenger. NO can induce numerous biochemical alterations in hepatocytes including inhibition of protein synthesis, mitochondrial respiration and CYP450 activities. In addition, NO can act as an antioxidant by scavenging reactive oxygen species and inhibiting lipid peroxidation. We investigated NO production in freshly isolated rat hepatocyte suspensions. Hepatocytes were isolated by standard collagenase perfusion techniques and incubated in modified Waymouths media at 37ûC for 6 hours. Extracellular nitrite levels (a breakdown product of NO) were measured by a fluorimetric assay. The level of extracellular nitrite remained at basal levels (<0.2 nmole/10^6 cells) for approx. 3 hours post-isolation, which was followed by a rapid increase in nitrite formation reaching 5 to 10 nmole/10^6 cells by 6 hours, constituting an up to 100-fold increase in nitrite level. The formation of nitrite was blocked by the nitric oxide synthase (NOS) inhibitors, N-methyl arginine or aminoguanidine. The nitrite burst was also inhibited by a nontoxic concentration of the protein synthesis inhibitor, cycloheximide. Collectively, these findings suggest that hepatocytes in suspension produce high levels of NO and that the inducible form of NOS may be involved. The NO production by hepatocyte suspensions was also inhibited by thiol depleting agents including diethylmaleate, ethylmethane sulphonate and N-ethylmaleimide, and the absence of extracellular calcium. In addition, antioxidants such as trolox and deferoxamine, were shown to both delay and attenuate nitrite production suggesting a relationship between the formation of reactive oxygen species and nitrite. These results provide the first evidence of marked NO production by rat hepatocyte suspensions that may have important implications for both mechanistic and metabolic studies conducted with this model system. (This work was supported by NIEHS/NIH grant # R01ES 05452.)

915 VALPROIC ACID HEPATOTOXICITY AND PROTECTION BY GLYCINE.

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The anticonvulsant drug valproic acid (VPA) is metabolized via the cytochrome P450-dependent pathway to a hepatotoxic compound 4-ene-VPA. Glycine, on the other hand, potentiates the action of anticonvulsant drugs in rats (Neuropharmacology 29, 399, 1990). Our group showed that glycine protected the isolated perfused rat liver against the damage induced by several hepatotoxic agents (Toxicology 128, 63-72, 1998). Therefore we questioned whether glycine was able to antagonize the VPA-induced hepatotoxic response in a human cell line (HepG2) and in the isolated perfused rat liver. As a measure of cytotoxicity the neutralred uptake and the release of LDH into the medium were tested. Glycine (12 mmol/l) completely protected HepG2 cells against the hepatotoxic response to VPA (4 mmol/l). The same concentration of VPA (4 mmol/l) was toxic to the isolated perfused liver of fasted rats (enzyme release of GPT, LDH and GLDH into the perfusate). Addition of glycine (12 mmol/l) to the perfusate counteracted the VPA induced enzyme release and increase of liver weight. Moreover, we studied the effect of glycine on the metabolism of VPA to the hepatotoxic intermediate, which is under current investigation.

916 TOXICITY OF ACRYLATES IN COMBINATION - INVESTIGATIONS IN HUMAN CELL CULTURES AND THE ISOLATED PERFUSED RAT LIVER.

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A review of the toxicological and ecotoxicological profile of 16 different acrylate and methacrylate compounds was published recently (Greim et al., Chemosphere 31, 2637-2659, 1995). Some of these compounds are common excipients in pharmaceutical preparations serving at least two different functions. In accordance with actual Pharmacopoeia polyacrylates may contain small amounts of the highly reactive monomeric acrylates. The question arose whether these monomeric acrylates in the matrix of coated tablets might be responsible for or at least contribute to hepatotoxic effects of these drug preparations. 5 different commercially available substances (acrylic acid esters, methacrylic acid esters) were tested for their toxicity as monomers or as a mixture in 3 different cell lines (liver: HepG2, kidney: LLC-MK_2, intestine: I-407) as well as in the isolated perfused rat liver. As a measure of cytotoxicity the neutral-red uptake and the LDH-release into the medium were tested. The acrylate-mixture was more toxic in the kidney cells (IC_{50} 210 mg/l) than in hepatoma (IC_{50} 310 mg/l) and intestinal cells (IC_{50} 700 mg/l). In the isolated perfused rat liver the mixture was hepatotoxic at a concentration of 320 mg/l (enzyme release of GPT, LDH and GLDH into the perfusate). Moreover, a strong depletion of hepatic reduced glutathione and an increase of lipid peroxidation (MDA in liver tissue and perfusate) was observed. Conjugation with glutathione (GSH) seems to be the major pathway of metabolic detoxification of acrylates and methacrylates.

917 COCAINE-PROTEIN ADDUCTS IN MOUSE LIVER: COMPARISON OF DETECTION BY RADIOCHEMICAL AND IMMUNOCHEMICAL METHODS.

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Covalent binding of reactive metabolites to proteins has been proposed as mechanism of cocaine-induced hepatotoxicity. In this study, detection of cocaine-protein adducts by immunochemical and radiochemical methods was compared. ICR male mice were dosed with ^{14}C-labeled or unlabeled cocaine (60 mg/kg, i.p.). Livers were removed and fractionated, and the proteins in homogenate and fractions were separated by SDS-PAGE. For detection of radiolabeled cocaine-protein adducts by fluorography, gels were dried and exposed to x-ray film. Immunochemical detection of cocaine-protein adducts utilized an anti-cocaine antibody and Western blots. Results showed adducts present only in the microsomes and mitochondria, with the former accounting for most of the binding. Fluorography revealed binding to proteins with pls between 4-6 and approximate molecular masses of 25-33, 40, 44-60, and 100 kDa. Western blotting found cocaine binding to proteins with similar masses. Sequence analysis identified two of the target proteins (64 and 70-80 kDa) detected by Western blotting as hsp60 and transferrin. As has been observed previously, pretreatment of mice with the hepatic enzyme inducing agents phenobarbital or beta-naphthoflavone resulted in a shift in the intralobular site of the lesion – toward the portal triad in the case of phenobarbital pretreatment, and toward the central vein in the case of beta-naphthoflavone pretreatment. Western blots using 1-D SDS-PAGE gels suggest that the same proteins are adducted in induced and uninduced mice. Insight into the role of each adducted protein in cocaine hepatotoxicity may come from determining the effect of adduction on protein function. Supported by DA-06601.

918 CYTOPROTECTION FROM THIOACETAMIDE-INDUCED LIVER INJURY ASSOCIATED WITH HEAT SHOCK PROTEIN INDUCTION.

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Increased expression of the heat shock proteins (hsps) is a highly conserved response to cellular stress in both eukaryotic and prokaryotic cells. Elevated levels of hsps provide cytoprotection from many of the stressors that induce hsps. Thioacetamide is a model hepatotoxicant that causes both necrotic and apoptotic liver injury. In order to study the effects of hsp induction on thioacetamide-induced liver injury, male ICR mice were made hyperthermic with either thermal (42.5°C for 30 min) or amphetamine (15 mg/kg i.p.) treatment with a resulting transient elevation of hepatic hsps. Mice were subsequently challenged with thioacetamide (70 mg/kg i.p.). Serum aminotransferase (ALT) activities were measured and compared to controls without hsp induction 3, 6, 12, 24, and 48 hours after thioacetamide treatment. The thermal or amphetamine pretreated animals were protected from thioacetamide-induced hepatotoxicity as evidenced by significantly lower serum ALT levels. Histological examination revealed marked necrotic and apoptotic injury surrounding the central veins of thioacetamide-treated animals, which was attenuated by pretreatment with either hsp induction regimen. Western blot analysis of livers from mice treated with thioacetamide alone showed strong induction of hsp70i and hsp25, weak induction of hsp32 and hsp90, and no induction of hsp60. Immunohistochemical staining of liver slices using anti-hsp70i or anti-hsp25 antibody showed strong immunostaining in areas corresponding to regions of hepatocellular injury. Thioacetamide appears to cause strong induction of at least two hsps, and these hsps may afford protection against thioacetamide cytotoxicity. (Supported by ES-07213.)

919 ENHANCED BIOACTIVATION AND IMPAIRED TISSUE REPAIR IN THIOACETAMIDE HEPATOTOXICITY IN DIABETIC RATS.

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Previously we reported that a sublethal dose of TA (300 mg/kg, ip) caused 90% lethality in streptozotocin-induced diabetic rats. Further studies suggested that the increased lethality of TA in diabetic rats was due to dual effects of enhanced TA liver injury and impaired liver cell proliferation and tissue repair. Diabetes and Diet restriction are known to induce CYP 2E1 and Phase I bioactivation may play a role in the enhanced TA liver injury. Work in our laboratory also showed increased TA liver injury in diet restricted rats. Therefore, the objective of present study was to test our hypothesis that increased TA hepatotoxicity in diabetic rats might be due to enhanced TA bioactivation. Specific aims were: **(a)** test the effect of isoniazid, a CYP 2E1 inducer, on TA liver injury; **(b)** study change of CYP 2E1 expression in the diabetic rats; **(c)** investigate if and how this change affects TA bioactivation; **(d)** quantitate TA sulfoxide, a proximate reactive metabolite, formed from diabetic and control microsomes by HPLC. Male Sprague-Dawley rats were pretreated with STZ (60 mg/kg, ip), isoniazid (200 mg/kg, ip) or saline (1 ml/kg, ip). TA (300 mg/kg, ip) was injected 10 days after STZ and saline treatment or 18 hr after isoniazid pretreatment. Liver injury was measured by plasma alanine aminotransferase (ALT) at 0-36 hr after TA injection. ALT activities increased and reached peak at 24 hr in all three groups (STZ > isoniazid > saline). Western blot analysis revealed that CYP 2E1 was increased 8- and 2.5-fold in STZ and isoniazid pretreated rats compared to the control, respectively. CYP 2E1 activity as assessed by *p*-nitrophenol hydroxylase assay was increased 5- and 2-fold in diabetic and isoniazid groups compared to the control. Collectively, these studies suggest that CYP 2E1 may be responsible for the increased TA liver injury in diabetes. Ongoing studies are aimed to investigate whether flavin monooxygenase (FMO) system is involved in the mechanism-driven increase in liver injury of TA in diabetes. (Supported by Louisiana Board of Regents Support Fund.)

920 CHRONIC ENTERAL ETHANOL CAUSES TRANDUCTION OF ADENO-ASSOCIATED VIRUS IN THE LIVER.

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The transduction of adeno-associated virus (AAV) in the liver has been extensively studied using DNA damaging agents such as etopiside, hydroxyurea, or UV-irradiation. Also, helper adenovirus has been useful in AAV transduction in the liver. The precise mechanism by which these agents cause *in vivo* transduction is unknown but most likely involves an increase in DNA synthesis and repair. Ethanol is known to induce mild liver damage, leading to an increase in DNA repair and synthesis *in vivo*. Therefore, it was hypothesized that chronic delivery of ethanol would increase AAV transduction *in vivo*. To test this idea, recombinant AAV ($1x10^{10}$ particles) encoding the β -galactosidase reporter gene under a cytomegalovirus promoter (rAAV-CMV*lacZ*) was given to ~250 g male Wistar rats by tail vein injection. Rats then received either ethanol diet (12.5 g/kg/day) via the Tsukamoto- French enteral-feeding protocol or high-fat control diet for three weeks. This enteral ethanol feeding model induces steatosis, inflammation, and mild necrosis within the liver after three weeks. Pathology scores (0.4 ± 0.2 vs. 3.75 ± 0.2) and serum aspartate trans-aminase levels (36.2 ± 3.5 vs. 132.3 ± 11.0) were measured in control and ethanol-treated animals. The expression and activity of *lacZ* in the liver was measured by Western blotting and enzymatic cleavage of the substrate 2-nitrophenyl-β -D-galactopyranoside, respectively. Both expression and activity of *lacZ* was increased nearly 4-fold over values in high-fat controls by chronic exposure to ethanol. Interestingly, *lacZ* activity correlated (r^2=0.91) with liver injury measured both by pathology score and serum transamimase levels. These results support the hypothesis that chronic ethanol treatment increases AAV transduction *in vivo*. Thus, ethanol may be a useful tool in AAV gene therapy rather than more harmful and damaging transducing agents. Additionally, alcoholic liver disease is an attractive target for adeno-associated viral gene therapy, since excessive consuption of ethanol could be used to increase expression of therapeutic genes (e.g., superoxide dismutase).

921 INHIBITION OF FAS-RECEPTOR (CD95)-INDUCED CASPASE ACTIVATION AND APOPTOSIS BY ACETAMINOPHEN IN MOUSE LIVER.

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The mechanism of liver cell injury induced by an overdose of the analgesic acetaminophen (AAP) remains controversial. Recently, it was hypothesized that a significant number of hepatocytes die by apoptosis. Since caspases have been implicated as critical signal and effector proteases in apoptosis, we investigated their potential role in the pathophysiology of AAP-induced liver injury. Male C3Heb/FeJ mice were fasted overnight and then treated with 500 mg/kg AAP. Liver injury became apparent at 4 hr and was more severe at 6 hr (plasma ALT: 4110 ± 320 U/L; centrilobular necrosis). DNA

fragmentation increased parallel to the increase of plasma ALT values. At 6 hr there was a 420% increase of DNA fragmentation and a 74-fold increase of TUNEL-positive cells located predominantly around central veins. However, the activity of the proapoptotic caspase-3 was not increased at any time after AAP. In contrast, injection of the anti-Fas antibody Jo-2 (positive control) caused a 28-fold increase of caspase-3 activity and severe DNA fragmentation before significant ALT release. Treatment with the caspase inhibitor ZVAD-CHF_2 had no effect on AAP toxicity but completely prevented Jo-mediated apoptosis. In contrast, Jo-induced caspase activation and apoptosis could be inhibited by AAP treatment in a time- and dose-dependent manner. We conclude that AAP-induced DNA fragmentation does not involve caspases suggesting a direct Ca^{2+}-dependent activation of endonucleases. In addition, electrophilic metabolites of AAP may inactivate caspases or their activation pathway. Thus, AAP metabolism has the potential to inhibit signal transduction mechanisms of receptor-mediated apoptosis.

922 EFFECTS OF ACUTE ALCOHOL EXPOSURE ON HEPATIC PARTITIONING OF CARBON TETRACHLORIDE: AN ADDITIONAL MECHANISM FOR ALCOHOL-MEDIATED POTENTIATION.

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Possible mechanisms underlying isopropanol and ethanol potentiation of carbon tetrachloride (CCl_4) hepatotoxicity were investigated in male Fischer-344 rats (67-77 days old). In separate experiments, animals were dosed with isopropanol (0, 2.5, 5, 10 or 20 mmol/kg, p.o.) or ethanol (0, 5, 10, 20 or 40 mmol/kg, p.o.) in aqueous solutions. Eighteen hours later, animals were either terminated to determine the effects of alcohol alone or dosed with CCl_4 (0.0 or 0.5 mmol/kg in 10 percent Alkamuls solution, p.o.). Termination of alcohol plus CCl_4 animals occurred 24 hours post CCl_4 exposure. No increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and sorbitol dehydrogenase (SDH) activities were seen for either alcohol alone. Relative to CCl_4 alone, significant increases in AST, ALT, LDH and SDH were observed at all isopropanol dosages examined and only the two highest ethanol dosages. Isopropanol alone increased CYP2E1 activity beginning at 5 mmol/kg. The isopropanol dosage eliciting peak AST, ALT, LDH and SDH levels following isopropanol and CCl_4 exposure did not correspond with the dosage eliciting peak CYP2E1 induction. Two dosages of isopropanol resulted in similar CYP2E1 increases, but differed greatly in their potentiation of CCl_4 hepatotoxicity. Ethanol alone did not induce CYP2E1 activity. A dose-related increase in liver:blood CCl_4 partitioning was observed following exposure to isopropanol alone, but not to ethanol alone. In conclusion, it appears that CYP2E1 induction is not the sole mechanism responsible for acute alcohol potentiation of CCl_4 hepatotoxicity. The data from this study suggest both CYP2E1 induction and alteration of hepatic CCl_4 partitioning as mechanisms of acute isopropanol potentiation of CCl_4 hepatotoxicity. In contrast, neither appears to be involved in acute ethanol potentiation of CCl_4 hepatotoxicity, implying that another, as yet unidentified, mechanism is involved. (This research was funded by the US DOE, Industrial Hygiene Graduate Fellowship Program, administered by Oak Ridge Institute for Science and Education. This abstract may not reflect US DOE or US EPA policy.)

923 ANTIOXIDANT AND ANTIHEPATOTOXIC ACTIVITIES OF PRENYLATED FLAVONOIDS FROM HOPS.

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Eleven flavonoids (chalcones and flavanones) from hops were examined for their antioxidant and antihepatotoxic effects in vitro. The prenylated chalcones [xanthohumol (XN), 2',4',6'4'-OH-3'-prenylchalone(TP), dehydrocycloxanthohumol hydrate (DH), prenylxanthohumol (PX) and xanthogalenol (XG)] were more effective than the flavanones [isoxanthohumol (IX), 6-prenylnaringenin (6PN) and 8-prenylnaringenin (8PN)] in inhibiting lipid peroxidation induced by iron-ascorbate in rat liver microsomes. The non-prenylated chalcone, chalconaringenin (CN), had the same inhibitory activity as XN. XN, but not IX, inhibited tert-butylhydroperoxide(TBH)-induced lipid peroxidation in rat liver microsomes. XN, XG and CN, at 5, 10 and 25 (M, were effective in inhibiting lipid peroxidation induced by iron-ascorbate in rat liver mitochondria. At 5 and 10 (M, XN and PX protected cultured rat hepatocytes from the toxicity of TBH. Other chalcones (TP, DH and XG), with similar antioxidant activities as XN, were not hepatoprotective. These results indicate that prenylated chalcones are more effective antioxidants than the prenylated flavanones; however, the antioxidant activity of the chalcones (with or without prenyl groups) may not be predictive of their ability to protect the liver from injury caused by toxicants that induce lipid peroxidation. (Supported by the Hop Research Council and NIEHS grants ES00210 and ES03850.)

924 PROTECTION BY ZINC AGAINST ACUTE ETHANOL HEPATOTOXICITY AND ITS RELATION TO METALLOTHIONEIN PRODUCTION.

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Recent studies have shown that oxidative stress is critically involved in alcoholic liver injury. Metallothionein (MT), a low molecular weight and cysteine-rich protein, plays an important role in protection against oxidative injury in multiple organs including the liver. This study was undertaken to determine whether induction of MT in the liver by zinc, a potent MT inducer, can protect the liver from acute ethanol toxicity. Mice (24-30g) were randomly assigned into the control and Zn-treated groups (n=20 in each group). Animals in Zn-treatment group were given $ZnCl_2$ in a dose of 10 mg/kg via ip 24 hr prior to ethanol administration, and saline was given to the controls. A half of the animals in each group were then treated with ethanol by ip in a dose of 5g/kg. The animals were sacrificed 20 hrs after ethanol treatment. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were examined as indicators of liver injury. Liver pathological changes, lipid peroxidation and MT concentrations were also measured. The results showed that Zn significantly increased liver MT concentrations. Serum ALT and AST activities were markedly elevated by ethanol, but were significantly lower in the Zn-pretreated than in the saline-pretreated mice. Ethanol-induced pathological changes and lipid peroxidation in the liver were also significantly depressed in the Zn-pretreated mice. It is therefore possible that MT functions in hepatic protection against ethanol-induced oxidative injury.

925 DOSE RESPONSE OF ETHANOL IN RELATION TO THE ANTIOXIDANT DEFENSE SYSTEM OF THE LIVER, LUNG, AND KIDNEY IN RATS.

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This study investigated the alterations in levels of glutathione, lipid peroxidation, and antioxidant enzyme activity in the liver, lung, and kidney of rats treated with acute doses of alcohol. Male Fisher-344 rats were randomly divided into groups, and were treated as follows: 1) vehicle (saline) control; 2) ethanol 2 g/kg, p.o.; 3) ethanol 4g/kg, p.o.; and 4) ethanol 6 g/kg, p.o. The most significant changes in the antioxidant system were observed following 6 g/kg treatment. Ethanol administration resulted in a depletion of GSH levels (70% of control) and SOD activity (70% of control) in the liver, a increase of GSH levels (158% of control) in the kidney, an elevation of GSH-Px activity and lipid peroxidation levels in the lung (151% and 124% of control respectively) and kidney (141% and 134% respectively), and a decrease of GR activity in the lung (89% of control) and kidney (83% of control). These results showed that the antioxidant system was influenced by ethanol administration in a tissue specific manner. The degree of oxidative stress on these antioxidant systems was proportional to the amount of alcohol administered. (Supported by the American Heart Association, IL Affiliate Grant #9708751A.)

926 TIME COURSE RESPONSE OF ETHANOL ON ANTIOXIDANT DEFENSE SYSTEM OF BRAIN, HEART AND LIVER OF RAT.

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This study was undertaken to determine the effect of acute ethanol dose on time response of antioxidant defense system in brain, heart and liver of rat. Male Fisher 344 rats were administered ethanol 4g/kg p.o. and sacrificed at 1.5; 2.0; 4.0 and 6.0 hr. The SOD activity was significantly decreased in brain at 1.5; 2.0 and 4.0 hr(84%,80% and 82% of control), in heart at 2.0 and

6.0 hr (83% and 82% of control) and in liver at 4.0 and 6.0 hr(78% and 66% of control) respectively. CAT activity was significantly low in brain and heart , where as it was elevated in liver. GSH-Px activity decreased significantly at 1.5 and 4.0 hr, but increased at 6.0 hr in brain and a significant increase at 6.0 hr in heart. The liver GSH-Px activity showed a consistent increase from 1.5 to 4.0 hrs(145%,153%, and 154% of control). GR activity exhibited a significant decrease in heart at all time points. In liver GSH and GSSG values are decreased significantly at 4.0 and 6.0 hr. MDA, the marker of lipid peroxidation increased significantly at 1.5; 4.0 and 6.0 hr (123%,123% and 131% of control) in heart and at 6.0 hr(124% of control) in liver. The AOE ratios are significantly altered during the time course study in all tissues. This study suggests that the altered activity of AOE reflects the oxidative stress of ethanol in a time dependent manner for a specific tissue (Supported by American Heart Association, IL Affiliate Grant #9708751A.)

927 PERIPHERALLY ADMINISTERED SODIUM AZIDE STRONGLY BUT VARIABLY INHIBITS BRAIN CYTOCHROME OXIDASE ACTIVITY IN RATS.

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Sodium azide is a specific inhibitor of cytochrome oxidase (C.O.) activity, which is the terminal enzyme in the respiratory chain that allows cells to use oxygen. In the present study we investigated the metabolic effects in brain of systemically administered sodium azide on the brain of male Sprague-Dawley rats. Osmotic minipumps subcutaneously delivered sodium azide at 400 μ g/hr for 14 days. Fresh-frozen sections (40 μ m) were stained for C.O. activity. Sodium azide was found to significantly decrease gray matter activity in all imaged areas (73 regions with a range of decreases between control and azide-treated of 14-53%). The average brain C.O. activity for the control group was 212 μ mol/min/g tissue wet weight and 144 for the azide-treated group resulting in a treatment-induced decrease of 32.32% across all analyzed regions. Thalamo-striatal projection areas exhibited the largest overall decreases in C.O. activity. The parafasicular thalamic nucleus, subthalamic nucleus, and rostral and caudal caudate-putamen all showed relative decreases of 45-53%. The caudate-putamen and thalamus, along with the cerebellum and visual structures, have been implicated in visuospatial motor integration (Lalonde, 1997), which has been shown to be consistently dysfunctional with chronic sodium azide administration (Bennett and Rose, 1998). The pattern of behaviors seen in this model parallel Balint's syndrome, a disorder affecting orchestration of oculomotor and frontostriate circuits often diagnosed in humans before the onset of Alzheimer's disease.

928 A COMPARATIVE STUDY OF A TYPICAL ANTIPSHYCHOTIC DRUG HALOPERIDOL AND AN ATYPICAL ANTIPSYCHOTIC OLANZAPINE IN AMELIORATING OXIDATIVE STRESS.

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Haloperidol, a typical anti-psychotic drug, is used for the treatment of schizophrenia and other psychotic diseases. Its use is however limited because of the severe extrapyramidal side effects. The atypical antipsychotics, such as olanzapine, have not been associated with extrapyramidal symptoms. Because oxidative stress has been suggested as a causative factor of schizophrenia, we investigated the different oxidative profiles of these drugs. Interestingly, we found that both haloperidol and olanzapine are effective hydroxyl radical scavengers. In hydroxyl radical-dependent deoxyribose degradation assay, olanzapine was found to be more efficient in scavenging hydroxyl radicals than haloperidol. Thus, as little as 0.5mM olanzapine inhibited deoxyribose degradation by 50 percent whereas 1mM of haloperidol was required to inhibit deoxyribose degradation by just 30 percent. Because iron was used to generate hydroxyl radicals in a Fenton's type reaction, we studied the chelating ability of these drugs. In fluorescence studies, using calcein as a fluorescent probe, neither haloperidol nor olanzapine were found to chelate iron. In lipid peroxidation assay, 10μM of haloperidol was found to cause a 100 percent increase in lipid peroxidation relative to the controls, whereas olanzapine up to 0.5mM had trivial effects. These data indicate that the antipsychotic effects of both drugs may, at least in part, be due to their ability to scavenge hydroxyl radicals; and oxidative stress, as evidenced by the lipid peroxidation caused by haloperidol may, in part, be responsible for the extrapyramidal side-effects.

929 DOSE DEPENDENT PROTECTION BY LIPOATE AGAINST CISPLATIN-INDUCED OTOTOXICITY IN RATS: ANTIOXIDANT DEFENSE SYSTEM.

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This study investigated the alterations in auditory brainstem evoked responses (ABRs) with the changes in the cochlear antioxidant defense system in cisplatin-induced ototoxicity and dose dependent otoprotection by lipoic acid. Male Wistar rats were divided into different groups and were treated as follows: 1) vehicle (saline) control; 2) cisplatin (16 mg/kg, i.p.); 3) lipoate (100 mg/kg, i.p.) plus saline; 4) cisplatin plus lipoate (25 mg/kg); 5) cisplatin plus lipoate (50 mg/kg), and 6) cisplatin plus -lipoate(100 mg/kg). Post-treatment ABRs were performed after three days and the rats were sacrificed, cochleae harvested, and analyzed. The cisplatin injected rats showed ABR threshold elevations above the pretreatment thresholds. Rats treated with lipoate plus cisplatin did not show significant elevation of hearing threshold. Cisplatin administration resulted in a depletion of cochlear GSH concentration, superoxide dismutase (SOD), catalase (CAT), GSH-peroxidase (GSH-Px), and GSH-reductase (GR) activities (69%, 57%, 78%, 59% and 58% of control), respectively, and an increase in malondialdehyde (MDA) concentration (196% of control). Cochlear SOD, CAT, GSH-Px and GR activities, GSH and MDA concentrations were restored in the rats injected with cisplatin plus graded doses of lipoate, compared to cisplatin alone. The data suggest that cisplatin-induced ototoxicity is related to impairment of cochlear antioxidant defence system. The dose dependent otoprotection conferred by an antioxidant lipoate against cisplatin ototoxicity is associated with sparing of the cochlear antioxidant defense system. (Supported by NIH,R01DC0239602.)

930 EXAMINATION OF HYDROGEN PEROXIDE FORMATION IN COCHLEAR OUTER HAIR CELLS AND SUPPORT CELLS USING 2',7'- DICHLOROFLUORESCIN DIACETATE.

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The cochlea has been shown to participate in reactive oxygen species (ROS) generation following various ototoxicant administrations. Exposure of isolated cochlear outer hair cells (OHCs) to direct ROS generating systems leads to cytoplasmic blebbing, shortening and calcium disequilibrium; instillation of these generating systems into the cochlea alters auditory function. To see whether OHCs might participate in ROS generation, this study examined the abilities of OHCs and Deiters' support cells to convert the superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2). Cells were isolated in a 600 μ l chamber of artificial perilymph mounted on an inverted fluorescent microscope with videomicrography/ image analysis capabilities. 2',7'-dichlorofluorescin diacetate (DCF-DA, 100 μ M), a fluorescent marker for H_2O_2, was superfused, followed by a direct O_2^- generating system which included xanthine (100 μ M) and xanthine oxidase (8.0 mM). O_2^- production was spectrophotometrically confirmed by following cytochrome *c* reduction at 550 nm. Cell images were captured at 1 min intervals using excitation and emission wavelengths of 488 and 530 nm, respectively. OHCs fluoresced robustly on O_2^- generating system administration, prior to observable morphological changes. Fluorescence intensity exceeded that of control OHCs in which the O_2^- generating system or DCF-DA was omitted, or, superoxide dismutase (50,000 U/l) was included. Deiters' cells fluoresced more intensely during O_2^- generating system exposure than did control cells or similarly treated OHCs. These results suggest that OHCs can participate in cochlear ROS generation under conditions which lead to morphological and functional changes, but that the more numerous support cells may contribute more to total cochlear ROS generation.

931 EXAMINATION OF THE ANTIOXIDANT PROPERTIES OF TEA IN SMOKERS AND NONSMOKERS.

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Tea has been shown to exhibit chemopreventive activity against chemically induced carcinogenesis in rodent species. This chemopreventive activity appears to be based, in part, on the antioxidant properties of tea. In contrast to animal and in vitro investigations, no evidence of the antioxidant proper-

ties of tea in humans has been described. Oxidative stress has been implicated as a causative or participating factor in several human diseases including cancer, neurodegenerative diseases, and heart disease. Increased oxidative stress and oxidative damage have been associated with certain lifestyle behaviors, including smoking. The present investigation was performed to determine if tea consumption reduced oxidative stress in humans. Smokers and non-smokers were given tea or placebo for up to a week in duration. Blood and urine samples were taken before and after tea drinking. Biomarkers of oxidative stress including urine and WBC oh8dG and MDA, plasma antioxidants and reactive oxygen radicals (ROS) were analyzed. Basal levels of oh8dG and MDA (WBC and urine) and ROS were higher in smokers than in non-smokers. In most individuals, tea consumption resulted in a decrease in oxidative damage with this effect being more pronounced in smokers. While tea consumption increased the plasma total antioxidant capability, no changes in individual antioxidants were observed. Tea also reduced ROS. These results confirm that smoking increases oxidative stress and that tea functions as an antioxidant in humans.

932 LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN LUNGS AFTER GRADED SMOKE INHALATION IN SHEEP.

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In our efforts to elucidate the mechanisms associated with smoke inhalation injury, the present study investigated evidence for lipid peroxidation and the antioxidant status in sheep lungs after graded exposure to smoke. Adult, male sheep (n=4-5/gp) were anesthetized and received 0, 5, 10 or 16 units of a 1:1 mixture of cooled Western bark (fir and pine) smoke and 100% O_2 through an orotracheal tube. Sheep were euthanitized 48 hr after smoke exposure and lung samples from left and right apical, middle and diaphramatic lobes were analyzed for thiobarbituric acid reactive substances (TBARS), antioxidant capacity, glutathione (GSH) concentrations and myeloperoxidase (MPO) activity. Smoke inhalation induced a dose-dependent 3-fold increase in TBARS (16±2 to 48±3 nmol/g; $p<0.05$) and a 30% decrease in antioxidant capacity (8.5±0.5 to 5.8±0.6 μmol/g; $p<0.05$) in the severe smoke group than in controls. Lung GSH concentrations were not significantly different from controls in the mild or moderate smoke groups, but were about 30% lower in the severe smoke group than in controls. MPO activity tended to be higher in all smoked groups than controls, but statistical significance ($p<0.05$) was achieved only in the sheep that received 16 units of smoke. These data support the hypothesis that oxygen radical mechanisms are associated with smoke inhalation injury and that leukocyte sequestration into lung may be an important component.

933 THE STRESS RESPONSE OF CULTURED CELLS INDUCED BY AQUEOUS EXTRACTS OF CIGARETTE SMOKE (CS) CAN BE MIMICKED BY THE COMBINED ACTION OF CS-RELATED ALDEHYDES AND PEROXYNITRITE.

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Previous studies[1] have identified peroxynitrite ($ONOO^-$) as a possible stress gene- (*e.g.*, c-*fos*) inducing compound formed by O_2^-• and NO• in phosphate-buffered saline solutions (PBS) bubbled with CS ('smoke-bubbled PBS'). However, these studies can not explain the discrepancy between the calculated maximum concentration of ~ 15 μ M $ONOO^-$ that forms in 0.045 puffs/ml smoke-bubbled PBS and the concentration of ~ 500 μ M that is required to elicit a detectable c-*fos* response in cultured 3T3 fibroblasts when synthetic peroxynitrite is used. Here we demonstrate that CS-related aldehydes - a mixture of formaldehyde, acetaldehyde, and acrolein at the concentrations calculated to appear in smoke-bubbled PBS - in combination with as little as 20 m M $ONOO^-$ are sufficient to mimic the stress-specific c-*fos* response seen with smoke-bubbled PBS, although these aldehydes are incapable of inducing c-*fos* by themselves. Mechanistic investigations revealed that: (i) CS-related aldehydes lower the intracellular glutathion (GSH) content in much the same way as smoke-bubbled PBS, and (ii) 3T3 cells artificially depleted of GSH by pretreatment with BSO require significantly lower amounts of smoke-bubbled PBS to produce a detectable c-*fos* expression, whereas (iii) *N*-acetyl-cysteine is an efficient inhibitor of CS-induced c-*fos* response. Therefore, we conclude, that lowering the intracellular GSH content with these aldehydes may allow $ONOO^-$ to interfere with critical components that feed into stress signal transduction resulting in the expression of c-*fos*. (Sponsored by Philip Morris USA). [1] Müller et al. (1997) Carcinogenesis, **18**, 295-301.

934 DOSE DEPENDENT PROTECTION BY LIPOIC ACID AGAINST CISPLATIN-INDUCED NEPHROTOXICITY IN RATS: ANTIOXIDANT DEFENSE SYSTEM.

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This study elucidated the role of antioxidant defense system in cisplatin-induced nephrotoxicity and dose dependent nephroprotection by an antioxidant lipoate. Male Wistar rats were divided into different groups and were treated as follows: 1) vehicle (saline) control; 2) cisplatin (16 mg/kg, i.p.); 3) lipoate (100 mg/kg, i.p.) plus saline; 4) cisplatin plus lipoate (25 mg/kg); 5) cisplatin plus lipoate (50 mg/kg), and 6) cisplatin plus lipoate(100 mg/kg). The rats were sacrificed three days post-treatment, blood and kidneys were isolated and analyzed. The cisplatin injected rats showed elevation of plasma creatinine (677% of control) which significantly decreased by lipoate in a dose dependent manner. Cisplatin administration resulted in a depletion of renal GSH, increased GSSG concentrations and decreased GSH/GSSG ratio (62%, 166% and 62% of control) which were restored by lipoate pretreatment. Cisplatin significantly decreased superoxide dismutase (SOD), catalase (CAT), GSH-peroxidase (GSH-Px), and GSH-reductase (GR) activities (62%, 75%, 62% and 80% of control), respectively, and an increase in malondialdehyde (MDA) concentration (204% of control). Renal SOD, CAT, GSH-Px and GR activities, and MDA concentrations were restored in the rats injected with cisplatin plus graded doses of lipoate, compared to cisplatin alone. The data suggest that cisplatin-induced nephrotoxicity is related to depletion of renal antioxidant defense system, enhanced lipid peroxidation and plasma creatinine concentrations . The dose dependent nephroprotection conferred by an antioxidant lipoate against cisplatin-induced nephrotoxicity is associated with sparing of the renal antioxidant defense system, reduction of plasma creatinine, renal platinum concentration and lipid peroxidation. (Supported by NIH,R01DC0239602.)

935 THE ROLE OF PIRFENIDONE IN SCAVENGING HYDROXYL RADICALS.

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Pirfenidone (Deskar; PF), a broad-spectrum anti-fibrotic agent, is known to offer protection against bleomycin-induced lung fibrosis in hamsters. Because reactive oxygen species (ROS) are thought to be involved in these events, we investigated the mechanism(s) by which PF reduces oxidative stress. PF was found to be ineffective as a superoxide radical scavenger in cytochrome c reduction and epinephrine oxidation assays. In deoxyribose degradation assay, PF was found to be a potent scavenger of hydroxyl radicals with a rate constant of 5.39 e6/M/s . EPR spectroscopy, in combination with spin trapping techniques, was performed to verify the ability of PF to scavenge hydroxyl radicals. In a Fenton reaction, utilizing DMPO as a spin trapping agent, PF was found to scavenge hydroxyl radicals in a dose-dependent manner. The concentration of PF required to inhibit 49% signal height was found to be approximately 10 mM. PF was also found to inhibit NADPH-dependent lipid peroxidation in sheep liver microsomes in a dose-dependent manner. The concentration of PF required to cause 57% inhibition of lipid peroxidation was approximately 6mM. In an assay utilizing the fluorescent probe, calcein, it was concluded that PF does not chelate free iron in solution. This is of importance since the above assays used iron dependent systems to generate ROS. Based upon the above findings, we conclude that PF ameliorates oxidative tissue injury by scavenging hydroxyl radicals.

936 TOTAL OXIDANT SCAVENGING CAPACITY OF ANTIOXIDANTS TOWARD PEROXYNITRITE AND HYDROXYL AND PEROXYL RADICALS.

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Cellular balance between oxidant challenge and antioxidant response is complex because antioxidants exhibit marked differences in their capacities to scavenge different oxidants. We used the Total Oxidant Scavenging Capacity (TOSC) assay, developed in our laboratory (Free Rad. Biol. Med., 24: 480, 1998), to quantify the ability of benchmark antioxidants to scavenge 3 potent oxidants. 3-Morpholinosydnonimine (SIN-1), the iron/ascorbate-driven Fenton reaction, and thermal homolysis of 2,2'-azo-*bis*- amidinopropane (ABAP) were used to generate, respectively peroxynitrite (PXN),

hydroxyl radicals (· OH), and peroxyl radicals (ROO·). After normalizing controls to yield equivalent time courses of ethylene production from α -keto-γ -methiolbutyric acid (KMBA), the endpoint of the TOSC assay, with the 3 oxidant-generating systems, antioxidants were compared for their abilities to compete for oxidants with KMBA. Glutathione (GSH) was ca. an order of magnitude more effective as a scavenger of ROO· than of PXN or · OH, which were similarly absorbed by GSH. Uric acid was a potent scavenger of PXN and ROO· ; the efficiency toward these oxidants was >10-times that toward · OH. The water-soluble vitamin E analog, Trolox®, most potently absorbed ROO· , followed by PXN and · OH. The TOSC of 3 benchmark · OH scavenging agents, showed mannitol, benzoic acid and dimethylsulfoxide (DMSO) to be at least 10 times more potent as scavengers of · OH. Mannitol and benzoic acid, but not DMSO scavenged ROO· . The results indicate the need to characterize chemicals and biological samples toward different oxidants when evaluating their antioxidant potential.

937 EFFECT OF ENCAPSULATED SUPEROXIDE DISMUTASE (SOD) AND NITRIC OXIDE SYNTHASE (NOS) INHIBITORS ON ACETAMINOPHEN HEPATOTOXICITY IN MICE.

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Acetaminophen (APAP) induced liver toxicity is believed to be mediated by CYP450 metabolism to N-acetyl-*p*-benzoquinone imine which covalently binds to proteins. We recently reported that a toxic dose APAP produced protein adducts and nitrated tyrosine residues in centrilobular cells. Tyrosine nitration occurs by reaction of tyrosine with peroxynitrite (PN), generated from nitric oxide (NO·) and superoxide (O_2^-). Kupffer cells are important in their formation. We have investigated the effect of SOD and NOS inhibitors on APAP hepatotoxicity in mice as determined by increases in serum alanine aminotransferase (ALT) levels (IU/L). APAP (300 mg/kg) plus liposomes increased serum ALT from 21 ± 3 (control; mean±SE) to 3418 ± 1200 at 4 hrs in mice (n=5 /group). APAP plus liposome encapsulated SOD had ALT levels of 102 ± 10. Serum levels (μM) of NO_2 + NO_3 (from NO·) increased from 30 ± 2 in control to 59 ± 8 in APAP plus liposomes and further to 88 ± 13 in APAP plus SOD. The effects of the nontoxic iNOS inhibitors L-NIL (3 mg/kg, 2 doses) at -0.5hr and +1.0hr and AG (aminoguanidine; 15 mg/kg, 2 doses) at 0.5hr and 2.0hr, and the nontoxic nonselective NOS inhibitor L-NMMA (30 mg/kg, 2 doses) at -0.5hr and +1.0hr on APAP hepatotoxicity were examined. APAP (300 mg/kg) increased ALT to 3225 ± 891 at 4 hrs. L-NIL plus APAP increased ALT to 6040 ± 1185. AG plus APAP increased ALT to 6470 ± 2005. L-NMMA plus APAP increased ALT to 6709 ± 1185. Thus, relative formation of O_2· and NO· are important determinants of toxicity.

938 PEROXYNITRITE SCAVENGING ACTIVITY OF NITRIC OXIDE SYNTHASE (NOS) INHIBITORS.

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NO and superoxide can combine to generate peroxynitrite, a potent and versatile oxidant that can attack a wide range of biological targets. To examine the relative roles of NO and peroxynitrite in biology, specificity of inhibitors must be determined. Peroxynitrite-mediated protein tyrosine nitration was used as a measure of peroxynitrite activity to examine the ability of NOS inhibitors to also scavenge peroxynitrite. We tested the inducible NOS inhibitors 2-amino-5,6-hydro-6-methyl-4H-1,3-thiazine (AMT), aminoguanidine (AG), and L-N-1-iminoethyl -lysine (LNIL), the non-selective NOS inhibitor L-N-monomethyl -arginine (LMMA), the NO scavenger [2(4-carboxyphenyl-4,4,5,5-tetramethylimidazoline oxyl-3-oxide) (CPTIO) and the iron chelator/peroxynitrite scavenger deferoxamine (DFO) for their abilities to inhibit nitration of bovine serum albumin (BSA) by peroxynitrite. Peroxynitrite (1mM) was added to a solution of BSA (2mg/ml) in 500mM phosphate buffer (pH 7.4) at 37°C. After 5 min absorbance was measured at 430nm, (E=4500$M^{-1}cm^{-1}$) for nitrotyrosine). Untreated BSA contained 0.7±0.6 nmol/mg. Peroxynitrite treatment produced 19±1 nmol/mg. Inhibitors were preincubated with BSA at 0.1mM, 1mM and 10mM. Of the iNOS inhibitors tested, only AG inhibited nitration (39±5% at 10mM; n=3). LMMA and CPTIO were not inhibitory at any concentration. DFO was a potent inhibitor even at 0.1mM (68±8%; n=3). These data indicate that the protective effects of AG, and the iron chelator DFO in NO-mediated biology may be due in part to their ability to scavenge peroxynitrite.

939 INACTIVATION AND HEME ALTERATION OF RECOMBINANT NEURONAL NITRIC OXIDE SYNTHASE BY GUANABENZ.

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Guanabenz, an antihypertensive drug, is an active site-directed metabolism-based inactivator of rat brain and penile nitric oxide synthase (NOS). The inactivation of NOS is concomitant to a decrease in immunodetectable NOS protein *in vivo*. To better understand these *in vivo* effects, we examined the mechanism of inactivation, with the use of recombinant neuronal NOS protein obtained by baculovirus-mediated expression in Sf9 cells. The kinetics of inactivation were followed by measuring rates of nitric oxide-mediated conversion of oxyhemoglobin to methemoglobin. *In vitro* studies with the use of a cytosolic fraction from Sf9 cells overexpressing neuronal NOS, the inactivation was found to depend on NADPH, time and concentration of guanabenz, similarly to that found for the native enzyme. The inactivation could be described by a pseudo-first order rate constant (k_{inact}) and an apparent dissociation constant (K_i) of 0.027 min^{-1} and 1.2 μ *M*, respectively. The inactivation of NOS activity in the presence of cytosol involved the alteration of the prosthetic heme group of NOS. The presence of L-arginine protected NOS from the loss of activity and alteration of heme. Interestingly, purified preparations of neuronal NOS were less sensitive to guanabenz inactivation and heme loss. The characterization of the mechanism of inactivation *in vitro* will likely lead to a better understanding of the regulation of NOS *in vivo*. This may be of importance as NO has a variety of biological roles and the inactivation of NOS may explain the toxicological effects of some chemicals. (Supported by NIH grant ES08365. YO is a recipient of the Burroughs Wellcome New Investigator Award in Toxicology.)

940 INDUCIBLE NITRIC OXIDE SYNTHASE KNOCKOUT MICE ARE LESS SUSCEPTIBLE TO THE TOXIC EFFECTS OF ACETAMINOPHEN THAN WILD TYPE MICE.

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Metabolism of acetaminophen (APAP) by CYP450 to N-acetyl-*p*-benzoquinone imine and covalent binding to proteins is the accepted mechanism of hepatotoxicity. We recently reported that a toxic dose APAP produced APAP-protein adducts and nitrotyrosine protein adducts in the hepatic centrilobular cells of mice. This product is formed by nitration of tyrosine by peroxynitrite (PN). PN is formed by reaction of nitric oxide (NO·) with superoxide. Also, we recently showed that the Kupffer cells inhibitors gadolinium chloride and dextran sulfate decrease APAP hepatotoxicity and hepatic nitrotyrosine protein adducts. In this work we show that APAP is less toxic in mice lacking inducible nitric oxide synthase (iNOS) (C57BL/6-$Nos2^{tm1Lau}$). APAP (300 mg/kg) administration to wild type male mice (n=4) resulted in significant hepatotoxicity by 4hrs, increase in serum alanine aminotransferase (ALT). ALT levels (IU/L) were 3389 ± 766 at 4 hrs, 8954 ± 654 at 6 hrs, and 7354 ± 963 at 8 hrs. In the iNOS knockout mice ALT levels were 1838 ± 432 at 4 hrs, 4021 ± 960 at 6 hrs, and 2824 ± 1011 at 8 hrs (saline =8 ± 2). ALT levels were significantly less in the APAP treated iNOS knockout mice at each time point than the wild type. APAP treatment did not cause serum levels of NO_2 + NO_3 (from NO·) to increase in the iNOS knockout mice. In the wild type serum levels of NO_2 + NO_3 (μM) were 53 ± 2 in saline treated controls. APAP treatment of mice produced significant increases to 70 ± 2 at 2 hrs, to 96 ± 5 at 4 hrs, and to 168 ± 27 at 6 hrs. At 8 hrs the levels were 49 ± 8. Thus toxic doses of APAP lead to increased synthesis of NO· from iNOS and NO· is important in APAP hepatotoxicity.

941 MICE LACKING INDUCIBLE NITRIC OXIDE SYNTHASE ARE PROTECTED FROM OZONE-INDUCED LUNG INJURY.

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Ozone is a highly reactive oxidant present in photochemical smog. Inhalation of ozone causes epithelial cell necrosis and Type II cell hyperplasia and proliferation. This is associated with an accumulation of macrophages in the lower lungs. We have previously shown that these cells contribute to ozone induced tissue injury. Macrophages are known to release a number of mediators with cytotoxic potential. One mediator of particular interest is nitric oxide which is produced in macrophages in response to inflammatory stimuli via an inducible form of nitric oxide synthase (NOSII). To study the role of nitric oxide in ozone induced tissue injury we used

knockout mice lacking the gene for NOSII. Treatment of wild type control mice with ozone (0.8-1 ppm) for 3 hr resulted in a time dependent increase in protein in bronchoalveolar lavage fluid (BAL) reaching a maximum 24-48 hr after exposure. Alveolar macrophages isolated from animals treated with ozone were found to produce increased amounts of nitric oxide. This was correlated with induction of NOSII protein and mRNA which was identified in macrophages *in situ* in histologic sections and in isolated cells. Alveolar macrophages from ozone exposed animals also produced increased amounts of peroxynitrite which was associated with nitrotyrosine staining in the lungs, a marker of peroxynitrite induced tissue injury. In contrast to cells from wild type control mice, alveolar macrophages from NOSII knockout mice did not produce nitric oxide or peroxynitrite even after ozone treatment of the animals. Moreover, these mice were protected from ozone induced tissue injury, as evidenced by the finding that BAL protein levels were at control levels. In addition, there was no evidence of peroxynitrite mediated injury in these animals. Taken together these data demonstrate that nitric oxide produced via NOSII plays a critical role in ozone induced tissue injury. (Supported by NIH grants ES04738, ES06897 and the Burroughs Wellcome Fund.)

942 KINETICS OF NITRIC OXIDE INHIBITION IN SINGLE CELLS MEASURED USING A SELF-REFERENCING NITRIC OXIDE SENSOR.

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Nitric oxide produced by macrophages is known to be critical in mediating toxicity induced by a variety of chemicals including acetaminophen, carbon tetrachloride, inhaled ozone and asbestos. In macrophages this reactive nitrogen intermediate is produced via the high output cytokine-inducible form of nitric oxide synthase (NOS2). Although the production of nitric oxide has been quantified using measurements of the stable oxidation products nitrite and nitrate, direct real time measurements of nitric oxide flux from single cells have not been reported. Using a novel self-referencing nitric oxide sensor we found that single cells or clumps of RAW 264.7 macrophages readily release nitric oxide following stimulation with gamma-interferon. Release of nitric oxide could be detected at distances up to 10-20 microns from single cells and remained constant for up to 3 hours, even when cells were examined in serum-free medium. Nitric oxide flux was dependent on the presence of L-arginine in the cell culture medium and was completely removed by oxy-hemoglobin. The nitric oxide synthase inhibitor L-arginine methyl ester (L-NAME, 1 mM) suppressed nitric oxide production within 7 min, an effect that was rapidly reversed. Taken together our data indicate that measurement of nitric oxide release can be obtained using single isolated cells. In addition, the inhibitory action of L-NAME is reversible and the cells do not sequester the drug. (Supported by NIH grants ES 03647, ES 06897 and RR 01395.)

943 ELUCIDATION OF SUPEROXIDE DISMUTASE (SOD1) COPPER LOADING WITH LYS7 FROM *S. CEREVISIAE*.

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Superoxide dismutase 1 (SOD1) is a cytosolic protein that functions in cellular antioxidant defense. This enzyme catalyzes the dismutation of superoxide radical (O_2^-) to hydrogen peroxide (H_2O_2) and oxygen (O_2). For proper activity and function, SOD1 requires a copper chaperone, either yeast LYS7 or its human homologue CCS, to deliver copper to the enzyme. LYS7 is a small, soluble protein containing a single consensus metal binding sequence MT/HCXXC. Studies are currently underway to characterize the LYS7 gene product, and to ascertain the manner in which LYS7 controls copper incorporation into SOD1. It has been shown that LYS7 can be proteolytically clipped into three distinct domains (I, II, III). Domain I is homologous to ATX1, another small copper chaperone, and domain II is highly homologous to SOD1. Recent data suggests that a protein containing only domains II and III of LYS7 (but missing domain I) can very poorly complement *lys7* null mutants under normal copper conditions; however, this protein does not complement at all under copper starvation conditions. Conversely, a protein containing only domains I and II does not complement the *lys7* null phenotype. Furthermore, site directed mutagenesis of possible metal binding histidines to alanines at positions 130/134, 151, and 198 in domain II of LYS7 do not diminish the ability of the protein to rescue the *lys7* null phenotype. Work is under way to mutagenize two possible metal binding cysteines in domain III. These studies suggest a possible model for copper delivery to SOD1. Domain I captures the copper, perhaps from the copper transporter, while domain II acts in a "docking" fashion to properly align LYS7 and SOD1 for copper transfer; furthermore, actual insertion of the copper ions is accomplished through the flexible domain III.

944 HYPEROXIC LUNG INJURY IS POTENTIATED BY SPC-PROMOTOR DRIVEN EXPRESSION OF AN HO-1 TRANSGENE IN MICE.

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Heme oxygenase (HO) catalyzes the first step in metabolism of heme to biliverdin and bilirubin, with concomitant production of CO and liberation of heme-bound iron. Some investigators have proposed that the inducible isoform, HO-1, may contribute to adaptive responses to oxidant stresses, in part through the production of bilirubin, which can exhibit antioxidant activities. To test this hypothesis in vivo, we generated a line of transgenic mice that express the rat spleen HO-1 cDNA driven by a human surfactant protein C promoter. HO activities (660+/-71 vs. 181+/-15 pmol/min/g tissue) and HO-1 protein and mRNA levels were greater in the lungs of the transgenic animals than in lungs from control animals The transgenic animals were more susceptible to lung injury in response to exposure to >95% O_2, as assessed by lung weights (12.17+/-2.80 vs 7.76+/-0.78 mg lung/ g animal by 96 h). Hyperoxia increased lung HO activities and HO-1 mRNA levels in the control animals, as has been reported previously, but message levels and HO activities were diminished by hyperoxia in the lungs of transgenic animals (242+/-82 pmol/min/g after 48 h). HO-1 expression in air-breathing transgenic animals appeared by immunohistochemistry to be predominantly in lung type II cells, but expression in type II cells was largely lost by 48 h or more of hyperoxia. Increased HO-1 protein in both transgenic and wild-type animals exposed to hyperoxia appeared to be associated almost exclusively with macrophages. (Supported by HD01124 and HD27823.)

945 QUINOL-THIOETHER-MEDIATED CYTOTOXICITY IN A RENAL PROXIMAL TUBULAR EPITHELIAL CELL LINE: EVIDENCE FOR MULTIPLE REACTIVE OXYGEN SPECIES.

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Treatment of LLC-PK1 cells with 2,3,5-*tris*-(glutathion-*S*-yl)-hydroquinone (TGHQ) causes the generation of reactive oxygen species (ROS) and subsequent induction of prostaglandin E2 (PGE2) synthesis. PGE2, and its analog 11-deoxy-16,16-dimethyl prostaglandin E2 (DDM-PGE2), protects LLC-PK1 cells against TGHQ-induced cytotoxicity. In this study we investigated the mechanisms by which by TGHQ elicits cytoxicity in order to delineate the pathways by which DDM-PGE2 evokes cytoprotection. Rutin hydrate (1 μM), a scavenger of nitric oxide (NO), increased cell viability by 20% in LLC-PK1 cells that were pretreated for 30 min prior to TGHQ. In addition, 10 μM ebselen, a peroxynitrite scavenger blocked the cytotoxic effects of TGHQ. Pre-treatment of LLC-PK1 cells for 30 min with 20 μM U-83836E ([2-methyl]aminochroman; a scavenger predominately of lipid radicals), significantly blocked TGHQ-mediated cytotoxicity. These data suggest that multiple ROS may mediate the cytotoxic effects of TGHQ in LLC-PK1 cells. The synthesis of PGE2 is catalyzed from arachidonic acid by one or both of the two isoforms of cyclooxygenase (COX). COX-1 is constitutively expressed in a number of tissue types and cell lines, while COX-2 is induced by a variety of stimuli, including ROS. Resveratrol (10 μM), a specific inhibitor of COX-1, or NS-398 (10 μM), a COX-2 inhibitor, blocked DDM-PGE2-mediated cytoprotection by 51% and 57%, respectively. Taken together, NO and peroxynitrite appear to contribute to TGHQ-induced cytotoxicity, and the cyclooxygenase pathway may, in part, mediate the cytoprotective effects of DDM-PGE2. (GM56321, ES07247).

946 IMMUNODETECTION OF NAD(P)H:QUINONE OXIDOREDUCTASE 1 (NQO1) IN HUMAN TISSUES.

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Human tissues were examined by immunoblot analysis and/or immunohisto-

chemistry for NQO1 expression. High levels of NQO1 expression were detected in many tissues including lung, breast, stomach, and eye, while low levels of NQO1 were detected in bone marrow and liver. In lung and breast tissues, intense staining for NQO1 was observed in columnar epithelium, vascular endothelium and adipocytes. In the stomach, intense staining for NQO1 was observed in chief cells localized in the gastric pits. In the small intestine and colon, moderate staining for NQO1 was observed in areas of columnar epithelium. Throughout the GI tract intense staining for NQO1 was observed in vascular endothelium and adipocytes. In the eye intense staining for NQO1 was observed in corneal, lens and retinal epithelium and vascular endothelium. Moderate staining for NQO1 was observed in optic nerve fibers. In the myocardium only trace levels of NQO1 were detected by immunoblot analysis, however, NQO1 was detected in the aorta. In immunohistochemical studies with monkey aorta, NQO1 was localized to vascular endothelium. No NQO1 could be detected by immunoblot in human bone marrow aspirates or isolated haematopoetic cells. In addition, only trace levels of NQO1 could be detected in liver by immunoblot analysis or immunohistochemistry. In tissues from individuals genotyped as homozygous for the NQO1 *2 (609C-T) allele, NQO1 protein was either non-detectable or present at only trace levels. The high levels of NQO1 observed in epithelium and vascular endothelium of many tissues and the low levels of NQO1 detected in liver suggests that NQO1 may function primarily as an antioxidant enzyme, and in contrast to rodents, NQO1 may not play a major role in hepatic xenobiotic metabolism in humans (Supported by EPA grant R82-5281-010 and CA 51210.)

947 PHARMACOLOGICAL RESCUE OF THE *14CoS/14CoS* MOUSE AS A MODEL FOR ENDOGENOUS OXIDATIVE STRESS.

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Mice homozygous for the radiation-induced *14CoS/14CoS* albino deletion die within 24 h post partum, due in large part to the disruption of the *Fah* gene encoding fumarylacetoacetate hydrolase, which catalyzes the last step in tyrosine catabolism. It has been hypothesized that death in *14CoS/14CoS* animals is the result of accumulation of electrophilic precursors of fumarylacetic acid and acetoacetate. Our lab has previously demonstrated that *14CoS/14CoS* mice exhibit a robust oxidative stress response both *in utero* and after birth. We show here the rescue of *14CoS/14CoS* mice by treatment with 2-(2-nitro-4-trifluoromethyl-benzoyl)-1,3-cyclohexanedione (NTBC), a potent inhibitor of *p*-hydroxyphenylpyruvate dioxygenase, the second enzyme in the tyrosine catabolic pathway. On a dose of 10 μg NTBC / mL in the drinking water, ~70% of *14CoS/14CoS* mice survive past day 20, whereas removal of NTBC results in death within 48 h. Growth, as measured by weight gain through day 28, is impaired in rescued mice compared with that in *ch/14CoS*, *Fah(+/-)* or *Fah(-/-)* mice. Cataracts occur in at least one eye of ~80% of the rescued *14CoS/14CoS* mice, but are not observed in *ch/14CoS*, *Fah(+/-)* or *Fah(-/-)* mice on NTBC, indicating that cataractogenesis involves at least one gene in addition to the *Fah* deletion. Finally, *14CoS/14CoS* mice on NTBC continue to exhibit a significant oxidative stress response over a 60-day time course: Northern blots of liver and kidney RNA demonstrate striking elevations in mRNA levels of the oxidative stress-responsive genes glutamate cysteine ligase (structural and regulatory subunits, *Gcls* and *Gclr*), and NAD(P)H:quinone reductase (*Nqo1*). These results suggest that *14CoS/14CoS* mice on NTBC might serve as a useful model for aging associated with the generation of endogenous oxidative stress. (Supported in part by NIH Grants RO1 AG09235, RO1 ES06321 and P30 ES06096.)

948 OXIDATIVE STRESS, ANTIOXIDANT ACTIVITY AND MECHANISM OF HEPATOTUMORIGENESIS IN PCB-DOSED RATS.

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We have recently described our toxicological findings in male and female Sprague Dawley rats undergoing chronic, parallel, multi-dose bioassays of Aroclors 1016, 1242, 1254, and 1260. Our correlations among those data are suggestive of two routes to hepatotumorigenesis: a liver TEQ-dependent one that was predominant in the females and a TEQ-independent route predominant in the males. Our detailed biochemical post mortem examinations of cryopreserved livers after the intermediate and terminal sacrifices, indicate a strong empirical correlation between hepatotumorigenesis and production of the reactive oxygen species (ROS) superoxide and peroxide, via redox cycling of cytosolic low molecular weight species. By liquid chromatographic methods we have tentatively identified some of these redox cyclers in the female rats as glutathionylated estrogen catechols. Antioxidant and phase II enzyme activities were measured to evaluate effects of elevated peroxide and superoxide production. In contrast to unaltered catechol-o-methyltransfease activity, superoxide dismutase activity showed limited increases while glutathione S-tranferase (GST) and quinone reductase (QR) showed increases with tumor yields. Regression analyses indicated that the GST/QR ratio was positively correlated with tumor yield. Finally, glutathione peroxidase activities showed a strong negative correlation with tumor yields. These results are consistent with stabilization of a catechol/semiquinone redox couple resulting in increased oxidant production by redox cycling. In addition, the limited antioxidant activity could not adequately scavenge the resulting elevated ROS. Mechanisms linking a series of events from chronic MTD exposure to oxidative stress via quinone-glutathione coupling, redox cycling and mutant cell promotion would appear capable of explaining the observed carcinogenic activities.

949 HEPATOTUMORIGENESIS IN PCB-DOSED AND CONTROL SPRAGUE-DAWLEY RATS PARALLELS CYSTOSOLIC REDOX CYCLING ACTIVITY.

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In order to identify the events leading from chronic, MTD administration of a non-genotoxic promoter to rodent liver tumors, we first characterized the dependencies of hepatotumorigenesis on sex, PCB internal dose, and composition via parallel, multidose, chronic, near-MTD bioassays of Aroclors 1016, 1242, 1254, and 1260 in male and female Sprague-Dawley (SD) rats (Mayes *et al.*, *Toxicol. Sci.* 41, 62-76, 1998), and then examined the progression of changes in liver biochemistry that occurred during the process of hepatotumorigenesis. These studies turned up one biochemical parameter (out of >20) that showed the same distinctive pattern of responses to the experimental variables as did the tumor count: namely, the production of superoxide by low MW redox-cycling agents in the liver cytosols. Such superoxide, or the derived peroxide, is believed to promote clonal expansion of spontaneously initiated cells, possibly via activation of NF-κ B. At least two routes for the formation of the redox cycling activity were identified. The first, predominant in the SD females, correlated with the hepatic levels of coplanar PCB congeners, which, presumably via the Ah-receptor, induced CYP1A, CYP1B, and associated oxidase activities. The second, predominant in the males, correlated with total adipose PCB (mostly non-coplanar congeners), which, presumably via an immunophilin-like receptor, induced CYP2B, CYP3A, and other oxidases. The oxidation products thus far identified as responsible for the tumorigenesis-correlated redox-cycling activity include a family of glutathionylated estrogen 2, 3- and 3, 4- quinones.

950 CHARACTERIZATION OF NORMAL AND CIRRHOTIC FAT-STORING CELL ALCOHOL AND ALDEHYDE METABOLISM.

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Chronic liver injury initiated by alcohol, iron or chlorinated hydrocarbons produces enhanced lipid peroxidation and activation of fat-storing cells (FSC). *Trans*-4-hydroxy-2-nonenal (4-HNE), an α-β unsaturated aldehyde produced by ω-6-polyunsaturated fatty acid peroxidation, reportedly stimulates FSC collagen gene expression and type 1 collagen production. An important question concerning the ability of α–β unsaturated aldehydes to modulate FSC collagen production is the potential for these cells to detoxify 4-HNE. In this study, we have characterized the ability of FSC lines derived from a normal (NFSC) and cirrhotic (CFSC) rat liver to metabolize 4-HNE by reductive (alcohol dehydrogenase; ADH) oxidative (aldehyde dehydrogenase; ALDH) and conjugative pathways (glutathione S-transferase; GST). Spectrophotometric analysis indicated that both cell lines had negligible ADH activity. ALDH specific activity was 0.90 ± 0.2 and 2.31 ± 0.55 nmoles min^{-1} mg^{-1} protein for NFSC and CFSC, respectively. The capacity of ADH and ALDH was also evaluated in cell suspensions by headspace gas chromatography using either ethanol or acetaldehyde as substrates. Both cell types lacked significant ADH activity but displayed ALDH activity of 44.57 and 53.51 μmoles hr^{-1} 10^6 cells for the NFSC and CFSC lines, respectively. Glutathione-S-transferase (GST) activity measured in NFSC and CFSC for CDNB conjugation were of 26.18 ± 10.97 (NFSC) and 53.72

± 10.59 (CFSC) nmoles min^{-1} mg^{-1} protein, and 4-HNE conjugation 9.53 ± 2.16 (NFSC) and 3.63 ± 2.53 (CFSC) nmoles min^{-1} mg^{-1} protein. Immunoblots for GST classes Ya, Yb and P1-1 demonstrate CFSC possess greater quantities of Yb and P1-1 GST; Ya was not detected. HPLC measurement of glutathione (GSH) reveled that CFSC lines displayed 3-fold higher concentrations of GSH (4.72 nmols/10^6 cells) as compared to NFSC (1.6 nmols/10^6 cells). Taken together this data suggests that CFSC have a greater capacity than NFSC to deactivate aldehydes through glutathione conjugation and oxidation to acid metabolites. (Supported by AA09300.)

951 PURIFICATION AND CHARACTERIZATION OF A MITOCHONDRIAL THYMINE GLYCOL ENDONUCLEASE FROM RAT LIVER.

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Mitochondrial (mt) DNA is exposed to damage caused by oxygen radicals produced during oxidative phosphorylation or during exposure to chemical or physical agents. Structural alterations in mtDNA and associated mt dysfunction have been correlated with aging and diseases. Accumulation of oxidative mtDNA damage may be responsible for these mtDNA alterations. mtDNA repair may therefore be important in preventing mt dysfunction. Here, we report the purification of a novel rat liver mt thymine glycol (TG) endonuclease (mtTGendo). Initially, we observed that crude protein extracts from purified rat liver mitochondria incised a radiolabeled oligonucleotide duplex containing one TG. Utilizing subsequent DEAE, cation exchange, hydrophobic interaction and size exclusion (SE) chromatography, a ~450-fold purification of the mtTGendo activity was achieved. Active SE fractions displayed a single band of ~37 kDa on a silverstained gel. mtTGendo is EDTA resistant and active within a broad KCl concentration range. Further, mtTGendo has an associated AP-lyase activity, making a similar incision into the TG-containing DNA as *E. coli* endonuclease III, the bacterial enzyme that repairs TG. Duplexed oligonucleotides containing 8-oxodeoxyguanosine or uracil and single-stranded oligonucleotide containing one TG are no substrates for mtTGendo. Given the fact that TG blocks DNA replication, this novel DNA repair enzyme may help to prevent accumulation of structural mtDNA changes, mt dysfunction and associated (patho)-physiological alterations *in vivo* that are associated with oxidative stress.

952 EFFECTS OF N-*TERT*-BUTYL-α-PHENYL NITRONE (PBN) ON DIISOPROPYLPHOSPHOROFLUORIDATE (DFP) OR KAINIC ACID (KA) INDUCED SEIZURES, FASCICULATIONS AND MUSCLE NECROSIS.

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Indirect evidence suggests that reactive oxygen species (ROS) may mediate DFP and KA induced neuronal and muscle fiber necrosis following seizures and fasciculations. Therefore the spin trapping agent PBN was used *in vivo* to assess formation of ROS during DFP and KA treatment. Pretreatment with PBN (150–300 mg/kg, i.p.) completely prevented seizures, fasciculations and necrosis induced with DFP (1.3 mg/kg, s.c.), but did not prevent seizures and fasciculations when given following DFP, nor did pretreatment with PBN prevent seizures induced by KA or pilocarpine, a muscarinic receptor agonist. DFP alone reduced AChE activity in brain to 3% and in diaphragm to 25% of control. Pretreatment with PBN attenuated this inhibition in brain to 37% and diaphragm to 80% of control. PBN given after DFP neither protected AChE from DFP inhibition nor prevented seizures and fasciculations. It is suggested that PBN has a direct, dose dependent reversible inhibitory effect on AChE of brain and muscle and thus protects the enzyme from critical irreversible inhibition by DFP. While the role of PBN as an effective antioxidant is well established its protection against DFP induced seizures, fasciculation and necrosis are not due to its antioxidant action. (Supported by NIH Grant, ES04597.)

953 ISOTOPIC DILUTION METHOD FOR THE IDENTIFICATION AND QUANTIFICATION OF 2-AMINO 6,8-HYDROXYPURINE (8ohG).

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Oxidative injury to DNA is a common cellular insult following exposures to many xenobiotic agents. The hydroxyl adduct of guanine at the C8 position (8ohG) of DNA has been associated with mutagenic base transitions and has been identified as a potential biomarker for human cancer risk. Analytic methods for identification of 8ohG include high pressure liquid chromatography (HPLC), gas chromatography mass spectroscopy (GC-MS), P^{32} postlabeling, and alkaline unwinding assays, each with inherent strengths and weaknesses. Our laboratory has evaluated the performance of an isotopic dilution method for GC-MS identification of 8ohG using a C^{13} N^{15} ring labeled internal surrogate ($8ohG_L$) with emphasis on quantifying artifactual oxidation of guanine. Data from repeat injections of the compound and replicate calibration curves over a four week period indicates that $8ohG_L$ is stable in 10mM sodium hydroxide. Precision of the method to reproducibly hydrolyze 8-hydroxyguanosine and then derivatize and quantify 8ohG was indicated by a 6.8% coefficient of variation (CV) in multiple replicate analyses of the base-sugar compound. Recovery of the 8OH-guanosine was 99%, and sensitivity was 20 pg (120 femptomoles). The detection limit (with 50 ug of DNA in a final extract volume of 100ul) was 20pg of 8ohG per ug of DNA. Replicate samples containing 50 ng of HPLC purified guanine were derivitized with bis(trimethylsilyl)-trifluoroacetatamide (BSTFA) and analyzed using this method to quantify artifactually produced 8ohG. Levels of 8ohG ranged from 500pg (1/2 detection limit) to 1200 pg, suggesting that artifactual oxidation during derivitization accounts for less than 2% of total guanine. Replicate analyses of MCF-7 carcinoma cell DNA and calf thymus DNA samples prepared by a high salt extraction procedure resulted in CVs of 22% and 23%, respectively. Ongoing work is comparing the method to HPLC using DNA samples extracted from cells and animal tissues treated with pro-oxidant agents. (Support from NIEHS #ES09455-01 and USACEHR.)

954 CORRELATION BETWEEN THE FORMATION OF 8-HYDROXY-2'-DEOXYGUANOSINE (OH8dG) AND MORPHOLOGICAL TRANSFORMATION IN SYRIAN HAMSTER EMBRYO (SHE) CELLS.

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The formation of OH8dG is one of the most prevalent oxidative DNA modifications. Previously reported studies have suggested an association between OH8dG formation and carcinogenesis. However, it is not clear whether OH8dG formation results in the necessary genotoxic events for cancer development. In the present study, the formation of OH8dG and its ability to transform SHE cells was examined. Methylene blue, a photosensitizer which in the presence of light can generate singlet oxygen by a type II mechanism, was used to generate oxidative DNA damage. This model has been shown to predominantly produce OH8dG. Methylene blue plus light produced a dose-dependent increase of OH8dG as well as morphological transformation in SHE cells. SHE cells transfected with DNA that contained increasing concentrations of OH8dG, produced a dose-dependent increase in morphological transformation. Co-treatment of SHE cells with both β-carotene, a singlet oxygen quencher, and methylene blue and light inhibited the morphological transformation (in a dose dependent manner). These results suggest that formation of OH8dG through methylene blue and light can induce morphological transformation. In addition, these data provide further support for a role of OH8dG formation in the carcinogenesis process.

955 OXIDATIVE STRESS AND 8-HYDRODEOXYGUANOSINE IN RATS FOLLOWING ACUTE EXPOSURE TO TRICHLOROETHYLENE OR PERCHLOROETHYLENE.

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8-Hydroxydeoxyguanosine (8OHdG) is a product of DNA oxidation and has been employed in occupational studies as a marker of biologically effective dose. Trichloroethylene (TCE) and perchloroethylene (PERC) are used in the dry cleaning industry and their metabolism can produce reactive oxygen compounds with the potential to oxidize DNA. The objective of the present study was to determine if acute exposure to TCE or PERC caused oxidative DNA damage in rats that could be detected as an increase in lymphocyte

8OHdG/dG or urinary excretion of 8OHdG. Thiobarbaturic acid reactive substances (TBARS) and 8-epi -prostaglandin 2 alpha (8epiPGF2) were also measured as biomarkers of increased oxidative stress. Male Fisher rats were administered a single i.p. injection of 0, 100, 500, or 1000 mg/kg of PERC or TCE. Control rats received only vehicle (1:4 v/v of Alkamuls/water) and a positive control group received 100 mg/kg 2-nitropropane (2NP). Rats were sacrificed 24 hrs after dosing. In rats receiving 2NP or TCE, TBARS and the 8OHdG/dG ratio were significantly elevated in liver. Lymphocyte 8OHdG/dG was also elevated, but not significantly. In rats receiving 2NP, urinary excretion of 8OHdG and 8epiPGF2 were significantly increased at 12 and 24 hrs. In rats receiving TCE, no change in urinary excretion of 8epiPGF2 was evident, but an increase in 8OHdG was noted at 24 hr. Results indicate that a single high dose of TCE, can result in detectable elevation in 8OHdG. Despite an absence of statistically significant effects in rats receiving PERC, dose-dependent trends in several endpoints suggest a slight increase in oxidative stress.

956 EFFECT OF SOY ISOFLAVONE DIETARY SUPPLEMENTATION ON LEVELS OF OXIDATIVE DNA DAMAGE IN BLOOD OF WOMEN.

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Dietary intake of soy has been linked with decreased cancer risk. Some of the active compounds in soy have been identified and include the isoflavones genistein and daidzein. As part of a pilot study to assess the potential benefits of a soy extract, NovaSoy® (Archer Daniels Midland Co.), we examined levels of oxidative DNA damage in blood of six women before and after soy supplementation daily. The NovaSoy® supplement is a soy extract tablet that contains 50 mg of soy isoflavones, chiefly genistein and daidzein in a 1:1 ratio. Blood samples were obtained at weekly intervals for three weeks from the women taking this tablet once daily. DNA was isolated from the blood nuclei and analyzed for levels of 5-hydroxymethyl-2'-deoxyuridine (5-OHmdU). Levels of 5-OHmdU decreased in four of the six women. The subjects who displayed decreased levels tended to have higher baseline levels of 5-OHmdU. Average levels of 5-OHmdU were 93 $\pm$ 36 at baseline and 48 $\pm$ 15 after 3 weeks of intervention (given as mean $\pm$ SD in pg 5-OHmdU/ng thymidine, n=6 in each case). This decrease was noted as soon as 2 weeks after starting on the soy supplement, indicating a fairly rapid decrease in levels of oxidative stress. Since increased levels of 5-OHmdU have been associated with increased breast cancer risk, these results indicate that soy supplementation could potentially be useful to decrease breast cancer risk in women.

957 OXIDATION OF LOW DENSITY LIPOPROTEIN BY HYDROGEN PEROXIDE-ALTERED MYOGLOBIN.

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Oxidized low density lipoprotein (Ox-LDL) is believed to play a significant role in the pathogenesis of atherosclerosis; however, the exact mechanism by which Ox-LDL is formed *in vivo* is not yet known. A variety of biological molecules, including the hemoproteins myoglobin and hemoglobin, have been shown to induce the formation of Ox-LDL *in vitro*. We have discovered, in *in vitro* studies with purified LDL, that hydrogen peroxide-altered myoglobin was approximately 2.5-fold more efficient than native myoglobin (Mb) at oxidation of LDL, as demonstrated by the formation of lipid peroxides, TBARS, and conjugated dienes. The oxidation of LDL appeared to be catalyzed by a peroxide-altered heme group covalently-bound to apomyoglobin (Mb-H). We have also discovered that Mb-H was formed during the reaction of Mb with purified LDL. Therefore, the previously observed capacity of Mb to oxidize LDL may be due, in part, to the generation of Mb-H. These results are consistent with the known redox activity of Mb-H. The formation of covalently-bound heme adducts has been shown to occur *in vivo* with a variety of hemoproteins. Thus, these observations suggest a potential role for oxidatively-modified hemoproteins in the initiation and progression of atherosclerosis. (Supported by BWF New Investigator Award in Toxicology (YO), Training Grant GM07767 (JLV), and ES08365.)

958 H_2O_2 INDUCES HYPERTROPHY AND APOPTOSIS IN RAT CARDIOMYOCYTES.

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Cardiovascular disease is the leading cause of nature death in U.S. Many heart diseases involve cardiomyocyte hypertrophy and cell death. The trigger and mechanism of these two cellular changes are unknown under most pathological conditions. Oxidants are by-products of aerobic metabolism and increase in the heart in many heart diseases associated with ischemia-reperfusion. Using H9C2 and primary cultured rat cardiomyocytes, we test whether H_2O_2 can induce hypertrophy and apoptosis. The majority of cells survived a 2-hr treatment with H_2O_2 up to 250 μM. These cells developed hypertrophy over 6 days judging by increases in cell surface area, protein content per cell and protein to DNA ratio. The enlarged cells showed enhanced actin filaments. Myosin was presented in a filament structure instead of myofibrils. Protein degradation rate showed a transient reduction but increased at 24 hr after H_2O_2 treatment, suggesting that increased protein content was not a result of reduced protein degradation. Cycloheximide prevented cell enlarging, indicating that new protein synthesis was critical for hypertrophy. In contrast, the same treatment, i.e. H_2O_2 =250 μM, 2 hr, caused a maximum of 45% cells to undergo apoptosis over 24 hr. These cells detached, showed nuclear condensation, and activated caspase-3. Thus, our data indicate that H_2O_2 can cause apoptosis in one fraction of the cardiomyocytes and hypertrophy in the other fraction.

959 CARDIOSELECTIVE OXIDATION OF MITOCHONDRIAL DNA FOLLOWING SUBCHRONIC ADMINISTRATION OF DOXORUBICIN.

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Acute intoxication of rats with doxorubicin causes the preferential formation of 8-hydroxydeoxyguanosine (8OHdG) in mitochondrial DNA (mtDNA) compared to nuclear DNA (nDNA) of cardiac tissue (Palmeira et al., 1997). The present investigation was designed to assess the persistence of these adducts and to determine whether they accumulate with repetitive injections of clinically relevant doses of doxorubicin. Adult male Sprague-Dawley rats received weekly intraperitoneal injections of either 2 mg/kg doxorubicin or isotonic NaCl. Selected animals were killed at weekly intervals, the nDNA and mtDNA isolated from both heart and liver, and the concentration of 8OHdG measured by LC/ESI-MS/MS. The concentration of 8OHdG in nDNA and mtDNA from heart or liver of saline-treated rats was less than 5 per 100,000 unmodified deoxyguanosine (dG) bases at all time points examined. However, the abundance increased to $65/10^5$ dG in cardiac mtDNA after 8 weekly injections. The rank-order abundance of 8OHdG adducts was cardiac mtDNA > hepatic mtDNA > cardiac nDNA > hepatic nDNA. Interestingly, the concentration of 8OHdG did not decline in any of the DNA fractions after allowing as much as 3 weeks of drug-free recovery. This preferential oxidation of cardiac mtDNA is consistent with the bioenergetic failure and the cumulative and irreversible cardiomyopathy that limits the clinical utility of this important antineoplastic drug. (Supported in part by HL 58016.)

960 PYRIDOSTIGMINE AND EXERCISE INTERACTION ON CARDIAC ANTIOXIDANT DEFENSE SYSTEM IN MICE.

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This study investigated the interactive effects of pyridostigmine and exercise on cardiac antioxidant defense system in mice. Male NIH Swiss mice were divided into four groups and treated as follows: 1) sedentary control (4 weeks sitting on treadmill belt followed by two weeks saline treatment and 4 weeks sitting on treadmill belt); 2) pyridostigmine [4 weeks sitting on treadmill belt followed by pyridostigmine (1.2 mg/kg, p.o) daily for two weeks and 4 weeks sitting on treadmill belt]; 3) exercise daily for ten weeks (saline treatment only at 5th and 6th week) and 4) pyridostigmine plus exercise for ten weeks. The animals were sacrificed 24 hours after the last treatments, hearts were isolated and analyzed. Pyridostigmine significantly increased cardiac SOD activity (125% of control) while GSH-Px activity

significantly decreased (78% of control). Pyridostigmine depleted cardiac GSH and GSSG concentrations (40% and 45% of control), respectively while lipid peroxidation was increased (116% of control). Exercise significantly decreased cardiac GSH-Px activity (78% of control) and depleted GSH concentration (75% of control). The combination of pyridostigmine and exercise decreased cardiac CAT and GSH-Px activities (56% and 72% of control), GSH concentration and GSH/GSSG ratio (73% and 47% of control), respectively and increased GSSG concentration (133% of control). The data suggest that pyridostigmine and exercise interaction perturb the cardiac antioxidant defense system leading to oxidative stress in mice (Supported by US Army contract # DAMD17-97-C-7066.)

961 COMBINATION OF PHENOLIC COMPOUNDS WITH ARACHIDONIC ACID INDUCES "FUTILE THIOL PUMPING" AND OXIDATIVE STRESS IN EPIDERMAL HUMAN KERATINOCYTES.

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In human epidermis, active metabolism of arachidonic acid to prostaglandins is regulated by prostaglandin H synthase (PHS) which is also known to catalyze xenobiotic biotransformation. In particular, peroxidase activity of PHS may catalyze one-electron oxidation of phenolic compounds to their phenoxyl radicals. The phenoxyl radical may attack intracellular thiols to regenerate the phenolic compound that may repeatedly undergo peroxidase-catalyzed oxidation. This thiol-supported redox-cycling of phenolic compounds (i.e,. "futile thiol pumping") has been demonstrated in keratinocytes exposed to acetaminophen (Mason et al., 1989). n the present work, we tested whether redox-cycling of phenolic compounds can induce peroxidation of membrane phospholipids and deplete antioxidants in keratinocytes. We used a fluorescence-HPLC assay for quantitation of membrane phospholipids metabolically prelabelled with oxidation-sensitive natural fluorescent fatty acid, cis-parinaric acid (PnA). We found that a combination of phenol/arachidonic acid or cresol/arachidonic acid caused a pronounced oxidation of major phospholipids: phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine in keratinocytes. Exposure to phenol (cresol) + arachidonic acid decreased total antioxidant reserves in keratinocytes as measured by luminol-enhanced chemiluminescence in the presence of a water-soluble azo-initiator, AAPH. Measurements with a thiol-reagent, ThioGlo-1™ showed that phenol (cresol) + arachidonic acid depleted GSH and oxidized protein sulfhydryls in keratinocytes. Electron microscopy revealed characteristic apoptotic nuclear fragmentation in keratinocytes exposed to a combination of phenolic compounds with arachidonic acid. These results suggest that peroxidase activity of PHS may be involved in phenol (cresol)-induced "futile thiol pumping" in human epidermal keratinocytes.

962 ROLE OF p53 TUMOR SUPPRESSOR GENE IN THE TOXICITY OF TCDD, ENDRIN, NAPHTHALENE AND CHROMIUM (VI) IN THE LIVER AND BRAIN TISSUES OF MICE.

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It has been postulated that tumor suppressor genes are involved in the cascade of events leading to the toxicity of diverse xenobiotics. Therefore, we have assessed the comparative effects of 0.01 LD_{50}, 0.10 LD_{50} and 0.50 LD_{50} doses of TCDD, endrin (EN), naphthalene (NAP) and chromium (VI) (CrVI) on lipid peroxidation (LP), DNA fragmentation and enhanced production of superoxide anion (cytochrome c reduction) in the liver and brain tissues of TSG-P53 deficient mice in order to determine the role of p53 in the toxic manifestations produced by these diverse xenobiotics and compare these data with C57BL/6J mice. In general, p53 deficient mice are more susceptible to all four xenobiotics. Dose-dependent effects were produced by the four xenobiotics which were studied in both the p53 deficient mice and the C57BL/6J mice. At a 0.50 LD_{50} dose, TCDD, EN, NAP and CrVI induced 2.5-, 2.3-, 3.7- and 3.3-fold greater increases in hepatic LP in p53 deficient mice as compared to the C57BL/6J mice. At this dose in brain tissues 1.8-, 2.4-, 3.0- and 2.1-fold greater increases in LP occurred in p53 deficient mice as compared to the C57BL/6J mice. Similar results were observed with respect to DNA fragmentation and production of superoxide anion. The results support the hypothesis that p53 tumor suppressor gene plays a role in the toxicity of structurally diverse xenobiotics.

963 AUTOFLUORESCENCE IN PRIMARY RAINBOW TROUT HEPATOCYTES INTERFERES WITH MEASUREMENT OF OXIDATIVE ACTIVITY VIA THE EXOGENOUS PROBE, DCF, BUT PROVIDES INTRINSIC MEASURE OF CELLULAR OXIDATIVE STATE.

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The compound 2',7'-dichlorodihydrofluoroscein diacetate (DCF-DA; Molecular Probes) is a probe commonly used to detect oxidative activity in live cells. Studies were undertaken to measure reactive oxygen species generated in freshly isolated rainbow trout hepatocytes exposed to a variety of redox cycling compounds, including 1,4-napthoquinone (NQ) and diquat (DQ). During our studies we found unusually high background levels of fluorescence (Ex: 490nm; Em: 520nm) in control, NQ- and DQ-treated cells loaded with DCF-DA. Similar levels of fluorescence were observed in freshly isolated cells which were not loaded with DCF-DA, indicating that the fluorescence in not due to the oxidized metabolite, DCF. It was determined that the background fluorescence apparent immediately upon isolation correlates with cell density, indicating that it is endogenous autofluorescence. The fluorescence was observed to increase with time in culture up to 24 hours post-isolation, reaching a maximum fluorescence up to an equivalent of 50 pmol DCF. This temporal change in autofluorescence confounds interpretation of measurements of DCF fluorescence as an indicator of oxidative activity. However, the autofluorescence itself may be a useful endogenous indicator of the oxidative state of the cells. Examination of the rainbow trout hepatocyte autofluorescence using multi-photon excitation microscopy indicates that it is consistent with previous reports of autofluorescence of flavoproteins and pyridine nucleotides in mammalian cells. Exploitation of these intrinsic fluorescent molecules will provide a means of monitoring cellular oxidative activity in future mechanistic studies of cellular responses to oxidants in rainbow trout heptocytes.

964 MAITOTOXIN-1 INDUCES LIVER AND PLASMA LIPID PEROXIDATION IN CF-1 MICE TREATED WITH SUBLETHAL ORAL DOSES.

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Human consumption of tropical fish containing polyether marine toxins (e.g. maitotoxins) can cause acute and chronic ciguatera fish poisoning. We tested the hypothesis that oral ingestion of maitotoxin-1 (MTX-1) causes significant increases in liver and plasma lipid peroxidation. Mice (CF-1 strain, n=5/dose/time interval) were gavaged with 0% (control), 0.1%, 1%, 10%, 25%, 50%, 75% of the MTX-1 LD_{50} (130 ng/kg, Calbiochem) and groups were sacrificed after either 3, 6, 24 or 72 h. Analysis was done of liver and plasma lipid peroxidation (HPLC), plasma protein, ALT and AST activities. MTX-1 caused a significant increase ($p<0.01$, 2-way ANOVA) in liver and plasma lipid peroxidation in many animals. This effect was more pronounced in liver than in plasma and varied substantially depending on dose utilized and time interval sampled. In the 3 h group a maximum effect was found; liver lipid peroxidation increased nearly 1800% relative to controls in animals treated with 75% of the LD_{50}. MTX-1 caused an increase of at least 300% in plasma lipid peroxidation in the 3 h group treated with 50% of the LD_{50}. These effects were abolished in animals treated with alpha tocopherol (100 μ M) after MTX-1 exposure. Collectively, these studies suggest that lipid peroxidation is a mechanistic component of acute ciguatera fish poisoning.

964A SHIFT IN FTIR (FOURIER TRANSFORM INFRARED) ABSORPTION SPECTRA OF AMIDE BOND-VIBRATION IN METHYL-CARBAMATE (METHOMYL) EXPOSED RAT SPLEEN CELLS IS RELATED TO DISRUPTION OF CYTOSKELETON.

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Oxidative stress caused by many toxicants to red blood cells can lead to apoptosis and release of lactate delydrogenase enzymes in plasma of exposed rats. We earlier reported that rats exposed to 8 mg/kg methomyl (s-methyl N -[(methylcarbamoyl) oxy] thioacetimidate) showed transient increase of lactate dehydrogerase isozyne (type 4) in plasma and the elevated level which subsided on day 2 after exposure. N-acetyl-cysteine, a free

radical scavenger which promotes glutathione anti-oxidant capacity can block this effect (Lohitnavy et al. 1997). We report a small significant shift in infrared absorption spectra detected by fourier transformed infra-red spectroscopy (Wong, 1995), in spleen cells of rats exposed to 8 mg/kg methomyl. It is known that cysteine groups of ~ tubulin can be cross-linked by a bivalent sulfhydryl reagent and that the cross-link can be inhibited by colchicine (Little and Lu Dena 1985). This result is discussed in terms of mechanism which -SH cysteine agents may alter cell growth and development. Methomyl binding to specific protein in mutant maize which alters cellular oxidative status is also discussed.

965 ORAL INGESTION OF TRICHLOROACETATE IN DRINKING WATER OF RATS AND MICE.

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A physiologically based pharmacokinetic (PBPK) model is under development for oral ingestion of neutralized trichloroacetic acid in drinking water of rats and mice. To develop a drinking water TCA rodent model, previously existing solvent PBPK models that described TCA as a metabolite were used in addition to kinetic data collected from conducting drinking water studies with TCA. Male Fischer 344 rats were placed on drinking water for up to 14 days containing either 2.0, 0.5 or 0.1 g/L TCA, and male B6C3F1 mice were administered drinking water containing either 0.8 or 0.08 g/L TCA. Water consumption rates for rats were 15.1, 19.2 and 19.0 ml/day/rat for the 2.0, and 0.5 and 0.1 g/L dose groups and in mice, 3.8 and 4.2 ml/day/mouse for the 0.8 and 0.08 g/L dose groups. Rats and mice were taken off treatment on Days 3 and 14. In rats, tail vein blood samples were collected immediately after removal from treatment water and then at selected times for up to 6 hours to monitor systemic clearance of TCA. Mice were killed by CO_2 on Days 3 and 14 of treatment, immediately after removal from water containing TCA and then selected times up to 16 hrs after removal from treatment. Blood and liver were removed at each time point for analysis of TCA. Peak blood concentrations of TCA were 4.3, 30.6 and 93.6 ug/ml in rats drinking 0.1, 0.5 and 2.0 g/L of TCA, respectively, and 10.3 and 72.9 ug/ml in mice drinking 0.08 and 0.8 g/L of TCA, respectively. A PBPK model for TCA in rats and mice will be used to calculate dosimetry of TCA after oral ingestion in drinking water.

966 SIMULATION OF TRICHLOROACETIC ACID KINETICS IN THE ISOLATED PERFUSED RAT LIVER USING A BIOLOGICALLY BASED KINETIC MODEL.

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Trichloroacetic acid (TCA) is a contaminant of groundwater. It induces peroxisome proliferation in livers of rats and mice and is hepatocarcinogenic in the latter species. To gain more insight into the mechanistic processes controlling TCA kinetics in the liver: 1) the isolated perfused rat liver (IPRL) was used to experimentally investigate TCA kinetics at two doses, and 2) a biologically based kinetic (BBK) model for the IPRL was used to analyze the experimental data. This BBK model integrates mechanism of protein binding, membrane transport, metabolism and biliary excretion in order to simulate the kinetic behavior of water-soluble chemicals. The IPRL was exposed to 25 and 250 μ M TCA for 2h in a recirculating system. These doses were not cytotoxic as determined by LDH leakage and bile formation. The BBK model simulated the TCA concentration in perfusion medium, the liver concentration at the end of the experiment, and the biliary excretion of TCA. Separate protein binding studies showed that over 90% of TCA was bound to albumin in the perfusion medium whereas binding in liver homogenate was negligible. Integrating the information on protein binding into the BBK model, the hepatic uptake of TCA and its biliary excretion could be fitted assuming active transport and passive diffusion, respectively. Additional experiments using a higher dose of TCA (1000 μ M) were conducted to validate the BBK model. This approach illustrates the usefulness of BBK modeling for analyzing experimental data and gaining insight in mechanisms involved in chemical kinetics.

967 PHARMACOKINETICS AND METABOLISM OF DICHLOROACETATE (DCA) ADMINISTERED IN DRINKING WATER IN RATS AND MICE.

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As a by-product of water chlorination dichloroacetic acid (DCA) is a ubiquitous contaminant of drinking water supplies. DCA is a complete hepatocarcinogen and a tumor promoter in B6C3F1 mice. To describe the pharmacokinetics of orally ingested DCA, male Fischer 344 rats and B6C3F1 mice were given DCA in drinking water at 3 different dose levels (rat – 0.1, 0.5 and 2.0 g/l; mice - 0.08, 0.8 and 2.0 g/l) for 3 or 14 days. Blood and liver samples were collected at selected time points up to 8 hr after being taken off treated drinking water. Cytosol was prepared from the remaining rat and mouse livers for investigation of metabolic competency. At the end of 14 days of treatment the blood DCA concentrations for mice and rats were 18.5 and 73.3 ug/ml, respectively, at the 2.0 g/l dose level. Liver concentrations at the same time point and dose level for mice and rats were 8.3 and 11.6 ug/ml, respectively. At the 0.08 and 0.8 g/l DCA dose level DCA was not measured in mouse blood or liver. Cytosol from control rat liver had a Km of 206 nmol and a Vmax of 3.8 nmol/min/mg protein, with Km and Vmax from the 0.1 g/l dose level being similar. Alteration of metabolic degradation of DCA was substantial at the 0.8 and 2.0 g/L drinking water dose levels in both rats and mice. These results may play an important role in interpreting DCA and drinking water dose-response studies that have been conducted with rodents.

968 INHIBITION OF METABOLISM BY CHLORINATED AND BROMINATED DI-HALOACETATES AND DIFFERENTIAL RECOVERY IN B6C3F1 MICE AND F344 RATS.

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Chloro, bromo and mixed bromo- chloro haloacetates (HAs) are by-products of drinking water disinfection and exhibit both carcinogenic and reproductive/developmental toxic properties in rodents. Previously, dichloro-acetate (DCA) was demonstrated to auto-inhibit its metabolism in mice and rats, with rats being noticeably more sensitive to this effect than mice. Presently, we were interested if other di-haloacetates, bromochloro (BCA) and dibromo (DBA) also caused metabolic inhibition. We pretreated male F344 rats with DCA, BCA or DBA at 2 g/L in their drinking water for 2 weeks. After a 16 hr recovery period, rats were euthanized and hepatic S9 fractions prepared. The rate of metabolism of each HA (measured from the loss of added substrate) was determined at several initial concentrations using S9 prepared from the various di-HA treated rats. The observed data was fitted to the Michaelis-Menten equation using non-linear regression methods to obtain estimates of the apparent K_m and V_{max}. The results indicated that pretreatment with a di-HA caused a substantial decrease in the metabolism all di-HAs. In general, the K_m tended to remain unchanged or increased, while the V_{max} was reduced 50-75 %. In additional experiments, we investigated the recovery time necessary to restore normal metabolic activity in mice and rats following a 2 week pretreatment with DCA at 0.2 or 2 g/L. These experiments used both in-vivo (mice- blood elimination after i.v. injection; rats- CO_2 formation after gavage dosing) and in-vitro methods to quantify metabolic activity. Results showed that mice completely recover from DCA treatments within 48 hours while rats require a considerably longer period of time. These results indicate that rats more closely resemble humans in regards to the persistence of di-HA induced metabolic inhibition. (Supported by EPA grant R825954.)

969 DOSE-DEPENDENT PHARMACOKINETICS OF MONOCHLOROACETIC ACID (MCA) IN ADULT MALE SPRAGUE-DAWLEY RATS.

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This study was conducted to determine the distribution, metabolism and excretion of MCA in male rats at a moderately toxic dose of 10 mg/kg and a highly toxic dose of 75 mg/kg (approximate LD_{50}). Rats were injected with ^{14}C-MCA (2.5-3.0 μCi/animal) into the tail vein and housed individually in

metabolism cages. Urine and feces were collected separately. Animals were euthanized at different time intervals (5, 15, 45 min, 2, 4, 8, and 16 hrs) and a whole array of tissues were collected by gross dissection. Radioactivity in weighed aliquots of tissues was determined by scintillation counting following digestion. The injected radioactivity was very rapidly distributed to tissues leaving 0.1 and 0.6 percent/ml of the dose in blood at 45 minutes after injection of the low and high doses, respectively. Concentrations of MCA in plasma, liver, heart, lungs, and brown fat were parallel to each other, whereas that in brain remained almost constant from 15 min until the end of the experiment (16 hrs). At later time points (2–16 hrs), concentration in thymus was noticeably higher than in other tissues including liver. Very large amounts of radioactivity (47 and 23% of the low and high dose, respectively, at 45 min) were found in the GI tract, almost all of which were reabsorbed. Only a negligible fraction was excreted in the feces (<0.6% of the low and ≈ 2% of the high dose) or recovered in the GI tract (only 0.4 and 1.4 percent of the low and high dose, respectively, at 16 hrs). A very large fraction of both doses was found in urine (73% of the low and 59% of the high dose at 16 hrs). There was no dose proportionality in tissue concentrations of radioactivity.

970 INFLUENCE OF ORAL ADMINISTRATION OF A QUATERNARY MIXTURE OF TRIHALOMETHANES ON THEIR BLOOD KINETICS IN THE RAT.

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Trihalomethanes (THMs) are disinfection by-products in drinking water which are formed by the reaction between chlorine and organic materials (e.g. humic and fulvic acids). THMs are metabolized by cytochrome P-450 that may lead to metabolic interactions. The purpose of this study was to verify the potential metabolic interactions, following oral administration of THMs in quaternary mixture on their blood kinetics in rat. A single dose of 0.25 mmol/kg or 0.5 mmol/kg b.w. of each THM alone, and of a quaternary mixture containing 0.25 mmol/kg of each THM/kg b.w (total dose of 1.0 mmol/kg) were administered by gavage. The venous blood concentrations of the THMs were measured by headspace gas chromatography at several time points (40, 60, 120, 180, 270 and 360 minutes) following administration. Results showed a non-linear relationship between area under the venous blood concentration time curves (AUCs) and doses for individual THMs, which suggests that the metabolism is saturated in this dose range. Administration of THMs in quaternary mixture resulted in higher AUC values by comparison with single administration. The ratios of increase for chloroform, dibromochloromethane, dichlorobromomethane and bromoform were 6.5, 8.1, 4.1 and 2.6, respectively. Additional experiments using binary mixtures of various THMs showed that mutual metabolic interaction occur between THMs. The likelihood of the occurrence of this interaction, at low doses in humans, will be evaluated using mechanism-based kinetic models. (Supported by CNTC.)

971 IN VITRO METABOLISM OF DIAZEPAM BY HORSE LIVER SLICES.

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The information resulting from the use of horse liver slices to determine the metabolism of an unknown drug has potential to be very helpful for laboratories regulating illegal drug use in performance animals. Use of in vitro horse liver slice systems for metabolism studies has not been validated. The drug, diazepam, was selected for in vitro validation because the multiple metabolic pathways - hydroxylation, demethylation and glucuronidation - could be evaluated. Freshly prepared horse liver slices from six horses were incubated with 68 mg of diazepam for 6 hours in a rotating 37°C incubator in Waymouths medium under 95% O2/5 % CO2. Slices and medium were homogenized and analyzed for diazepam and its metabolites by HPLC. Identities and mean (±SD) quantities of the four metabolites produced were 9.4 (± 3.5) nmoles of oxazepam, 14.7 (± 2.5) nmoles of temazepam, 26.2 (± 11.3) nmoles of nordiazepam and 27.7 (± 9.1) nmoles of temazepam glucuronide. Diazepam and the four metabolites accounted for 96.7% of the drug added to the incubation mixture. The in vitro metabolites identified from horse liver slices in this study are consistent with those reported from in vivo studies in the horse.

972 KINETICS OF BROMOSULFOPHTHALEIN CONJUGATION AND BILIARY EXCRETION AT VARYING ALBUMIN CONCENTRATIONS IN THE ISOLATED PERFUSED RAT LIVER.

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A better understanding of the kinetics involved in bromosulfophthalein (BSP) metabolism will enhance its utility as a test compound, and also provide a framework for the kinetic analysis of other chemicals. The biliary excretion of BSP was studied using a recirculating isolated perfused rat liver system. The initial BSP concentration was 20 μ M and BSP clearance was studied for 150 min at 0, 1, or 4% bovine serum albumin (albumin, w/v) in perfusion medium. With this design, different clearance kinetics of BSP were observed without changing the initial total BSP concentration. Bile was collected at 30 min intervals and BSP and its glutathione conjugate were determined by HPLC. The appearance of total BSP in bile reflected its disappearance from perfusion medium. With no albumin present, the concentration of total BSP in bile was >10 mM until BSP was depleted from the perfusion medium, whereas in the presence of albumin the total biliary BSP concentration was lower and more constant throughout the experiment. Regarding the conjugation of BSP, for 0% albumin, the fraction of BSP conjugate in bile was between 70 and 85% at all times. For the 1% albumin case, the percentage of conjugate was initially 62% and rose to 83% by the end of the experiment. For 4% albumin, the conjugation fraction was initially 47% and rose to 61%. The data indicate that BSP conjugation is not saturated even at high BSP uptake rates and provide insight into the relative kinetics of metabolism and excretion of BSP and its conjugates.

973 PRECLINICAL PHARMACOKINETICS AND INTERSPECIES SCALING OF A NOVEL VITRONECTIN RECEPTOR ANTAGONIST.

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Allometric scaling may be used in risk assessment or drug development to predict from animal data the pharmacokinetics of xenobiotics in man. Although allometry may be successful for compounds that are excreted unchanged or are oxidatively metabolized (with corrections for metabolic capacity), it is more challenging for compounds excreted primarily as conjugates in bile. SB-265123 (S-10,11,dihydro-3-[3-(pyridin-2-ylamino)-1-propyloxy]-5H-dibenzo[a,d]cycloheptene-10-acetic acid) is a novel α vβ 3 (vitronectin receptor) antagonist. In this study, the *in vivo* pharmacokinetics and *in vitro* plasma protein binding of SB-265123 were examined in mice, rats, dogs, and monkeys. In monkeys and dogs, SB-265123 exhibited moderate clearance, while low clearance (< 20% hepatic blood flow, Qh) was observed in the rat, and high clearance (> 70% Qh) was seen in the mouse. Visual inspection of the concentration-time profiles indicated the possibility of enterohepatic recirculation; subsequent studies in bile duct-cannulated rats demonstrated extensive biliary excretion of an acyl-glucuronide of SB-265123. Allometric scaling was used to attempt to predict the disposition of SB-265123 in man. Various standard correction factors for protein binding, maximum lifespan potential, or neoteny were employed; each failed to produce adequate scaling of clearance across the animal data set (r2 < 0.88). Thus, a novel correction factor incorporating bile flow and microsomal UDPGT activity in each species was used; this correction substantially improved the interspecies scaling (r2 = 0.96). This study demonstrates the use of allometry in interspecies scaling of compounds that undergo Phase II and Phase III metabolism and excretion, and describes a new correction technique that may improve interspecies scaling of such xenobiotics.

974 ABSENCE OF DIFFERENTIAL SENSITIVITY TO CHOLINESTERASE INHIBITION IN DEVELOPING RATS COMPARED TO DAMS TREATED PERINATALLY WITH CHLORPYRIFOS.

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Pregnant Sprague-Dawley rats were exposed to chlorpyrifos (*O,O*-diethyl-*O*-[3,5,6-trichloro-2-pyridinyl] phosphorothioate; CPF) by gavage (in corn oil) from gestation day (GD) 6 to postnatal day (PND) 10. Dosages to the dams were 0 (control), 0.3 (low), 1.0 (middle) or 5.0 mg/kg/day (high). On GD 20, the blood CPF content in fetuses was about one half to one third the

level found in their dams, indicating about a 2 to 3-fold level of maternal protection. Consistent with maternal:fetus differences in blood CPF, cholinesterase (ChE) inhibition occurred in dams at 0.3 mg/kg/day, but not in fetuses from dams given 1 mg/kg/day. Interestingly, no blood CPF could be measured (limit of detection 0.7 ng/g) in dams given 0.3 mg/kg/day, while fetuses of dams given 1 mg/kg/day had a blood CPF level of about 1.1 ng/g. Thus, based on these data, pups were more tolerant to ChE inhibition than were dams at these dosage levels. Inhibition of ChE occurred at all dosage levels in dams, but only at the high-dosage level in pups. At the high dosage, ChE inhibition was greater in dams than in pups, and the relative degree of inhibition was RBC≈plasma>heart>brain (least inhibited). Pup exposure via milk from dams given 5 mg/kg/day was estimated to be roughly 120 μg/kg/day, which would be sufficient to inhibit blood ChE if pups were extra sensitive. Instead, ChE levels of all tissues of high-dosage pups rapidly returned to near control levels by PND 5, further indicating a lack of differential sensitivity at this dosage level.

975 IN VIVO METABOLISM, ACETYLCHOLINESTERASE INHIBITION, AND TOXICOKINETICS OF ALDICARB IN CHANNEL CATFISH (*ICTALURUS PUNCTATUS*).

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The carbamate pesticide, aldicarb, demonstrates significant acute toxicity in mammals, birds, and fish through the inhibition of acetylcholinesterase (AChE) and may present high potential for exposure of aquatic organisms during periods of runoff. Toxicity studies have shown that channel catfish are approximately 10 times less sensitive to the acute toxic effects of aldicarb than are rainbow trout or bluegill. It is suggested by an earlier *in vitro* study, that the aldicarb resistance may be related to a low level of bioactivation to the potent aldicarb sulfoxide in catfish. The current study examines the plasma kinetics and *in vivo* metabolism of aldicarb in mature catfish in order to further understand this resistance. The dorsal aorta of 4 channel catfish (1 kg) were cannulated, and animals were given a 0.32mg (2 μCi) intravascular dose of ^{14}C-aldicarb. Blood samples were taken at time points ranging from 0.04 h to 48 h. Plasma was removed and analyzed for total plasma radioactivity, AChE activity, and parent:metabolite ratio. HPLC analysis showed the occurrence of a single polar metabolite (not aldicarb sulfoxide) which accounted for 100% of the total radioactivity by 12 h. The 2-compartment kinetics of total aldicarb equivalents displayed a rapid distribution phase ($t_{1/2}$ = 0.14 h) followed by a prolonged elimination phase ($t_{1/2}$ = 27.82 h) of similar duration to trout; whereas the 2-compartment model of parent compound gave α and β half-lives of 0.044 h and 1.67 h respectively. Inhibition of plasma AChE mirrored the profile of total ^{14}C, with maximal inhibition at 2.5 min, and return to control activity by 48 hours. These data support the hypothesis that a low rate of aldicarb bioactivation is a significant contributor to aldicarb resistance in catfish.

976 PHARMACOKINETICS OF DANTROLENE IN THE HEN: FOR TREATMENT OF ORGANOPHOSPHORUS CHEMICAL EXPOSURE.

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The hen is the model of choice for the investigation of organophosphorus-induced neuropathy. A proposed therapeutic intervention is the blocking of neuronal intracellular calcium release with dantrolene. Repeated drug administration can be achieved more efficiently and with less trauma to the animals using the oral route as opposed to intravenous administration. This report describes data after giving dantrolene orally at 25 mg/kg. Oral doses were administered via gavage as dantrolene sodium dissolved in propylene glycol. Blood samples were taken at selected time intervals (0.5, 1, 2, 4, 6, 8, 12, 24 hrs), extracted, and analyzed via high performance liquid chromatography (HPLC) for dantrolene. The data were then applied to model pharmacokinetic parameters (WinNonlin). Blood levels of dantrolene sodium exhibited considerable variability after oral administration (data given as mean ± standard deviation [SD]). Peak blood concentration (Cmax) was 29.34 ug/mL (SD = ±7.14) at 4.7 hrs (Tmax) and declined to 18.1 ug/mL (SD = ±9.83) at 23.4 hrs. The area under the curve (AUC 0-24 hrs) was 517 mg-hr/L. The estimated apparent oral clearance (CL/F) was 67.37 mL/hr. Though the average time course for absorption is similar to that expected from other animal studies, the results suggest a best fit for a two-compartment model with a long terminal elimination phase not previously reported.

977 DISPOSITION OF 3-CHLORO-4-(DICHLOROMETHYL)-5-HYDROXY-2(5H)-FURANONE (MX) IN B6C3F1 MICE.

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MX is a potent mutagen formed as a by-product of chlorination of drinking water, particularly where the water contains humic matter. MX has been estimated to account for 50% of the mutagenic activity in some drinking water. A recent bioassay in rats demonstrated an increased tumor incidence, primarily in liver and thyroid. An NTP study in mice is underway to complement the rat study. A distribution/metabolism study of MX in mice is being conducted in support of this study. MX is rapidly absorbed in mice when administered orally and is excreted in urine and feces as several polar metabolites. At doses from 0.2 to 20 mg/kg about 50 and 42% are excreted in urine and feces, respectively, in 72 hours. Most excretion occurs in the first 24 hours. Tissue/blood ratios are highest in forestomach (> 100), glandular stomach, intestine, and kidney, in that order. After iv administration of 0.2 mg/kg 72 h excretion in urine and feces accounts for 73 and 18 % of the dose. Intravenous administration results in high, prolonged levels of radioactivity in blood compared to oral dosing. MX disposition appears to be dominated by its chemical reactivity with highest concentrations of radioactivity being found at the site of administration.

978 THE USE OF A VALIDATED METHOD FOR THE ANALYSIS OF 3,3',4,4',5-PENTACHLOROBIPHENYL (PCB-126) IN PRELIMINARY TOXICOKINETIC RAT SAMPLES.

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A gas chromatography mass spectrometry method was developed to analyze tissue samples for a preliminary toxicokinetic study, which provided information to set doses, time points and limits of quantification (LOQ) for the definitive toxicokinetic study. This method will also be used in the National Toxicology Program's (NTP) chronic toxicity studies that comprise the Dioxin Toxicity Equivalency Factor Initiative. Female Harlan Sprague-Dawley rats were given a single gavage administration of PCB-126 at a dose of 3 or 3000 ng/kg and a dose volume of 2.5 or 5 mL/kg. The vehicle was corn oil that contained 1% acetone. Tissue samples were taken from a single animal per time point at 0.5, 2, 3, 5, 7, 11, 16, 21, 31, 41, 56, 71, 91 and 119 days after administration. Serum concentrations (ranged from 12 to 129 pg/mL; 3000 ng/kg dose) were 2 to 3 times higher than blood for a given time point and dose. Half-life values for blood and serum ranged from 95 to 112 days. Liver concentrations ranged from 34 to 94 pg/g (3 ng/kg) and 4.2 to 27.3 μ g/g (3000 ng/kg); lung concentrations ranged from 2.4 to 4.4 pg/g (3 ng/kg) and 152 to 476 pg/g (3000 ng/kg); and fat concentrations ranged from 56.7 to 190 pg/g (3 ng/kg) and 3.3 to 14.0 μ g/g (3000 ng/kg). The lower LOQ values were 5 pg/mL (or pg/g) in blood, serum or lung and 10 pg/g in liver or fat. The lower LOQ is limited by environmental factors such as the presence of small amounts of PCB-126 in the feed, vehicle, bedding and process carry over. (This work was supported by the NTP Chemistry Support Services Contract N01-ES-55395.)

979 THE USE OF A VALIDATED METHOD FOR THE ANALYSIS OF 2,3,4,7,8-PENTACHLOROCIBENZOFURAN (PeCDF) IN PRELIMINARY TOXICOKINETIC RAT SAMPLES.

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A gas chromatography mass spectrometry method was developed to analyze tissue samples for a preliminary toxicokinetic study, which provided information to set doses, time points and limits of quantitation (LOQ) for the definitive toxicokinetic study. This method will also be used in the National Toxicology Program's (NTP) chronic toxicity studies that comprise the Dioxin Toxicity Equivalency Factor Initiative. Female Harlan Sprague-Dawley rats were given a single gavage administration at a dose of 10 or 1000 ng/kg and a dose volume of 2.5 or 5 mL/kg. The vehicle was corn oil that contained 1% acetone. Tissue samples were taken from a single animal per time point at 0.5, 2, 3, 5, 7, 11, 16, 21, 31, 41, 56, 71, 91 and 119 days

after administration. Serum concentrations (ranged from 2.1 to 29.2 pg/mL; 1000 ng/kg dose) were 2 to 3 times higher than blood for a given time point and dose. Half-life values for blood and serum ranged from 106 to 166 days. Liver concentrations ranged from 118 to 210 pg/g (10 ng/kg) and 11.2 to 19.5 μ g/g (1000 ng/kg); lung concentrations ranged from 28.9 to 68.5 pg/g (1000 ng/kg); and fat concentrations ranged from 6.9 to 22.4 pg/g (10 ng/kg) and 0.5 to 0.9 μ g/g (1000 ng/kg). The lower LOQ values were 5 pg/mL (or pg/g) in blood, serum or lung, 10 pg/g in fat and 50 pg/g in liver. The lower LOQ is limited by environmental factors such as the presence of small amounts of PeCDF in the feed, vehicle, bedding and process carry over. (This work was supported by the NTP Chemistry Support Services Contract N01-ES-55395.)

980 COMPARATIVE PHARMACOKINETICS OF SARAFLOXACIN IN RAINBOW TROUT AND CHANNEL CATFISH.

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Sarafloxacin HCl ([6-fluoro-1-(4-fluorophenyl)-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid hydrochloride) is a broad spectrum fluoroquinolone potentially effective in controlling a number of bacterial infections in fish. Of the aquaculture therapeutants that are under consideration for approvals by the U.S. Food and Drug Administration, sarafloxacin, a flouroquinolone antibacterial is an example of an orally administered drug that would be readily metabolized, and therefore a model compound to study the concept of crop grouping in fish. The concept hypothesizes that species of fish can be grouped based on defining characteristics such as activity level, phylogeny, and temperature preference. As an initial test of the crop grouping concept, the similarities and differences in the pharmacokinetics of sarafloxacin after a single intraarterial dose of 5 mg/kg were tested in two surrogate fish species (rainbow trout and channel catfish) that differ greatly in group defining characteristics. The mean apparent volume of distribution of individual trout was approximately 10 L/kg and 4 L/kg for catfish while total body clearance was 0.05 L/kg/h for trout and 0.17 L/kg/h for catfish. Rainbow trout had an extended terminal elimination with a half-life of approximately 158 h, compared to 18 h for channel catfish. The results indicate that the two surrogate fish species differ dramatically in their ability to distribute, store, and eliminate sarafloxacin.

981 EFFECT OF OCTANOL:WATER PARTITION COEFFICIENTS OF ORGANOPHOSPHORUS COMPOUNDS ON BIODISTRIBUTION AND PERCUTANEOUS TOXICITY.

S E Czerwinski, J P Skvorak, D M Maxwell, and D E Lenz. *US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, USA.* Sponsor: J A Romano, Jr.

In amplifying our understanding of the pharmacokinetic and pharmacodistribution properties of chemical warfare agents (CWA), knowledge of partition coefficient (log P) data can play a critical role. Using a recently published gas chromatographic method, the octanol:water log P for the CWA tabun, sarin, soman, GF, and VX were determined to be 0.384 ± 0.033, 0.299 ± 0.016, 1.824 ± 0.109, 1.038 ± 0.055, and 0.675 ± 0.070 respectively. Based on these data, the theoretical fluorophosphonate fragment value was determined to be -2.256 ± 0.273. There is a well established inverse relationship between log P and the rate of percutaneous penetration. In this study, using the time to onset of local fasciculations as an indicator of the percutaneous penetration rate, guinea pigs exposed to equitoxic doses of either soman or VX showed signs of intoxication at times (6.3 and 29.0 minutes respectively) inversely proportional to the derived log P values (1.824 and 0.675 respectively). Equilibrium tissue:blood log P may be estimated from octanol:water log P by assuming a direct correspondence between octanol:water partitioning and the partitioning between the lipid and aqueous phases of biological samples. Comparison of the estimated and directly measured tissue:blood log P revealed a correlation of 0.98 for soman in liver, muscle, and adipose tissue. Our results demonstrate that log P data can be used to both predict rates of absorption or determine the biodistribution of CWA in selected tissues. This will help us to identify possible therapeutic compounds and treatments for CWA exposure and investigate potential interactions between multiple therapeutic regimens in various mammalian species.

982 CROSS-SPECIES PHARMACOKINETIC COMPARISON OF AN ICAM-1 ANTISENSE OLIGONUCLEOTIDE, ISIS 2302, FROM MOUSE TO MAN.

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ISIS 2302 is a 20-mer phosphorothioate antisense oligodeoxynucleotide (P=S ODN) targeted to the 3' UTR of human ICAM-1 mRNA. This compound has been studied in mouse, rat, rabbit, dog, and monkeys. ISIS 2302 is currently being developed as an anti-inflammatory therapeutic. The pharmacokinetics and metabolism of ISIS 2302 are remarkably similar across this broad range of species. Following IV dosing, the pharmacokinetics of ISIS 2302 are characterized by rapid distribution ($t_{1/2}$ = 30-60 minutes) to a wide variety of tissues followed by slow metabolism and elimination from these tissues. Plasma clearance (Cl) and steady-state volume of distribution (V_{ss}) are both related across species by body weight, not surface area. This relationship suggests that the plasma disposition is driven primarily by distribution to tissues. In fact, metabolism and urine excretion in plasma are minor contributors to plasma clearance of ISIS 2302. The plasma disposition half-lives and metabolism rate constants are similar between species and allow for direct prediction of plasma exposure in human patients. Finally, the pattern of distribution to tissues is similar across species. The similarity between the animal and human plasma pharmacokinetics give confidence in the predictions of exposure to human tissues derived from preclinical models.

983 4-METHYLIMIDAZOLE TOXICOKINETIC STUDY USING F344 RATS AND B6C3F$_1$ MICE.

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Male and female F344 rats and B6C3F$_1$ mice were given a single intravenous (10 mg/kg BW) or gavage (10, 50 or 100 mg/kg BW) administration of 4-methylimidazole (4-MI), using 0.05 M phosphate buffered saline as the vehicle. Dosing volumes were 2 (rats) and 4 (mice) mL/kg for IV administration; 5 (rats) and 10 (mice) mL/kg for gavage administration. Animals were 14± 2 weeks of age when dosed. Serial blood samples were collected at specified time points after dosing (range 2 min to 24 hours). The plasma was analyzed for 4-MI using a validated gas chromatography method, which utilized a nitrogen phosphorus detector. The limit of quantitation was 1 μ g/mL. The IV plasma concentration time profiles were monophasic for the rats and mice. The $Conc_{(2\ min)}$, V_d and Cl values were ~ 11 μ g/mL, 750 mL/kg and 700 mL/hr, respectively, for the rats and 7.5 μ g/mL,1575 mL/kg and 2600 mL/hr, respectively, for the mice. After gavage administration, the C_{max} values for the 10, 50 or 100 mg/kg groups were 4.2, 11.9 and 20.5 μ g/mL (male rats); 6.6, 15.3 and 20.2 μ g/mL (female rats); 3.9, 18.6 and 30.2 μ g/mL (male mice) and 3.6, 18.7 and 30.2 μ g/mL (female mice), respectively. Oral doses of 50 and 100 mg/kg resulted in non-linear kinetics. (Chemistry Support Services Contract No. N01-ES-55395.)

984 PLASMA PROTEIN BINDING OF 2,4-DICHLOROPHENOXYACETIC ACID (2,4-D) AND 2-METHYL-4-CHLOROPHENOXYACETIC ACID (MCPA) IN THE DOG.

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The purpose of this study was to determine plasma protein binding of 2,4-D and MCPA in dogs. Determinations of protein binding of 2,4-D and MCPA were performed by ultracentrifugation of freshly obtained pooled beagle plasma supplemented with 42.5 - 574 μg/mL 2,4-D or 45.0 - 515 μg/mL MCPA. Total 2,4-D and MCPA plasma concentrations and unbound 2,4-D and MCPA ultrafiltrate concentrations were determined by HPLC with fluorescence detection. The percent of unbound 2,4-D was 4 % and 27 % at total 2,4-D concentrations of 48.2 μg/mL and 574 μg/mL, respectively. Determinations of the percent unbound of MCPA resulted in similar findings with the percent unbound of MCPA being 3 % and 29 % at total MCPA concentrations of 45.0 μg/mL and 515 μg/mL respectively. Non-linear regression of the hyperbolic curve of bound 2,4-D versus free 2,4-D, and bound MCPA versus free MCPA were best fit as 2 classes of binding sites. From the fit of these data, the sites were determined to be a low capacity, high

affinity binding site, and a high capacity, low affinity binding site. Protein binding of 2,4-D was also determined in plasma supplemented with 2,4-D + MCPA. At a plasma concentration of 50 µg 2,4-D/mL, fraction of unbound 2,4-D was 4% after no supplementation of MCPA and increased to 16% after supplementation of 200 µg MCPA/mL. When the plasma concentration of 2,4-D was 400 mg/mL, the fraction of unbound 2,4-D increased from 21% to 31% after co-supplementation of zero or 200 µg MCPA/mL.

985 MEASURING BIOAVAILABILITY OF DIESEL SOOT-ADSORBED BENZO(A)PYRENE IN DOGS FOLLOWING A SINGLE-BREATH INHALATION EXPOSURE.

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Exposure to inhaled diesel soot is suspected to increase the risk of lung cancer in humans. Whether it is the carbonaceous core of the diesel particle or its coat of adsorbed organics that poses the greater lung cancer risk is a matter of controversy. The extent to which carcinogenic hydrocarbons on diesel soot are bioavailable in the lungs is also disputed. Here we report the alveolar absorption in the Beagle dog of tritiated benzo(a)pyrene (BaP) adsorbed on the carbonaceous core of diesel soot particles. The concentration of BaP on the soot was 0.015 g/g, which corresponds to 25% of a monomolecular coating of BaP on the soot surface. A novel method was used to expose the dogs to a bolus of resuspended diesel soot in a single breath. Following inhalation into the deep lung, the appearance of BaP in the systemic circulation was monitored by repeatedly sampling blood from the ascending aorta and the posterior vena cava. The particle-adsorbed BaP falls into two categories in terms of bioavailability: an instantaneously available fraction desorbed quickly from the soot particles upon deposition in the lungs; a second—either slowly desorbing or permanently adsorbed— fraction gave a low dose rate to surrounding lung tissues. The findings suggest that the rapidly desorbed fraction in the lungs poses the greater lung cancer risk. (Research sponsored by HEI Grant EPI 1351901, and the Swedish Council for Working Life Research, Grant 95-0192 in facilities provided by the U.S. DOE/OHER under Cooperative Agreement DE-FC04-96AL76406.)

986 BUTADIENE RESOLVED CHIRAL EPOXIDES: REACTIONS WITH DNA.

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Butadiene (BD), a major environmental/occupational toxicant, is both mutagenic and carcinogenic in rodents and is classified by IARC as a Group 2A carcinogen. BD is metabolized to the reactive epoxides butadiene monoepoxide (BO) and butadiene diepoxide (BDO_2). Each epoxidation of BD introduces an asymmetric carbon, thus producing five possible stereoisomers: (S)-BO, (R)-BO, (2S,2S')-BDO_2, (2R,2R')-BDO_2, and diastereomeric *meso* BDO_2. Stereoisomers of chiral monofunctional alkylating agents like BO react with DNA to form diastereomeric adducts. Each diastereomeric adduct is a distinct chemical species that will be both formed and repaired at a unique rate. In addition, if the diastereomeric adduct contains a second chiral electrophilic group, as with BDO_2, each second group also will have a unique reaction rate with chiral nucleophiles. To examine the role of stereochemistry in BD-DNA adduct formation, we have synthesized, purified and characterized (S)-BO, meso-BDO_2 and (2R,2R')-BDO_2. Purity was determined by boiling points, GC/MS, and/or NMR analysis. Enantiomeric purity was determined by polarography and/or circular dichroism. The rate of reaction between butadiene-epoxide stereoisomers and DNA was determined using an isotope-dilution, multi-dimensional GC/GC/MS method. Initial results suggest that the half-life of the racemic BDO_2-DNA reaction is approximately 17 hr and follows first-order reaction kinetics. Further studies will determine the effect BD-epoxide stereochemistry has upon DNA interactions. (Supported by NIH Grant 1 F32 ES05836-01 from NIEHS and U.S. DOE cooperative agreement No. DE-FC04-96AL-76406.)

987 COMPARATIVE DI-(2-ETHYLHEXYL)PHTHALATE (DEHP) TOXICOKINETIC PROFILE IN FEMALE WISTAR RATS AND CD1 MICE.

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Di-(2-ethylhexyl) phthalate is extensively used as plasticiser for PVC. Developmental toxicity studies have shown that mice are more sensitive than rats. Mono-(2-ethylhexyl)phthalate (MEHP), the major DEHP-metabolite is excreted in the urine of mice but not in rats. Furthermore, glucuronidation phase-2 pathway is not involved in rats for the metabolism of MEHP. In this study, female mice and rats have received DEHP by oral gavage at the dose levels of 200 or 1000 mg/kg bw, either as a single dose of [^{14}C]-DEHP, or as a 5-day pre-treatment with unlabelled-DEHP followed by a single dose of [^{14}C]-DEHP on the 6th day. Blood was collected at defined time interval, and analysed quantitatively for ^{14}C-radioactivity. The major difference observed is a much higher level of ^{14}C-radioactivity in mice than in rats during the blood peak time (1-4 hours after administration) after a single administration at 200 and 1000 mg/kg, and after a repeated administration at 200 mg/kg. ^{14}C-radioactivity levels are quite similar after repeated administration at 1000 mg/kg. Repeated treatment have no effect in rats at both dose levels on the ^{14}C-blood levels compared to a single administration, and in mice at 200 mg/kg. In contrast, ^{14}C blood level is significantly decreased in mice after repeated administration of 1000 mg/kg. However, DEHP was toxic at that dose level. In conclusion, higher blood ^{14}C-radioactivity levels were observed in mice than in rats, after single or repeated oral administration of non-toxic DEHP dose levels.

988 COMPARATIVE DI-(2-ETHYLHEXYL)PHTHALATE (DEHP) TOXICOKINETIC PROFILE IN PREGNANT WISTAR RATS AND CD1 MICE.

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A "sister" study have highlighted some significant toxicokinetic differences between female rats and mice after single or repeated oral administration of DEHP. In the present study, pregnant rats or mice received DEHP by oral gavage at the dose levels of 200 or 1000 mg/kg bw, either as a single dose of [^{14}C]-DEHP on gestation day (GD) 7, or as a 5-day pre-treatment with unlabelled-DEHP followed by a single dose of [^{14}C]-DEHP on GD11. Blood was collected at defined time interval, and analysed qualitatively and quantitatively for ^{14}C-radioactivity and DEHP-metabolites. The concentration in ^{14}C-radioactivity during the peak time (1-4 hours after administration) was significantly higher in mice than in rats only after a single dose of 200 mg/kg. In the other cases, ^{14}C-radioactivity blood levels were comparable between the species. At 1000 mg/kg, repeated administration increased the ^{14}C-radioactivity blood level at 1000 mg/kg in both species compared to a single treatment. GC analysis have demonstrated that in all cases the majority of ^{14}C-radioactivity was associated with MEHP. After a single dose, MEHP peak blood levels were higher in mice than in rats. After a repeated administration, MEHP peak blood levels were comparable in both species at 200 mg/kg, but lower in mice than in rats at 1000 mg/kg. In both species, after a single administration of 1000 mg/kg and to a less extend at 200 mg/kg, a significant part of the blood ^{14}C-radioactivity comes from non-hydrolysed DEHP. After repeated administration the contribution of non-hydrolysed DEHP to ^{14}C-radioactivity level is minimal. In conclusion, the administration of high dose levels of DEHP affects significantly its toxicokinetic in pregnant mice and rats.

989 ELIMINATION AND DISTRIBUTION OF TETRYL FOLLOWING SUBCUTANEOUS ADMINISTRATION IN THE MALE SPRAGUE DAWLEY RAT.

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Tetryl is a booster explosive used in the production of detonators and blasting caps, and exposure to tetryl has been associated with numerous toxicities in humans such as dermatitis, respiratory irritation, gastric disorders, neuro-

logical disorders, anemia, and possibly, bone marrow suppression. While tetryl is no longer produced in the United States, large stores of tetryl are present in areas, which were once sites of tetryl production and use, such as, army depots, ammunition plants, and proving grounds. Currently, there are twelve sites on the National Priorities List, which are contaminated, with tetryl. Environmental exposure to these sites and potential threat to human health has yet to be determined. At present, there is very little information concerning the distribution and elimination of tetryl. To study the distribution and elimination, of tetryl, Sprague Dawley rats were administered tetryl subcutaneously at doses of 25, 100, and 300 mg/kg, which contained 14C-tetryl as a marker. The animals were placed in metabolism cages, and at twenty-four hours, urine, feces, blood, and various organs were collected and radioactivity was determined. The percent dose eliminated in the urine at 25, 100, and 300 mg/kg was 10.02 ± 2.48, 10.3 ± 1.66, and 13.24 ± 5.79 respectively

990 PHARMACOKINETICS OF RECOMBINANT HUMAN RELAXIN IN RATS: EFFECTS OF INDUCED ANTIBODIES.

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Sponsor: R Hill.

Relaxin (Rlx), a hormone of pregnancy in most mammalian species, is a 6 kD protein member of the insulin family. The pharmacokinetics (PK) of recombinant human Rlx (rhRlx), an investigational therapeutic, have been described previously in intravenously dosed naïve animals. However, the effects of anti-rhRlx antibodies (Ab) on serum rhRlx levels and PK parameters were not known. To study this, high titers of serum anti-rhRlx Ab were pre-generated in young adult female Sprague-Dawley rats (Ab-positive). Rlx naïve and Ab-positive rats received either 1 or 5 daily bolus subcutaneous doses of rhRlx, at 0.1, 0.5 or 1.0 mg/kg/day (N=4 per dose level). Blood was sampled at –0.5, 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 24 hours. Serum rhRlx levels and anti-rhRlx Ab titers were measured by ELISA. Data underwent non-compartmental analysis. Following a single bolus dose (1.0 mg/kg) to naïve rats, the maximal concentration (C_{max}) and area under the curve (AUC) were 1288 ng/mL and 3507 ng*h/mL, respectively. The time at which the highest concentration was observed (T_{max}) and the β-phase elimination half-life ($T_{1/2\beta:4-10\ h}$) were 60 and 52.5 minutes, respectively. The residual serum rhRlx level at 24 hours (C_{24}) was 0.33 ng/mL. A dose-proportional relationship was found in both C_{max} and AUC. In naive rats, there were minimal differences between single and repeat-dose groups. By comparison, in Ab-positive rats, a single bolus resulted in a reduced C_{max} (40%), increased AUC (10%), doubled T_{max} (120 min), while the C_{24} was 43 ng/mL, or about 100 times higher. However, repeat dosing in Ab-positive rats increased C_{max} (107%), AUC (270%), but not T_{max} values, over the repeat-dose, naïve group. $T_{1/2\beta}$ was extended by about 2.5 times, consistent with increased binding capacity for rhRlx. In conclusion, prolonged treatment of rats with rhRlx, like other recombinant human proteins, generates Ab that affect clearance and thus potentially, exposure. Rat anti-rhRlx Ab caused an increase in serum rhRlx levels and delayed clearance, especially following repeat dosing.

991 TOXICOKINETICS OF FORMAMIDE IN RODENTS.

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Sponsor: M L Cunningham.

The pharmacokinetics of formamide (FM) were assessed after a single i.v. or single oral administration to rats (Fischer 344) and mice ($B_6C_3F_1$) at 3 dose levels/route/species. Pharmacokinetic (PK) parameters, including dose normalized area under the concentration time curve (DNAUCs), peak plasma levels (C_{max}), and clearance (Cl), determined for rats after an i.v. dose suggest linear kinetics over the dose range. FM was rapidly absorbed into the systemic circulation after oral dosing to rats, with C_{max} determined at 1 to 2 hr. DNAUCs and Cl were comparable between sex and dose level. These data suggest linear kinetics after oral administration of FM over the dose range. The estimate of absolute bioavailability (F) for rats was greater than 1 at all dose levels. This observation, unexpected due to the apparent linear kinetics, may result from route-dependent first pass lung clearance, or route-dependent enterohepatic cycling, or from intergroup variability in the elimination half-life ($t_{1/2}$) which may have resulted in an overestimation of the AUC and ultimately F. F-values of greater than 1 were also observed in mice. However, the PK behavior of FM in mice appeared different than in rats. Trends in route dependent changes in parameters were not clear for mice. There was rapid absorption into the systemic circulation following oral administration with C_{max} occurring within 1 hour. Although Cl in mice appeared to remain constant at the different oral doses, DNAUCs, $t_{1/2}$ and mean residence time increased with increasing dose. Comparison of i.v. and oral data suggested a dose dependent decrease in Cl. The PK data obtained for mice are indicative of the saturation of metabolism (Michaelis-Menten type). If observed decreases in the elimination rate constant and Cl are real, the system is not linear. This is consistent with the observed increase in DNAUCs for the oral route, since a decrease in elimination capacity will result in a higher AUC for a given dose. In conclusion, FM is rapidly and completely absorbed after oral administration to rats and mice. Estimated parameters suggest a linear kinetic system for this compound in rats. PK data obtained for mice are suggestive of the saturation of metabolism, with evidence of a nonlinear kinetic system for this species.

992 BRANCHIAL ELIMINATION OF SUPERHYDROPHOBIC ORGANIC CHEMICALS BY FISH: DEPENDENCE ON CHEMICAL LOG K_{ow}.

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The development of PBTK models for fish has been slowed by a lack of understanding of factors that control branchial elimination of superhydrophobic compounds. Branchial efflux of high log K_{ow} compounds is difficult to measure directly because chemical concentrations in expired water are extremely low. A method was therefore developed based on the extraction of large volumes of expired water by Ambersorb® resin followed by high-resolution GC/MS analysis of toluene extracts using an isotope dilution technique. Data for a model PCB (2,2',5,5'-tetrachlorobiphenyl; tetraCB, log K_{ow} = 5.84) were reported previously (The Toxicologist, 42:1-S, no. 1039). In this poster we present branchial efflux data for two additional PCBs, 2,2',3,3',5,5',6,6'-octachlorobiphenyl (octaCB, log K_{ow} = 7.24) and decachlorobiphenyl (decaCB, log K_{ow} = 8.18). OctaCB and decaCB were present in water at low (< 1 ppt) but measurable concentrations. These data were converted to apparent blood/water partition coefficients (K_{bw}) based on measured residues in blood. Estimated K_{bw} values for octaCB and decaCB were 228,000 and 630,000, respectively. A regression of partitioning data for the three PCBs against chemical log K_{ow} yielded a log-linear equation: log K_{bw} = 0.72 log K_{ow} + 0.02 (r^2 = 0.98). The slope and intercept terms in this equation do not differ significantly from those of an equation developed previously using less hydrophobic compounds (log K_{bw} = 0.63 log K_{ow} - .58; calculated using blood data for rainbow trout). It is suggested, therefore, that branchial elimination of high log K_{ow} compounds proceeds to an equilibrium between afferent blood and expired respiratory water, as assumed in current models for branchial flux at fish gills.

993 DERMAL PHARMACOKINETICS OF METHYL TERTIARY BUTYL ETHER (MTBE) AND TERTIARY BUTYL ALCOHOL (TBA) IN HUMAN VOLUNTEERS.

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The extensive use MTBE as a fuel additive has resulted in its contamination of water sources from run off, spills, and leaks from below ground sources. As a result of such contamination drinking water wells have been closed or remediated. MTBE contamination of domestic has raised the probability that it will result in dermal exposure. Dermal pharmacokinetics of MTBE is important to assess this risk. To that end, one arm of five males subjects was exposed to MTBE in an aqueous exposure system for one hr at a water temperature of 40°C. Each subject received one concentration of MTBE (30, 45, 60, 75, or 90 mg/L). Fourteen blood samples were obtained from each subject at intervals during exposure and up to 24 hrs post-exposure. The objective was to determine the uptake and elimination of MTBE and the elimination of a metabolite, TBA. The basic results indicated that, after a lag of about 15 min, MTBE appeared in the blood, rose rapidly, and was rapidly eliminated. The MTBE blood elimination data were well-described by a double exponential fit. The half life ranged from about 40 min to 2 hr for a first compartment and about 5 to 6 hrs for a second compartment. TBA slowly increased during and after the exposure and was slowly eliminated approaching baseline after 24 hrs. This is an abstract of a proposed presentation and does not reflect EPA policy.

994 TOXICOKINETICS OF METHYL-TERTIARY BUTYL ETHER INHALED ALONE AND IN COMBINATION WITH GASOLINE.

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Methyl-tertiary butyl ether (MTBE) is the most commonly used fuel oxygenate added to gasoline to decrease carbon monoxide and hydrocarbon emissions. Little is known of the toxicokinetics of MTBE inhaled over a range of concentrations relevant to human exposure or the effects of co-exposure to gasoline vapors on its toxicokinetics. The objectives of this study were to compare the toxicokinetics and metabolism of MTBE in rats inhaling 4, 40, or 400 ppm MTBE and to evaluate the effects of acute and repeatedly inhaled gasoline on the toxicokinetics and metabolism of 40 ppm MTBE. Groups of 51 male F344/Crl rats, 8-10 wk old, were acutely exposed nose-only for 4 hr to 4, 40, or 400 ppm ^{14}C-MTBE or to 200 ppm gasoline containing 40 ppm MTBE. A group of 51 male rats was similarly exposed for 7 consecutive days to 200 ppm gasoline containing 40 ppm MTBE, followed by an 8th day of exposure to 200 ppm gasoline containing ^{14}C-MTBE. Separate groups of five rats each were designated for determination of: 1) respiratory minute volumes; 2) initial MTBE body burden; and 3) rates of excretion and identification of metabolites in breath, urine, and feces up to 72 hr post exposure. The remaining rats were sacrificed after 0.5, 1, 2, and 4 hr of exposure and at selected time points after exposure for determination of tissue uptake, metabolism, and clearance. Concentrations of MTBE equivalents in liver, lung, kidney, testes, and brain were highest at the end of the 4-hr exposure. Uptake and retention of MTBE equivalents in these tissues were greatest for rats inhaling 4 ppm MTBE and least for rats inhaling 400 ppm. MTBE and/or its metabolites were chiefly excreted in breath and urine. The single co-exposure to gasoline vapor had little effect on the uptake, tissue clearance, and excretion of 40 ppm MTBE. Repeated exposure to gasoline in combination with 40 ppm MTBE altered the tissue uptake and clearance of MTBE and enhanced excretion of MTBE metabolites in urine. These results suggest that the tissue uptake and retention are dependent on MTBE concentration and that repeated inhalation of MTBE in gasoline may alter its toxicokinetics. (Research funded under DOE Cooperative Agreement No. DE-FC04-96AL76406.)

995 STUDIES OF THE INTESTINAL PERMEABILITIES OF HYDROCARBONS IN THE RAT.

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Petroleum products are complex mixtures of hydrocarbons. Understanding the absorption and distribution of individual hydrocarbons constituting these mixtures will aid in assessing the potential exposure to petroleum products. n-Nonane, an aliphatic hydrocarbon found as a component of some petroleum products, has demonstrated significant distribution to the rat brain after inhalation. However, little is known of the absorption and distribution associated with the ingestion of n-nonane. The purpose of the present study is to determine the intestinal permeabilities of n-nonane and isomers of n-nonane using the single pass intestinal perfusion (SPIP) procedure. Age matched male and female Sprague Dawley rats and Fisher 344 rats were perfused with either [^{14}C]-n-nonane or [^{14}C]-1,2,4-trimethylcyclohexane in an isoosmotic MES buffer. [^{3}H]-Polyethylene glycol 3350 was used as a nonabsorbable marker for the purpose of assessing net intestinal water transport. Outlet samples were collected and analyzed by liquid scintillation counting. Effective intestinal permeabilities were calculated using ratios of inlet and outlet concentrations of n-nonane and 1,2,4-trimethylcyclohexane corrected for water flux. 1,2,4-Trimethylcyclohexane demonstrated greater permeability than n-nonane in male Sprague Dawley (1.37 and 1.11, respectively), male Fisher 344 (1.68 and 1.18, respectively), and female Fisher 344 (1.37 and 0.93, respectively) rats. Female Sprague Dawley rats exhibited similar permeabilities for 1,2,4-trimethylcyclohexane (1.46) and n-nonane (1.45). These results suggest that both compounds would be systemically available following ingestion.

996 KINETICS AND ORAL BIOAVAILABILITY OF FUMONISIN B_1.

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Fumonisin B_1 (FB_1), mycotoxin from fungus *Fusarium moniliforme*, a common contaminant of corn worldwide, possesses hepatocarcinogenic and hepatotoxic properties in rats and has been statistically associated with an increased risk of oesophageal cancer in man. The risk assessment of FB_1 requires detailed information on kinetic properties. We investigated plasma disposition of FB_1 after iv (2.0 mg/kg) and oral (10 mg/kg) administration in Wistar male rats. After FB_1 dosing, serial blood samples were obtained and plasma samples separated. FB_1 levels in the plasma samples were analyzed by a HPLC method which involves solid-phase anion-exchange clean-up, precolumn derivatisation with *o*-phthaldialdehyde and reversed-phase HPLC (LiChrosorb RP-8 column) with fluorescence detection (excitation 335 nm, emission 440 nm). Plasma samples were deproteinised with methanol and then centrifuged. The sample supernatant applied to a SAX cartridge. FB_1 eluted with 5% acetic acid in methanol. The eluate was taken to dryness under N_2 at < 60° C and the residue was finally taken up in 0.1M sodium borate prior to derivatisation and HPLC analysis. The mobile phase was methanol- 0.1M sodium di-hydrogenphosphate (63:34) adjusted to pH 3.4. FB_1 kinetic behaviour was best described by a 2-compartment open model. FB_1 was rapidly, but incompletely absorbed ($t_{1/2}a$, 0.13h). T_{max} and C_{max} in plasma were 0.58 h and 0.23 μ g/ml. Oral bioavailability was 3.6 %. FB_1 distributed more quickly after iv than after oral dosing ($t_{1/2}\alpha$, 0.23 and 0.33h. The apparent volume of distribution [$V_{d(area)}$] and at steady state [$V_{d(ss)}$] were 0.11 and 0.10L, after iv dosing, suggesting a considerable diffusion of FB_1into tissue. The elimination half-lives ($t_{1/2}\beta$) for iv and oral dosing were 1.07 and 3.46 h, indicating rapid disappearance of the FB_1 from blood. The fate of FB_1 is still unclear, but our results might to support the hypothesis that a FB_1 portion undergoes metabolic activation. Further studies will be necessary to provide this information. This work has been supported by the *European Union*, Brussels, Project AIR3-CT94-1325.

997 TOXICOKINETICS OF CYHALOTHRIN IN THE RAT.

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Cyhalothrin is a synthetic pyrethroid insecticide which is used topically for the control of ectoparasites. It is also widely used in agriculture and in-home desinsection. Pyrethroids are neurotoxic compounds. In humans, intoxications are usually very light, and neurological symptoms, such as tremor and seizures, are found only in animals. Sensory facial irritation is a very common syndrome (paraesthesia and dysaesthesia). Dermal irritative and allergic effects are very often reported following occupational exposure. The data on toxicokinetics of insecticide pyrethroids are very sparce. Due to toxicokinetic data generated from experimental animal studies are an important component in the overall assessment of human health risk, the objective of this work was to determine the toxicokinetic profile of cyhalothrin. Wistar rats were treated with single iv dose of 3 mg cyhalothrin/kg. Serial blood samples were obtained, plasma was separated and concentrations of cyhalothrin were determined by HPLC assay. Plasma samples were extracted in three 5-ml portions of acetonitrile. After shaking and centrifugation, the combined acetonitrile extracts were rinsed with hexane. The acetonitrile phase was taken to dryness under N_2 at < 30° C, and the residue was finally taken up in 0.5 ml of mobile phase (acetonitrile and distilled-deionized water 80:20) for HPLC analysis using a Nucleosil 120-5 C_{18} column. Detection was effected using ultraviolet absorption at 266 nm. Cyhalothrin plasma kinetics were adequately described by a two-compartment open model with a relatively rapid distribution ($t_{1/2}\alpha$ = 0.11h) and a slow elimination phase ($t_{1/2}\beta$ = 8.15h). The kinetic variables obtained after iv administration confirm the belief that cyhalothrin is well distributed to the tissues ($V_{d(area)}$ and $V_{d(ss)}$ are 0.14 and 0.11 liters). The value of the K_{12} / K_{21} (found to be 11.7) is also indicative of good tissue penetration. (This work has been supported by the *Dirección General de Enseñanza Superior e Investigación Científica, Ministerio de Educación y Cultura*, Spain, Project No. PB97-1236.)

998 TOXICOKINETIC AND INDUCTION OF HEPATIC P 450 1A1/1A2 ENZYME ACTIVITIES IN MALE RATS AFTER SINGLE S.C. DOSES OF 3,3',4,4'-TETRACHLOROBIPHENYL (PCB 77).

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The polychlorinated biphenyls (PCBs) found in blood and other tissues of humans, wildlife, and fish exposed via the food chain are mainly non-coplanar congeners, thus toxicokinetic data of such PCBs are much needed. We have conducted a toxicokinetic study of a non-coplanar PCB (PCB 77) and determined whether it induces 7-ethoxyresorufin O-deethylase (EROD) and 7-methoxyresorufin O-deethylase (MROD) activities. The animals were treated subcutaneously with single doses of 18 mg (PC18) or 60 mg/kg PCB 77/kg bw (PC60). The concentration of PCB 77 was determined in the blood, liver, fat, testis 1, 4, and 8 weeks following treatment, whereas EROD and MROD activities in the liver were only determined 1 and 4 weeks after treatment. The concentration of PCB 77 in the whole blood 1 week after treatment was 1590 and 3800 in PC18 and PC60 groups, respectively. Four weeks following treatment, the blood concentration declined to 8.8 % in PC18 and 20 % in PC60. The concentration ratio: liver/adipose tissue 1, 4 and 8 weeks following treatment in PC60 group was 1.8, 2.6 and 1.9. Significant amount of PCB 77 corresponding up to 60 % of liver concentration was detected in the testis 1 week following treatment. PCB 77 elicits dose-dependent induction of EROD and MROD activities in the liver with a time-course of induction directly correlated to the concentration of the substance in this organ.

999 STRAIN-DEPENDENT DISPOSITION OF ARSENIC IN THE MOUSE.

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Inorganic arsenic (iAs) is methylated and excreted by most mammalian species. The role methylation has in As toxicity is not clear. Acutely, it appears to be a detoxication mechanism, but a methylated metabolite of iAs, dimethylarsinic acid (DMA), promotes tumors. There may be genetic polymorphisms in iAs metabolism. Potential differences between inbred mouse strains in iAs disposition was examined. Female mice (90 days old, inbred strains C3H/HeNCrlBR, C57BL/6NCrlBR and hybrid B6C3F1/ CrlBR) were administered [^{73}As]-arsenate (AsV) or -arsenite (AsIII) po (0.5 or 5.0 mg As/kg). The 24 hr clearance of ^{73}As in one group was monitored by whole-body radioassay. In a second group, excreta and tissues were collected at 24 hr. The clearance of 0.5 mg As/kg AsV and AsIII was similar in the three strains. By 4 hr, > 50% of the dose was eliminated and < 10% remained at 24 hr. ^{73}As retention was slightly higher in C57BL/6 mice up to 6 hr in mice dosed with 0.5 mg/kg AsV. In the 5 mg As/kg AsV group, the clearance was slower than in the low dose group. Elimination of 50% of the dose required 6-8 hr. ^{73}As retention at 4 and 6 hr was greatest in the C57BL/6 mice, followed by B6C3F1 and C3H mice. However, 10% of the dose remained in all strains at 24 hr. The three strains had similar clearance of 5.0 mg As/kg AsIII. It took more than 8 hr for 50% of the dose to be cleared and < 10% remained at 24 hr. Except in liver of AsIII-exposed mice, there was a trend of higher tissue ^{73}As concentration (%dose/gm) in the high AsIII and AsV dose groups. DMA was the major urinary metabolite. There was a trend of lower DMA and higher AsIII, AsV and monomethylarsonic acid excretion with increased dose of iAs. The results suggest a strain-dependent clearance of arsenate at time points less than 8 hr after an acute dose. Thus genotype may affect arsenate disposition in the mouse. Uncertainty in the arsenic risk assessment will be reduced with a better understanding of the factors that affect its disposition. (This abstract does not necessarily reflect U.S. EPA policy.)

1000 INTRATRACHEAL INSTILLATION OF GANCICLOVER AS AN ALTERNATIVE DRUG DELIVERY ROUTE.

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Ganciclovir, an antiviral drug, is usually given by the intravenous route. In this study we explored the possibility of administering ganciclovir directly to the lungs by characterizing the pharmacokinetics and tissue disposition of ganciclovir after pulmonary delivery. We first determined whether intratracheal (*it*) instillation of ganciclovir could elicit toxic or inflammatory responses in the lungs. Bronchoalveolar lavage analyses showed that ganciclovir at up to 20 mg/kg dose had neither toxic nor inflammatory effects in the lungs of male CD rats 24 hr post-instillation. To determine the fate of ganciclovir, rats with indwelling arterial cannulas were given 5 or 20 mg/kg by *iv* and *it* administration and followed for up to 7 hr. Serial blood samples as well as lung tissue and lung macrophages were taken and analyzed by HPLC to measure ganciclovir content. Ganciclovir was eliminated from the blood at approximately the same rate in *iv* and *it* groups at both doses, as reflected by similar elimination rate constants and half-life values. Ganciclovir was absorbed rapidly from the lungs; peak concentrations in blood of 1.93 and 11.13 μ g/ml were reached within 15-20 min after *it* dosing at 5 and 20 mg/kg, respectively. *It* instillation enhanced the drug levels in the arterial blood during the elimination phase. The absolute bioavailability of instilled ganciclovir was 0.99 ± 0.08 or 1.28 ± 0.14 (mean ± SE) for the respective doses. One hr after *iv* and *it* administration of 5 mg/kg, respective ganciclovir levels in the lungs after multiple lavages were 0.46 and 56.0 μ g/g tissue. From 30 min to 7 hr, the area under the lung tissue concentration-vs.-time curve was 107-fold higher for *it* than *iv* routes. Interestingly, ganciclovir levels in the lung macrophages were more than 150-fold higher in *it* instilled animals than in *iv* animals at both doses at all times examined. These findings imply that *it* administration of ganciclovir not only results in efficient delivery of the drug to lung tissue, but also offers the prospect of systemic drug delivery as well. (Supported by NIH grant HL43510.)

1001 KINETICS OF ZICONOTIDE GIVEN INTRATHECALLY IN DOG.

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Ziconotide is a peptide that blocks N-type calcium channels and exerts analgesic effects after intrathecal delivery. We characterized the pharmacokinetics of Ziconotide given intrathecally by bolus injection and infusion in dog. Male Beagles (N = 5) were prepared with chronic lumbar intrathecal injection and a cerebrospinal fluid (LCSF) sampling catheters, passed from the cisterna to the L_{2-3} and L_{1-2} level. Externalized portions of the catheters were connected to vest mounted pumps (100μL/hr). Each dog received: i) intrathecal bolus injection of Ziconotide-10μg + ^{3}H-Inulin-1μCi; ii) continuous intrathecal infusion for 48 hrs of Ziconotide-1μg/hr; iii) continuous intrathecal infusion for 48 hrs of Ziconotide at 5μg/hr; and iv) intravenous injection of Ziconotide-100μg/kg. After intrathecal bolus injection, both LCSF Ziconotide and inulin showed an initial peak and biphasic clearance (Ziconotide: $T_{1/2}\alpha$ = 0.4 and $T_{1/2}\beta$ = 1.8 hours; MRT=0.28 hrs). The Ziconotide/Inulin ratio for each animal fell over time from 0.007 to 0.0006, consistent with the more rapid disappearance of Ziconotide. After initiation of intrathecal infusion of 1 and then 5 g/hr, LCSF concentrations peaked at 8-24 hrs and were stable through 48 hrs at 343 and 1527 ng/mL, respectively. Corresponding plasma levels were 0.05 and 1.12 ng/mL. Upon termination of infusion, the terminal half-life of ziconotide in LCSF was $T_{1/2}$ β = 1.34 hrs. Intrathecal Ziconotide displays dose proportional kinetics after bolus injection or infusion, which are consistent with a molecule that is cleared similar to inulin from the spinal space.

1002 HEPATIC γ-GLUTAMYL TRANSPEPTIDASE-POSITIVE FOCI DEVELOPMENT IN RATS CO-TREATED WITH 17β-ESTRADIOL AND 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN.

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2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin) is a potent hepatocarcinogen in female, but not male rats. Several lines of evidence suggest a key

role of ovarian hormones, presumably estrogen, in the mechanism of TCDD-induced hepatocarcinogenesis. The aim of this current study was to test the hypothesis that estrogen is required for TCDD-induced formation of enzyme altered hepatocellular foci. Diethylnitrosamine-initiated female Sprague-Dawley intact and ovariectomized rats were co-treated with 17ß-estradiol and TCDD for 20 weeks and preneoplastic altered hepatocellular foci expressing γ-glutamyl transpeptidase (GGT) were measured. Increased formation of GGT positive foci was observed in TCDD-treated intact but not ovariectomized rats as compared to controls. These data confirm previous observations demonstrating the effect of ovariectomy on TCDD-induced events in hepatocarcinogenesis. No foci were observed in ovariectomized rats receiving 17β-estradiol alone. In ovariectomized rats, the number of foci/cm^3, percentage of liver occupied, and mean focus volume in rats co-treated with TCDD and 17β-estradiol were significantly greater than in rats that received 17β-estradiol alone, but significantly lower than rats receiving only TCDD. These data suggest that 17β-estradiol inhibits foci formation possibly through effects on initiation and/or growth of initiated foci in the liver. This may lead to the creation of an environment that is more permissive for growth of TCDD-responsive altered hepatocellular foci at later timepoints.

1003 MECHANISM OF INHIBITION OF ESTROGEN-INDUCED GENE EXPRESSION BY TCDD.

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17β -Estradiol (E2) induces progesterone receptor (PR) gene expression in MCF-7 human breast cancer cells, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) inhibits the hormone-induced response. PR gene promoter sequences required for aryl hydrocarbon (Ah)-responsiveness were investigated using the following constructs containing PR gene promoter inserts linked to the bacterial chloramphenicol acetyltransferase (CAT) reporter gene: pPR1 (-2762 to +788); pPR2 (-325 to +788); pPR3 (-165 to +788); pPR4 (-30 to +788); and pPR6 (+340 to +788). In transient transfection studies using pPR1 - pPR6, E2 induced a 3.5-, 10-, 5.8-, 4.2-, 8.0- and 26.2-fold increase, respectively, in CAT activity. The PR^{ERE} construct (+698 to +729) which contains a functional estrogen responsive element (ERE) (+707 to +719) is also induced by E2 (4.1-fold). The PR gene promoter contains two putative inhibitory dioxin responsive elements (iDREs) at +43 to +47 and +300 to +304; however, in MCF-7 cells treated with 10 nM E2 + 10 nM TCDD and transiently transfected with pPR1 - pPR6 or pPR^{ERE}, there was a 47 to 72% decrease in hormone-induced CAT activity. These results suggest that the iDREs are not functional genomic sequences required for the antiestrogenic activity of TCDD using PR gene promoter-derived constructs. TCDD significantly decreased basal and E2-inducible nuclear and cytosolic immunoreactive ER protein in MCF-7 cells, and the role of decreased ER and other nuclear proteins in mediating the antiestrogenic activity of TCDD will be discussed. (NIH ES04176)

1004 INHIBITION OF ESTROGEN-INDUCED RETINOIC ACID RECEPTOR α1 GENE EXPRESSION IN MCF-7 CELLS BY Ah RECEPTOR AGONISTS: MECHANISM OF Ah RECEPTOR-ESTROGEN RECEPTOR CROSSTALK.

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Retinoic acid receptor α (RARα) is a member of the nuclear steroid/thyroid/retinoic acid receptor superfamily and mediates effects of retinoids on gene expression in target cells. 17β -Estradiol (E2) induced RARα gene expression (2-fold) in MCF-7 human breast cancer cells lines and 10^{-8} M 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) inhibited basal and E2-induced RARα gene expression in these cells. Similar effects were also observed for retinoids and antiestrogens. pRARA12 is an estrogen-responsive construct containing an insert from the -509 to +105 region of the RARα1 gene promoter linked to a bacterial chloramphenicol acetyl transferase (CAT) reporter gene. In MCF-7 cells transiently transfected with pRARA12 (10 μ g) plus human estrogen receptor (hER) expression plasmid (1 μ g), 10^{-9} M E2 induced a 2.5-fold increase in CAT activity, and in cells cotreated with 10^{-9} M E2 + 10^{-8} M TCDD, induced CAT activity was decreased by 90%. In parallel experiments, 10^{-6} M α-naphthoflavone, an aryl hydrocarbon receptor(AhR) antagonist, reversed the antiestrogenic effects of TCDD, and TCDD did not exhibit antiestrogenic activity in Ah-nonresponsive benzo[a]pyrene-resistant MCF-7 cells. TCDD also inhibited E2-induced CAT activity in MCF-7 cells transiently transfected with pRAR5(-111 to -49) and pRAR5-m1 (mutated in a potential AhR binding motif at -110 to -106). These results demonstrate that the antiestrogenic activity of TCDD is AhR-mediated but is not dependent on the core binding motif that is functional in other E2-responsive gene promoters. Therefore, other possible mechanisms of AhR-mediated antiestrogenic responses are currently being investigated. (NIH ES04176)

1005 ESTROGEN AND ARYL HYDROCARBON RESPONSIVENESS OF ECC-1 ENDOMETRIAL CANCER CELLS IN CULTURE.

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ECC-1 human endometrial cancer cells were evaluated for aryl hydrocarbon (AhR) and estrogen receptor (ER) functionality. Nuclear AhR and ER were detected in the ECC-1 cells line by Western immunoblot analysis of nuclear extracts. 17β -Estradiol (E2) induced proliferation of ECC-1 cells (~7-fold); in cells cotreated with several AhR agonists including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), benzo[a]pyrene (BaP), 6-methyl-1,3,8-trichlorodibenzofuran (MCDF), and diindolylmethane (DIM), there was a significant inhibition (45 to 75%) of this hormone-induced response. TCDD significantly induced CYP1A1-dependent ethoxyresorufin *O*-deethylase (EROD) activity in ECC-1 cells. E2 and TCDD also induced reporter gene activity in ECC-1 cells transiently transfected with estrogen and Ah-responsive constructs. E2 induced cathepsin D and pS2 gene expression in ECC-1 cells, and in cells cotreated with E2 plus TCDD, there was a significant decrease in E2-induced gene expression. These results indicate that ECC-1 endometrial cancer cells are both Ah- and E2-responsive and current studies are focused on mechanisms of AhR-ER crosstalk. (NIH CA64081)

1006 INHIBITION OF E2-INDUCED c-*fos* PROTOONCOGENE EXPRESSION BY 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) IN MCF-7 HUMAN BREAST CANCER CELLS.

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17β -Estradiol (E2) induced c-*fos* protooncogene mRNA levels in MCF-7 human breast cancer cells and maximal induction was observed within 1 hr after treatment. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) inhibited the E2-induced response within 2 hr. The molecular mechanism of this response was further investigated using the pFC2-BLCAT plasmid which contains the -1400 to +41 5N-flanking sequence from the human c-*fos* protooncogene linked to a bacterial chloramphenicol acetyltransferase (CAT) reporter gene. In MCF-7 cells, transiently transfected with the pFC2-BLCAT plasmid and treated with 10 nM E2, there was an 8.5-fold induction of CAT activity. Cotreatment with 10 nM E2 + 10 nM TCDD resulted in a 46% decrease in E2-induced CAT activity; moreover the antiestrogenic effects of TCDD were blocked by the Ah receptor antagonist, α-naphthoflavone. Similar results were obtained with a construct (pF1) containing the E2-responsive (-1220 to -1155) sequence. This region of the *fos* promoter also contains two core dioxin-responsive elements (DREs); and transient transfection studies using constructs containing wild-type or mutant core DREs showed that the downstream core DRE (-1161 to -1157) was required for Ah-responsiveness. The nuclear Ah receptor complex from MCF-7 cells also crosslinked to the core DRE, confirming that the iDRE motif is required for Ah receptor-mediated antiestrogenicity. (NIH ES04176)

1007 IDENTIFICATION OF ESTROGEN-INDUCED GENES DOWNREGULATED BY 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) BY SUPPRESSION SUBTRACTIVE HYBRIDIZATION.

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The antiestrogenic activity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is mediated through crosstalk between the aryl hydrocarbon receptor (AhR) and estrogen receptor (ER) signaling pathways. Identification of 17β -estradiol (E2)-induced genes downregulated by TCDD utilized suppression subtractive hybridization (SSH) using poly(A)$^+$RNA from MCF-7 cells treated with 1 nM E2 and 1 nM E2 + 1 nM TCDD as targets and references, respectively. The subtraction efficiency was determined using DNA blot analysis and a subtractive cDNA library containing 396 clones was constructed.

Forty-eight clones were further characterized using E2 and E2 + TCDD treated cDNA probes by dot blot analysis. Twenty-one putative candidate clones were further subjected to sequence analysis and GenBank database search. Preliminary results indicate that some of these clones have sequence homology to previously described genes including heat shock protein 27, thymidylate synthase, Wilm's tumor-related protein, cytokeratin 18 (3 clones), early growth response gene, prothymosin-α, heparin-binding growth factor receptor, mitochondrial ATP synthase, Cdc28/Cdc2-associated protein and ribosomal protein L7 α (2 clones). In addition, two potential novel sequences were also identified. Northern blot analysis confirmed that levels of heat shock protein 27, early growth response gene, and Wilm's tumor-related protein mRNAs were down-regulated by treatment with TCDD. Expression patterns of the remaining genes and 2 novel sequences are currently under investigation and will provide further information on E2-regulated genes that are targeted by AhR agonists. (NIH ES04176)

1008 IN UTERO AND LACTATIONAL TCDD EXPOSURE ALTERS ESTROGEN INDUCED MAMMARY GLAND GROWTH IN THE RAT.

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In the mammary gland, estrogen triggers ductal cell proliferation. Intact estrogen signaling pathways are required for normal duct development. This study was initiated to determine whether in utero and lactational (IUL) 2,3,7,8–tetrachlorodibenzo- p–dioxin (TCDD) exposure alters estrogen signaling in the rat mammary gland. To test this hypothesis, pregnant Holtzman rats were administered a single oral dose of 1μ % g/kg, or vehicle, on gestation day 15 and offspring were weaned on postnatal day 21. TCDD exposed and control non-exposed female offspring were oophorectomized at 9 weeks of age. Two weeks later both the TCDD and control rats were divided into three groups: Group 1: 0.1 mg 17 Symbol estradiol implant, Group 2: 0.025 mg 17 Symbol estradiol implant, Group 3: placebo implant. After 48 hours all rats were euthanized and the mammary tissue removed for analysis. Whole mount studies revealed that IUL TCDD exposure resulted in baseline morphological abnormalities in the lobular alveolar structures. In addition, acute 17 Symbol estradiol exposure initiated enhanced ductal growth and accelerated lobular-alveolar development in IUL TCDD exposed as compared to control rats. Ki67 immunohistochemistry demonstrated that ductal cells from IUL TCDD exposed mammary glands have a higher rate of cell proliferation at the 0.025 mg 17 Symbol estradiol dose than the non-exposed control rats. Gene expression studies are focused on identifying effector genes which mediate the abnormal growth response. In conclusion, this study demonstrates that in utero and lactational TCDD exposure can alter the growth response to estrogen in the mammary gland.

1009 APPARENT MODULATION OF PRENEOPLASTIC FOCI BY OVARIAN HORMONES IN RESPONSE TO TCDD.

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Dioxin (2,3,7,8-tetrachloro-p-dibenzodioxin; TCDD) is a polychlorinated biphenyl. TCDD is a potent environmental toxin that is carcinogenic in two stage models of hepatocellular carcinogenesis in female rats. Ten week old female rats either intact sham-operated, or ovariectomized (OVX) were intraperitoneally initiated by diethylnitrosamine (DEN) [200mg/kg] in saline (S) followed by promotion with TCDD in corn oil (C) [1.5mg/kg every 15 days for 43 weeks] by gavage. Both intact and OVX rats were divided into four groups, S/C (controls receiving saline and corn oil), DEN/C (initiated only), S/TCDD (promoted only), and DEN/TCDD (initiated and promoted). Intact sham-operated and OVX animals from the DEN/TCDD group developed hepatic focal lesions indicative of preneoplastic foci. All TCDD treated animals showed significant ($P<0.05$) induction of hepatic microsomal cytochrome P450d (1A2/1A1) with the exception of the OVX-DEN/TCDD group. Immunohistochemical analysis of liver sections with placental glutathione-S-transferase revealed a significant reduction in preneoplastic foci in the OVX-DEN/TCDD group relative to it's control group. These data suggest that ovariectomy exerts a protective effect with regard to the promotion of liver tumors by TCDD. (Supported by NIH G12 RRO 3032 and NIEHS ES00287.)

1010 EFFECT OF ESTROGEN AND DIOXIN ON IL-1B ACTIVITY.

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We recently reported that endogenous and some environmental estrogens markedly enhanced lipopolysaccarhide (LPS)-induced IL-1β activity in a macrophage cell line stably transfected with the human estrogen receptor (ERα) and an IL-1β promoter-chloramphenicol acetyltransferase (CAT) reporter construct. In the present study we examined the effect of estrogens and the environmental contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on the ability of LPS to induce two different IL-1β promoter-CAT constructs transiently transfected into the RAW macrophage cell containing endogenous ER. Estradiol markedly enhanced LPS stimulated CAT activity 2-3 fold in RAW264.7 cells transfected with either a CAT reporter construct driven by 4000 bp of upstream IL-1β promoter sequence or the isolated NFIL-6 element found in the IL-1β promoter. The RAW264.7 cells contain the Ah receptor and TCDD alone also enhanced LPS stimulated CAT activity 2-fold in cells transfected with the 4000 bp upstream promoter. However, this was not the case with cells transfected with the NFIL-6 driven reporter. Most dramatic was the synergy observed when the cells transfected with either reporter construct were treated with both TCDD and estradiol prior to LPS stimulation. In these cases a two-fold additional increase was observed over LPS plus either estradiol or TCDD alone. These results demonstrate that TCDD potentiates the synergism between estrogen and LPS to markedly enhance IL-1β promoter activity, although the exact mechanism is not understood. (Support: NIH ES05968.)

1011 BIOLOGICALLY BASED MODELING OF ALTERED HEPATIC FOCI AFTER TREATMENT WITH 2,4,5,3',4'-PENTACHLOROBIPHENYL.

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A biologically based quantitative foci growth model was used to analyze the effect of 2,4,5,3',4'-pentachlorobiphenyl (PCB118) on foci development in rat liver initiated with partial hepatectomy and diethylnitrosamine (DEN). Six DEN-initiated PCB dosed groups + DEN-initiated control animals were treated weekly for 20 weeks. Three DEN-initiated PCB dosed groups + DEN-initiated controls were treated for 52 weeks. All groups were included in the computer simulation. Hepatocyte proliferation in foci and normal tissue was experimentally examined using bromodeoxyuridine as a marker of proliferating cells. The proliferation data were used in the selection of division rates for the computer simulations of foci growth. The labeling index in foci was generally larger than in non-focal tissue for PCB-treated as well as untreated initiated animals. A strong correlation was found between foci occurrence and proliferation in both foci and non-focal tissue. The foci data were adequately simulated by the foci growth model when certain assumptions were introduced. Given that PCB118 is a non-mutagenic compound, the foci data could not be modeled assuming one homogeneous cell population, but was adequately fitted assuming two separate cell populations that respond differently to the promotion stimulus. Another assumption was a selective growth advantage for initiated cells during and shortly after the initiation treatment. Furthermore, a transient increase of proliferation in focal hepatocytes at the first PCB administration was assumed in the higher dose groups to account for the large occurrence of foci in these groups at the end of the experiment. At high doses (640, 2500 and 10,000 μg/kg/week), an increased rate of focal cell death was also predicted by the simulation model. These assumptions are well supported by literature data for other carcinogens, indicating the importance of studying cell kinetics and balance between cell division and cell death in PCB induced hepatocarcinogenesis.

1012 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ALTERS CHICK EMBRYO CARDIAC ELECTRICAL CONDUCTION.

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We have previously shown that in the chick embryo TCDD induces a phenotype consistent with congestive heart failure: ventricular cavity dilatation, cardiac hypertrophy, and increased myosin synthesis, culminating in peripheral edema. To further characterize TCDD-induced cardiotoxicity, we

assessed the electrical conduction properties of the chick embryo heart in ovo following TCDD exposure. Fertile chicken eggs were injected with control (triolein) or 0.4 pmol TCDD/g egg in triolein prior to incubation. On day 11 or 12 of development, embryos were anesthetized with a chloralose/urethane mixture injected into the air cell. Silver wire electrodes were inserted through small holes made in the shell and EKGs were measured at ca. 1 min intervals. Following EKG measurements, embryos were collected and body and heart wet weights taken. EKG tracings were analyzed for heart rate (beats per minute), RR interval (one contraction/relaxation cycle), QT interval (ventricular repolarization time) and QT interval corrected for heart rate ($QT_c = QT/(RR)^{1/2}$). On incubation day 11, TCDD did not significantly alter heart weight or the heart/body weight ratio. In addition, TCDD did not alter heart rate or the RR interval. However, TCDD did significantly shorten the QT_c interval (TCDD: 0.283 ± 0.02; Control: 0.351 ± 0.14). On incubation day 12, TCDD significantly increased heart weight (TCDD: 69 ± 3.5; Control 48 ± 1 mg) as well as shorten the QT_c interval (TCDD: 0.277 ± 0.04; Control 0.369 ± 0.01). The shortened QT_c interval induced by TCDD was a more sensitive endpoint than changes in heart weight or heart/body weight ratio. In TCDD-exposed chick embryos a shortened QT_c interval reflects that the ventricles repolarize faster after contracting than in the hearts from control embryos. A shortened QT_c could reflect hyperkalemia, reduced Ca release from the sarcoplasmic reticulum, or a decreased proliferation of the myocardial wall. Future studies will test the relative contributions of these possible mechanisms to the altered cardiac conduction induced by TCDD.

1013 COMPUTER–ASSISTED ANALYSIS OF EPIDIDYMAL SPERM MOTILITY OF RATS EXPOSED MATERNALLY TO 2,3,7,8–TETRACHLORODIBENZO–DIOXIN (TCDD).

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Spermatogenesis is one of the most sensitive endpoints of toxicity of rat offspring maternally exposed to TCDD. TCDD reduced epididymal but not testicular sperm numbers in rats and caused epididymal lesion in rat and hamster (Gray et al, 1995, 1997). Epididymis is specifically altered by TCDD during sex differentiation. Within the epididymis, sperm acquires progressive motility and fertility capacity. Epididymal sperm motility might be susceptible to TCDD exposure in utero. However, no report has been published on evaluating sperm motility by CASA (computer assisted semen analysis) system. In the present study, epididymal sperm o²9DdÍ of rat offspring exposed maternally to TCDD was analyzed by the CASA system. Pregnant Holtzman rats (JCR) were given a single oral dose of 0, 12.5, 50, 200 t²9Dd 800 ng TCDD/kg body*²9De weight on gestational Day 15, and male offspring were sacrificed on Day 63 and 120 after birth. On Day 63, percentages of motile sperm and progressive sperm were decreased by 200 and 800 ng TCDD/kg body weight, but these tendencies were not statistically significant (ANOVA). Other parameters of sperm motility, path velocity (VAP), progressive velocity (VSL), track speed (VCL), lateral amplitude (ALH), beat frequency (BCF), straightness (STR) and linearity (LIN) were not affected. In conclusion, maternal dosage up to 800ng TCDD/kg body weight had no effects on epididymal sperm motility o u²9Dd Day 63 v²9Dd rat offspring.

1014 DOES VOLUME OF INJECTION MATTER WHEN CONCENTRATIONS ARE IDENTICAL.

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Studies with TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) in our laboratory have indicated that TCDD is induces an interhemispheric brain asymmetry with associated behavioral changes, when injected *in ovo* into fertilized chicken eggs in volumes of 0.1 μ m per egg. Other research facilities often use injection volumes of 1.0 μ m per egg. The purpose of this study was to determine whether differences in the volume of injection, when concentrations are identical, would differentially affect behavior. Fertile domestic chicken eggs were air sac injected prior to incubation with either 2, 20 or 200 picagrams of TCDD per gram of egg, diluted in a sunflower oil or corn oil vehicle. Stock solutions of TCDD were diluted so that either 0.1 μ m or 1.0 μ m per egg resulted in the appropriate concentration. Control eggs received the oil alone or no injection at all. One group of eggs received 0.1μ m of TCDD per gram prior to incubation, at embryonic day zero, whereas the other group received 1.0 μ m per gram of egg, also prior to incubation. Eggs were incubated until hatch and chicks were run through several reflexive, locomotor and perceptual tests within 24 hours of hatching. Analysis indicated that the volume of injection detestably alters the behavioral status of chicks. Activity was increased tenfold, timed movement in a fixed direction was nearly doubled, pecks made at food were reduced by almost half and a righting reflex was mildly suppressed in the high volume groups when compared to the behavioral responses in low volume groups.

1015 PRENATAL TCDD EXPOSURE AND PREDISPOSITION 9® MAMMARY CANCER IN RATS.

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We have previously reported that prenatal TCDD exposure enhances dimethylbenz(a)anthracene (DMBA) induced mammary cancer (Carcinogenesis 19:1623,1998). To gain insight into the mechanism of short–term TCDD exposure predisposing for mammary cancer, we have investigated specific mRNA and protein expressions. Pregnant Sprague Dawley CD rats were gavaged with 1 ug TCDD/kg body weight. At parturition, the offspring were transferred to surrogate dams. At 50 days post–partum, the female offspring were gavaged with DMBA or sesame oil (S.O.). At 100 days postpartum, mammary glands were dissected. Early increased lobular hyperplasia correlated with increased mammary tumorigenesis in rats exposed prenatally to TCDD and in adulthood to DMBA (TCDD/DMBA) as compared to S.O./DMBA treated rats. CYP1A1 as measured by western blot analysis was higher in mammary glands of S.O./DMBA and TCDD/DMBA treated rats as compared to non–DMBA treated rats. Estrogen receptor–alpha and beta mRNAs as measured by RT–PCR were similar in all groups. We conclude that prenatal TCDD exposure confers early permanent alterations to predispose the resulting adult for susceptibility to mammary cancer.

1016 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD)-DEPENDENT ALTERATIONS IN B CELL LYMPHOPOIESIS ARE MEDIATED AT THE LYMPHOCYTE LEVEL DIRECTLY.

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TCDD, acting through the aromatic hydrocarbon receptor (AhR) and its dimerization partner, arnt, elicits numerous toxicological effects including immunosuppression. Previous work from our laboratory has suggested that TCDD exposure in mice is associated with altered lymphopoietic development in the bone marrow compartment. Furthermore, it has been demonstrated that bone marrow stromal cells express functional AhR and arnt proteins. Because these cells play an important role in the support and direction of lymphopoiesis, it is possible that TCDD treatment could be altering lymphopoietic development through effects mediated at the stromal cell level. In order to determine what role, if any, bone marrow stromal cells play in such TCDD-dependent lymphopoietic alterations, a bone marrow stromal cell/B cell co-culturing system was established using the M2-10B4 cell line. When B220+ bone marrow lymphocytes derived from AhR-expressing mice were co-cultured with M2-10B4 cells, TCDD exposure was associated with dose-dependent decreases in the total number of hematopoietic cells, lymphocytes, and B220+CD43- pre-B cells. Similar alterations were not observed using B220+ cells derived from AhR-deficient mice. These results indicate that TCDD exposure alters B cell development *in vitro* and suggests that this effect is mediated at the lymphocyte level directly rather than through the bone marrow stromal cells. Furthermore, these results suggest that the B220+CD43- pre-B cell population may be particularly sensitive to the immunotoxic effects associated with TCDD exposure. (This work supported by NIH Grant ES04862, Center Grant ES01247, and Training Grant ES07026.)

1017 THE EFFECTS OF 2,3,7,8 - TETRACHLORODIBENZO-p-DIOXIN (TCDD) ON INSULIN IN A MURINE MODEL OF TYPE II DIABETES.

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Recent studies suggest an association between exposure to TCDD and increases in type II diabetes. When fed a high -fat high simple carbohydrate diet (HFHSC) C57/BL6J mice develop signs of type II diabetes. The goal of this project is to determine if TCDD alters the severity or decreases the time to onset of type II diabetes in this murine model. The effects of TCDD on serum glucose, cholesterol, and insulin concentrations in mice following

subchronic exposure to TCDD were determined. The subchronic studies include a four, eight, and twelve week exposure to TCDD (0,1.5 and 150 ng/kg/day) with mice either on the HFHSC diet or a normal diet . All animals were terminated three days after the last exposure. Blood was collected for cholesterol, glucose, and insulin analysis. The liver was removed for histological and lipid analysis. The HFHSC diet increased insulin concentration. TCDD exposure produced a dose dependent decrease in serum insulin in mice fed either the normal or HFHSC diet. The HFHSC diet increased resting serum glucose and cholesterol concentrations after 8 weeks. TCDD administration did not alter serum glucose concentrations in animals on either the normal or the HFHSC diet. TCDD decreased resting serum cholesterol concentrations only in animals on the HFHSC diet. Hepatic total lipid concentrations and cholesterol were increased by TCDD in both normal and HFHSC diet fed mice. However, the HFHSC diets led to a greater concentration of hepatic lipid. In conclusion, TCDD does appear to have a moderate effect on signs of type II diabetes. (This abstract does not reflect USEPA policy.)

1018 ENDOCRINE FUNCTION OF MALE RATS EXPOSED MATERNALLY TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD).

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The environmental pollutant 2,3,7,8-tetrachlorodibenzo-dioxin (TCDD) is known to disrupt vertebrate endocrine system. It is generally believed that the embryonic stage of development is highly susceptible to toxicity of TCDD. To evaluate of influence of maternal exposure to TCDD on neonatal development especially on endocrine function, pregnant Holtsman rats (JCR) were given a single oral dose of 0, 12.5, 50, 200 or 800 ng TCDD/kg body weight on gestation Day 15, and male offspring were sacrificed on Day 2, 49 or 63 after birth. There was no TCDD effect in litter size, birth weight and sex ratios of pups for all groups. The highest maternal dose of TCDD caused an increase in liver weight (15.3%) as well as significant thymic atrophy (20.1%) on Day 63. No changes in weight of body, brain, thyroid, pituitary gland and adrenal gland were observed in the offspring exposed to TCDD. Of hormones examined in this experiment, the highest dose of TCDD induced a decrease in serum T4 levels (14.7%) on Day 63 (postpubertal), but not on Day 2 and Day 49 (pubertal), whereas serum T3 levels were not affected in these offspring. TCDD did not affect serum levels of thyroid-stimulating hormone (TSH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in the offspring in all the dose groups. The present study showed that the earliest sign detectable in the offspring following maternal exposure to relatively low level of TCDD was a decreased serum T4, not in pubertal, but postpubertal stage.

1019 HEPATIC EFFECTS OF PRENATAL AND POSTNATAL EXPOSURE TO AROCLOR 1254 OR PCB 126.

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The present study examines the hepatic effects of developmental exposure to a PCB mixture, Aroclor 1254 (Lot 124–191) or to PCB 126. Hepatic EROD activity, retinyl palmitate, and porphyrin concentrations were determined. In the first study, female Long Evans(LE) rats were treated with PCB 126(0, 0.25 and 1 ug/kg/day) starting 35 days before mating and ending on PND 21. Pups were examined on PND 28 thru 30 and 115. There was a significant increase (10 fold) in EROD activity and a significant decrease(58%) in retinyl palmitate levels at the highest dose at PND 28–30. At PND 115, these responses were no longer present. In the second study, LE– rats were treated with Aroclor 1254 (0,1, and 6 mg/kg/day) starting on GD6¿ and ending on PND 21. Pups were examined on PND 4, 7, 14, 21, and 60. There were significant increases in EROD activity, up to 218 fold, at the highest dose on PND 4,7,14 and 21. At the highest dose, retinyl palmitate levels were decreased at PND14(80%) and PND 21(54%). On PND 60, no effects were observed for EROD activity or retinyl palmitate concentrations. There were moderate increases in porphyrin levels(50%) at all time points at the high dose. Finally, LE–rats were dosed to approximate steady state concentrations prior to mating by treating with Aroclor 1254(0, 0.3,1 and 3 mg/kg/day) starting 90 days before mating and ending on PND 21. Pups were examined on PND 14 and 21. EROD activity was significantly increased, up to 108 fold, at doses of 1 and 3 mg/kg/day at both timepoints. Retinyl palmitate levels were decreased by approximately 40% (1 mg/kg/day) and 70%(3 mg/kg/day) on both days. Porphyrin levels were moderately increased at both time points at 1 and 3 mg/kg/day. Previous studies in our laboratories using the same dosing regimens produce pronounced decreases in thyroid hormone concentrations and neuro behavioral effects. The present study indicates that moderate dioxin–like biochemical changes in hepatic tissue are occuring in these animals and that dioxin like effects may be useful markers for alterations in thyroid hormones or neurodevelopment. (This abstract does not necessarily reflect EPA policy.)

1020 QUANTITATIVE ANALYSIS OF LYMPHOCYTE CYP1B1 EXPRESSION IN INDIVIDUALS OCCUPATIONALLY EXPOSED TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN (TCDD).

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Cytochrome P450 1B1 mRNA is constitutively expressed and TCDD-inducible in human peripheral blood lymphocytes, as previously demonstrated in our laboratory using a competitive RT-PCR assay. The present study evaluated lymphocyte CYP1B1 expression as a potential biomarker of dioxin exposure and individual responsiveness in 95 individuals occupationally exposed to dioxin-like compounds. These individuals were employed at a German chemical plant and were highly exposed to dioxins during the production of chlorinated herbicides and pesticides; blood plasma dioxin toxic equivalents (TEQ) values ranged from 15-916 ppt lipid. Constitutive levels of CYP1B1, as measured in uncultured lymphocytes, ranged from 3×10^4 to 3×10^6 copies/μg RNA. For the subpopulation of individuals with elevated plasma TEQ (>40 ppt lipid), there was an increased frequency of individuals with high constitutive CYP1B1 mRNA levels. CYP1B1 mRNA levels were also measured in mitogen-stimulated cultured lymphocytes (MSL) from 82 of these individuals. Addition of TCDD to the MSL induced CYP1B1 mRNA levels in the individuals with varying magnitude. The induction (treatment/control) ranged from 1 to 18-fold, with an average induction of 6-fold. CYP1B1 induction *in vitro* did not appear to be related to plasma dioxin TEQ. These data indicate that CYP1B1 may be useful as a marker of *in vivo* human exposure to dioxin, and for characterizing interindividual variation in biological response.

1021 ORAL TOXICITY OF 1,2,3,4,6,7,8-HEPTACHLORODIBENZO-*p*-DIOXIN (HpCDD) OBEYS HABER'S RULE OF INHALATION TOXICOLOGY.

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Eight different doses (2.5 to 10.0 mg/kg) of 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD) were administered acutely to a total of 271 female Sprague-Dawley rats. The doses ranged from a NOAEL for wasting/hemorrhage to supralethal doses. Dose-and time-responses of wasting/hemorrhage, anemia and cancer were and are being studied as end points of toxicity. The experiments will be continued until the last rat dies. There was a very steep dose- and time-response between the LOAEL for wasting/hemorrhage (2.8 mg/kg) and the third highest dose (4.1 mg/kg) of HpCDD. The dose-and time-responses were symmetrical obeying Haber's Rule of inhalation toxicology ($c \times t$ = constant) even beyond 100% mortality. Introduction of a minimum of 25% body weight loss as a discriminatory criterion to separate wasting from hemorrhage as the primary cause of death reduced variability from 5.8 to 3.1%. An arithmetic plot of the dose and time data resulted in a nearly perfect hyperbola. A logarithmic plot of these data yielded a straight line of similar perfection. Surviving rats in all groups started developing anemia 120 days after dosing but no rat died of wasting/hemorrhage after day 74. Thus, although the experiment is completed as far as dose-and time-responses of wasting/hemorrhage are concerned, it will be about another 2 years before complete dose-and time-responses are available for anemia and cancer.

1022 CONSTITUTIVE AND 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN- INDUCED EXPRESSION OF CYP1A1 AND CYP1B1 IN HUMAN LYMPHOCYTES.

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a ubiquitous environmental contaminant which produces a spectrum of biological responses. We are using the dioxin inducible genes CYP1A1 and CYP1B1 to identify individuals who are highly responsive and possibly highly susceptible to the adverse health effects of dioxins. The study cohort consists of local volunteers (n=40) who provided blood samples and questionnaire information. Individual responsiveness was assessed by measuring CYP1A1 enzyme activity in peripheral blood lymphocytes after 3 days of *in vitro* incubation with 10nM TCDD. The effect of exposure to dioxins from environmental sources was evaluated by measuring CYP1B1 expression in uncultured peripheral blood lymphocytes by quantitative RT-PCR. *In vitro* TCDD treatment induced detectable levels of CYP1A1 enzyme activity in the lymphocytes of 38 (95%) of the individuals. Comparison of the 5th and 95th percentiles shows that the range of expression for constitutive CYP1B1 mRNA varied 26-fold compared to 9-fold for *in vitro* CYP1A1 enzyme inducibility. CYP1A1 enzyme activity showed a small, but significant negative correlation with age. Neither constitutive CYP1B1 mRNA nor *in vitro* TCDD-induced CYP1A1 enzyme activity correlated with plasma levels of TCDD, total dioxin toxic equivalents (TEQs) (range 3-33 ppt lipid), gender, or smoking. However, the 3 individuals with the highest levels of constitutive CYP1B1 expression had dioxin exposures within the lowest quartile for the cohort. These results combined with continuing studies of the *in vitro* inducibility of CYP1B1 demonstrate variation in human response to dioxins that may be associated with adverse health effects.

1023 EFFECTS OF TCDD ON MICE CHRONICALLY INFECTED WITH THE PROTOZOAN PARASITE, *TOXOPLASMA GONDII.*

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Toxoplasmic encephalitis is a life threatening condition observed in patients with AIDS. It is caused by reactivation of a latent infection with the protozoan parasite, *Toxoplasma gondii*. Cysts that contain latent bradyzoites become reactivated and progress into rapidly dividing stages, tachyzoites, which then begin to destroy tissue. This reactivation is possible because of the immunocompromised condition of the AIDS patient. TCDD exposure is known to cause immunosuppression. The present study was conducted to determine if treatment with TCDD would influence the course of infection in chronically infected C57/BL6 mice. Twelve female mice were fed 25 cysts of *T. gondii* and chronic infections established for 10 weeks. Control mice (n=11) were not infected. After the 10 week period, 6 *T. gondii* infected mice and 6 control mice were fed 20 μ g/kg TCDD and 6 *T. gondii* infected mice and 5 control mice were fed corn oil. One mouse with chronic toxoplasmosis fed TCDD died 12 days postinoculation (PI) while none of the other mice died. One-half the remaining mice in each group were killed and examined 3 weeks PI. Cyst counts in TCDD treated *T. gondii* infected mice were 200 ± 144 (mean ± SD) and the numbers of cysts in *T. gondii* infected nontreated mice were 173 ± 46. Brain lesions were similar in both groups of *T. gondii* infected mice. Results of this study suggest that TCDD may cause reactivation of latent toxoplasmosis in some mice but does not influence cyst numbers or lesions in the brains of mice.

1024 ROLE OF OXIDATIVE STRESS IN THE SUBCHRONIC TOXICITY OF TCDD IN C57BL/6J FEMALE MICE.

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Oxidative stress (OS) resulting from increase production of reactive oxygen species (ROS) have been established as one of the mechanisms of the acute toxicity of TCDD. However, no extensive studies have investigated the role of OS in the subchronic toxicity of this compound. To assess the involvement of OS in the subchronic toxicity of TCDD, effects of 13 weeks oral treatment with daily doses of 0.05 (high), 0.025 (medium) and 0.005 (low) LD_{50} TCDD/kg on animal weights, target organs weights and on measures of oxidative stress in C57BL/6J female mice was determined. The high dose of TCDD produced a significant body weight wasting that was not observed with the low and medium dose. The high dose produced approximately 60% mortality at three months and all the remaining animals in that group were sacrificed at that point. The low and medium dose had 100% survival at three months following TCDD treatment. TCDD produced a dose response increase in liver and kidney weight and a dose-response decrease in thymus and spleen weight. TCDD produced a dose dependent increase in hepatic and spleen lipid peroxidation following subchronic exposure. Furthermore, DNA-SSB was observed in the liver nuclei and production of ROS was enhanced by the liver mitochondria and microsomes. The results implicate a role for OS in the subchronic toxicity of TCDD.

1025 ROLE OF OXIDATIVE STRESS IN THE CHRONIC TOXICITY OF TCDD IN C57BL/6J FEMALE MICE.

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Oxidative stress (OS) plays a role in the acute toxicity of TCDD and may contribute to the toxic effects following chronic exposure to this compound. Therefore, to assess the involvement of OS in the chronic toxicity of TCDD, effects of 26 weeks oral treatment with daily doses of 0.05 (high), 0.025 (medium) and 0.005 (low) LD_{50} TCDD/kg on animal weights, target organs weights and on measures of OS in C57BL/6J female mice was determined. The low and medium dose produced no body weight wasting following chronic treatment. The high dose produced approximately 60% mortality at three months and all the remaining animals in that group were sacrificed at that point. The medium dose produced 20% mortality at four and a half month following TCDD treatment. TCDD produced a dose response increase in liver, kidney and spleen weight and a dose-response decrease in thymus weight. The low and medium dose of TCDD produced a significant increase in lipid peroxidation in the liver and spleen. The medium dose produced a significant increase in brain lipid peroxidation. The low dose produced 6.6 fold increase in liver nuclei DNA-single strand breaks. Furthermore, the low dose enhanced the production of ROS by peritoneal lavage cells; by liver mitochondria and liver microsomes. The results implicate a role for OS in the chronic toxicity of TCDD.

1026 VITAMIN SUPPLEMENTATION TO PREVENT DEVELOPMENTAL TOXICITY OF 2,3,7,8-TETRA-CHLORODIBENZO-*p*-DIOXIN (TCDD) IN TCDD-SENSITIVE RATS.

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Supplemetation with folic acid and other vitamins has been reported to have a protective effect against malformations and fetotoxicity. This developmental study was performed in TCDD-sensitive Long-Evans rats (L-E; LE/AB/KTL (SPF); LD_{50} about 10 μg/kg) to investigate whether the gestational multivitamin administration reduces the incidence of cleft palate and fetotoxic effects induced by TCDD. Pregnant rats were administered orally with different composition of group B vitamins (thiamin, riboflavin, pantothenic acid, pyridoxine, and cobalamin), folic acid and ascorbic acid on gestation days 1-19 and once with TCDD (2 μg/kg or 4 μg/kg) on gestation day 8. The dams were necropsied on gestation day 20. All fetuses were fixed in Bodian's solution and assessed for visceral anomalies. The high TCDD dose, with or without vitamin supplementation, was slightly maternotoxic (decreased corrected body weight gain but no clinical signs). The maternal thymus weight decreased in all TCDD-dosed groups. The mean fetal weight decreased at the high TCDD dose level. The anogenital distance in TCDD-dosed male fetuses with or without vitamin supplementation was less than in control animals. The number of resorptions increased at high TCDD dose level; this effect was slightly protected with vitamin supplementation. To the incidence of fetuses with cleft palate the vitamin supplementation had no effect. This preliminary study did not reveal any clear preventive effect of these vitamins to TCDD-induced teratogenicity in these TCDD-sensitive L-E rats. However, vitamin supplementation slightly modulated TCDD-induced fetotoxicity.

1027 TCDD INCREASES THE PRODUCTION OF VASOACTIVE EICOSANOIDS AND COFRACTIONATES WITH LIPOPROTEIN PARTICLES IN HYPERLIPIDEMIC MICE.

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Studies over the years document numerous adverse health affects to humans and experimental animals that result from exposure to aryl hydrocarbon receptor agonists such as TCDD or other environmental pollutants. Much of what we understand about the effect of this class of compounds on human health results from epidemiological studies of populations subjected to accidental high-level exposures. For example, exposure to halogenated aromatic hydrocarbons from a chemical plant explosion in Hamburg (Germany) resulted in a greatly increased incidence of death from ischemic heart disease (IHD). IHD is a progressive disease and all humans are affected to varying degrees as a function of age. TCDD may accelerate the onset of IHD either by affecting the levels of the molecular mediators of IHD (i.e. lipoprotein particles, lipid second messengers etc.) or by directly influencing the progression of the atherosclerotic lesion. We have tested aspects of these two alternatives using C57BL/6J and apolipoprotein E-null (ApoE) mice, respectively. Because ApoE mediates the clearance of lipoproteins, ApoE-null mice have elevated cholesterol levels. In C57BL/6J mice, we find that TCDD treatment results in a rapid increase in urinary levels of the vasoactive eicosanoids. Such lipid second messengers have been implicated in the manifestation of IHD. On the other hand, hyperlipidemic ApoE-null mice given a single IP injection (12.5 nmol/kg) of TCDD show a partition of TCDD to lipoprotein particles of all densities. This contrasts their wild-type counterparts. These data suggest that lipoprotein particles are likely a vehicle for TCDD delivery to atherosclerotic plaques. This process may exacerbate the severity of IHD. (Supported by NIH P30ES06096, ES08147, ES06273.)

1028 HISTOPATHOLOGICAL CHANGES FOLLOWING SUBCHRONIC AND CHRONIC EXPOSURE TO TCCD IN C57BL/6J FEMALE MICE.

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The aim of this studty is to evaluate the histopathological changes following the subchronic and chronic toxicity of TCDD. Groups of twenty C57BL/6J (bb) female mice were treated orally with daily doses of 0.05 (high), 0.0025 (medium) and 0.005 (low) LD_{50} TCDD/kg for thirteen and twenty six weeks. Control animals only received the corn oil vehicle. Animals were sacrificed on day 92 or 182 following TCDD treatment. Selected tissue samples from the different organs were fixed in 10% buffered neutral formalin and paraffin-embedded. Sections were stained with hematoxylin and eosin and trichrome stains and then evaluated. Selected samples were also analyzed by electron microscopy. Following subchronic and chronic treatment with TCDD, a dose response effect was observed with the low dose and the medium dose of TCDD. TCDD produced major histopathological changes in the liver with less dramatic changes in thymus, kidney, spleen, adrenals, and brain. A striking cholangiopathic and lobular hepatitis was seen in the medium dose following subchronic and chronic exposure with more severe changes present in the chronic treatment group. The thymus showed progressive loss of small lymphocytes in the cortex with a loss of demarcation between the cortex and medulla. The spleen showed foci of extramedullary hematopoiesis and loss of white pulp (small lymphocytes). Histopathology from the kidneys, adrenals and brain, will be presented. Thus, subchronic and chronic TCDD exposure resulted in similar histopathological changes in target organs of TCDD to what is observed following acute exposure.

1029 COMPARISON OF BIOMARKER SENSITIVITY IN PREPUBESCENT FEMALE DEER MICE FOLLOWING EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN.

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TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) causes a broad range of immunotoxic effects including immunosuppression of nonspecific as well as specific cell-mediated and humoral immunity. TCDD administration to experimental animals also results in induction of cytochromes P450 (CYP450). The objectives of this study were to determine the effects of TCDD on immune function and CYP450 activity and to determine which one is the more sensitive biomarker in a non-traditional mammalian species. In this study, 21-day old female deer mice (*Peromyscus maniculatus*) were dosed with 2,3,7,8-TCDD via oral gavage. Five concentrations of TCDD (0.1, 0.3, 1, 3,and 10 μ g/kg) and a corn oil vehicle (control) were used for the dose response study. Immune function was assessed using T- and B-lymphoblastogenesis, and macrophage phagocytosis to determine potential effects on cell-mediate immunity, humoral immunity, and non-specific immunity. Ethoxyresorufin-O-deethylase activity (a CYP450 enzyme), as expected, was increased in activity compared to control. Humoral immune function was suppressed compared to control. However, there was no obvious effect on T-cell proliferation nor macrophage phagocytosis. These results indicate that at levels as low as 0.1 μ g TCDD/kg EROD is significantly induced in this non-traditional mammalian species and that humoral immune function may be an adjunct descriptor of mammalian species exposure to xenobiotics.

1030 TOXICITY OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN (TCDD) UNDER CONDITIONS OF TOXICOKINETIC STEADY-STATE IN ADULT FEMALE SPRAGUE-DAWLEY RATS.

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Groups of ten adult female Sprague-Dawley rats (240-270 g) were administered by gavage 3 different oral doses of TCDD (35, 52.5, and 70 μg/kg of body weight) in corn oil (4 ml/kg). In order to maintain steady-state concentration, rats were injected into the tail vein every 4th day with a maintenance dose of TCDD contained in a stable emulsion (4.5, 6.8, and 9.1 μg/kg for the low, middle and high dose, respectively). The doses were selected to be equipotent to a total dose of 10,000, 7,500 and 5,000 μg/kg of 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (HpCDD). The total number of rats per dosage group (at least 30) and the number of doses administered (at least 8) were chosen in analogy to the HpCDD study (*ibid*). Currently, ten rats of each of the 3 dosage groups are available for evaluation. Rats were weighed and examined daily for signs of wasting/hemorrhage. Mortality was recorded every day between 8 am and 6 pm. As expected all 10 rats died in each of the 3 dosage groups. Time to first mortality and mean time to death, both were proportional to the dose. The product of dose *x* time appears to be reasonably constant although variability (~20%) is higher than in the HpCDD study. This is not surprising considering the smaller number of animals per dosage group and the smaller number of different doses administered as yet in this experiment. These preliminary data in conjunction with our other posters presented here and also in agreement with previous reports support the notion that Haber's Rule of inhalation toxicology probably is a fundamental Law of Toxicology which can be seen only under ideal conditions of experimentation (e.g., toxicokinetic/ toxicodynamic steady state).

1031 AN ALTERNATIVE MECHANISM FOR TCDD INDUCED NEUROTOXICITY.

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We have conducted studies to evaluate the effect of TCDD on perturbing normal developmental gene expression in the brain. The rationale is that differential gene expression is in part, regulated by transcription factors which may be targets for perturbations by TCDD. As reviewed by Zawia et al., (1998) an essential sequence of genes are required to coordinate the differentiation of cells and to promote migration, dendritic arborization, synapse formation, and myelination in the developing nervous system. As such, we hypothesized that intrauterine exposure to TCDD would have deleterious consequences on the developing nervous system. Timed pregnant Sprague-Dawley rats were given single oral doses on gestation day 15 of 0.25, 0.5, and 1.0 μg TCDD/kg body weight. Mobility gel shift analysis of nuclear extract incubations from various brain regions of the resulting pups on postnatal days 3, 5, 10, 15, 20 and 30 with an Sp1 consensus sequence oligonucleotide revealed perturbations in the developmental profile of Sp1 as a result of intrauterine exposure to TCDD. A secondary goal of this work was to relate TCDD induced changes in the Sp1 DNA-binding properties with that of protein product levels. Nuclear extracts from cortex and cerebelli

were analyzed by western blot analysis with anti-Sp1, Sp2, Sp3, and Sp4 antibodies. The results suggest that the observed alterations in the DNA-binding properties of target proteins may not always be the result of perturbations in the biosynthetic processes of that transcription factor. (Supported by NIH G12 RRO 3032 and NIEHS ES00287.)

1032 DOSE-RESPONSE RELATIONSHIP OF SUBCHRONIC DOSING WITH [3H]TCDD ON DOSIMETRY AND CYP1A ACTIVITIES.

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Subchronic dosing of low-level exposures of TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) mimics the most probable mode for human exposure, including dietary consumption. Steady-state kinetics resulting from repeated dosing permits more accurate assessment of the relative potency for TCDD. Ideally, TCDD dosimetry should be evaluated in an animal model under steady-state conditions. The present study was conducted to examine the disposition of TCDD following subchronic oral dosing in female B6C3F1 mice. Doses were 0.15, 0.45, 1.5, 4.5, 15, 45, 150, or 450 ng [3H]TCDD/kg/day (Mon-Fri) for 90 days. Endpoints examined were dosimetry in various tissues and hepatic CYP1A1 and CYP1A2 enzymatic activities. The present study demonstrated dose-dependency of [3H]TCDD in hepatic and non-hepatic tissues. Liver/fat (L/F) concentration ratios range from 0.2 to 3.4 (low to high dose). At higher doses (i.e., induced animals), greater TCDD concentrations were found in liver than in fat—indicating hepatic sequestration due to the inducible binding protein, CYP1A2. At 15 ng/kg/day, L/F conc ratio was 1; CYP1A2 enzymatic activity was ~5 fold ($p<0.0001$) greater than controls. Hepatic CYP1A1 activity was significantly increased ($p<0.02$) starting at the lowest dose (0.15 ng/kg/day) with L/F conc ratio of 0.2 and body burden of 2.7 ng [3H]TCDD/kg. This study provides direct evidence of low dose effects at steady-state conditions on a measured response and body burdens that are within background levels of the general human population. Futhermore, this study emphasizes that body burden or tissue dose for a persistent chemical such as TCDD is more important to consider than daily dose. (This abstract does not necessarily reflect EPA policy.)

1033 EXAMINATION OF GENE EXPRESSION IN CENTRILOBULAR AND PERIPORTAL CELLS AFTER TCDD EXPOSURE BY QUANTITATIVE RT-PCR.

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Previous immunolocalization studies revealed that the hepatic distribution of CYP1A1/1A2, and CYP1B1 was mainly localized to the centrilobular region after subchronic/chronic exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Therefore, in order to examine the differential inducibility of populations of hepatocytes, this study examined the dose-dependent effects of TCDD in unfractionated, centrilobular and periportal cells on gene expression after acute exposure (3 days) of female Sprague-Dawley rats to a single oral dose of 0.01, 0.3 or 10.0 μg TCDD/kg by quantitative RT-PCR. Whole hepatocytes were isolated by a collagenase perfusion technique; whereas centrilobular and periportal enriched-hepatocytes were isolated by a dual pulse digitonin-collagenase perfusion method. Actin mRNA expression in unfractionated, centrilobular and periportal hepatocytes was relatively constant over the dose-range tested. A dose-dependent increase in TCDD-induced CYP1A1, CYP1A2 and CYP1B1 mRNA expression was observed in all liver cell populations. The centrilobular hepatocytes exhibited a 7.0-fold higher concentration of TCDD-induced CYP1A1 mRNA as compared to the periportal hepatocytes within the 0.01 μg TCDD/kg treatment-group with an equal distribution of CYP1A1 mRNA in both cell types at higher doses. Furthermore, CYP1A2 and CYP1B1 mRNA concentrations were higher in centrilobular cells as compared to periportal cells isolated from animals dosed with either 0.01 or 0.3 μg TCDD/kg. This study demonstrates that acute exposure to TCDD results in a differential expression of multiple cytochrome P450 genes in isolated centrilobular and periportal hepatocytes. (Abstract does not necessarily represent USEPA policy.)

1034 COMPARISONS OF RELATIVE POTENCIES (REPs) FOR EROD INDUCTION AND DECREASES IN SERUM THYROXINE FOR TCDD AND PCBS 77, 126 AND 118.

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The TEF methodology has been used to assess the potential health effects of mixtures of dioxin-like chemicals and assumes that the REPs are the same for all endpoints. The present study examines this hypothesis by comparing the REP values for enzyme induction and decreases in serum T4 by TCDD, and two coplanar dioxin-like PCBs 77, 126 and one mono-ortho dioxin-like PCB, 118. Female Long-Evans rats (28 days old) were administered test chemicals by oral gavage on 4 consecutive days and killed on the fifth. Serum T4 and hepatic EROD activity were determined and REPs were estimated based on ED50 ratios. All chemicals induced EROD activity with a similar maximum of approximately 30-40 fold induction and the REPs are 0.07, 0.00005, 0.00003 for 126, 77 and 118 respectively. TCDD and 126 produced a maximal 40-45% decrease in T4 while 77 and 118 produced 80-90% decreases. The REP values for decreases in T4 are similar to those for EROD induction: 0.08, 0.00007 and 0.00005 for 126, 77, and 118. Because 77 and 118 are more efficacious for T4 decreases compared to TCDD, these REP values may not be valid for predicting T4 decreases using the dioxin TEF methodology. REP values based on ED50's do not take into consideration differences in efficacy between chemicals. These data suggest that PCBs 77 and 118 are decreasing T4 concentrations through both Ah receptor and non-Ah receptor mediated mechanisms and that the dioxin TEF methodology may not predict decreases in T4 in mixtures containing PCBs due to the different mechanisms of action of these chemicals. Future work will examine mixtures of these chemicals to examine their interactions (This abstract does not represent EPA policy).

1035 EFFECT OF IN VIVO TCDD EXPOSURE ON SECRETION OF ACTH AND CORTICOSTERONE BY PERIFUSED PITUITARY AND ADRENAL GLANDS.

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Although *in utero* maternal stress can have lasting effects on male rat offspring, chemical-induced alterations of the maternal/fetal hypothalamic-pituitary-adrenal axis (HPA) have not been well studied. This study examined the effects of *in vivo* TCDD exposure on pituitary-adrenal function in perifusion culture. Male and pregnant female (gestation day 8) Sprague-Dawley rats were gavaged with 10 μg/kg TCDD and euthanized 10 days later. Hemi-pituitary and right adrenal glands from the same animal were quartered, placed in separate chambers, perifused at 200 μl/min with Medium 199 (4.4 μM NaHCO3, 10 mM HEPES, 50 μM Bacitracin, 0.3% BSA, pH 7.4, 37° C) and samples collected every 10 min. After two hours of perifusion, glands were stimulated twice, 30 min each, with either 10-9 M CRH (pituitary) or 10-9 M ACTH (adrenal). In males, TCDD caused about a 1.7-fold increase in CRH-stimulated ACTH secretion (4.2 ± 0.69 μg/ml vs 6.8 ± 1.01; 3.9 ± 0.75 μg/ml vs 6.6 ± 1.91 μg/ml, control vs TCDD). No difference was observed in total pituitary or plasma ACTH (3.2 ± 0.69 μg/mg vs 3.5 ± 1.13 μg/mg tissue; 13.5 ± 4.10 ng/ml vs 8.6 ± 1.51 ng/ml, control vs TCDD). In females, there was no difference in CRH-stimulated ACTH secretion (9.5 ± 2.55 ng/ml vs 17 ± 4.05; 10.6 ± 3.84 vs 14 ± 5.07 ng/ml control vs TCDD) or total tissue ACTH (1.4 ± 0.15 μg/mg vs 1.2 ± 0.31 μg/mg tissue, control vs TCDD). ACTH-stimulated CORT secretion was not affected by TCDD in either sex. Adrenal tissue CORT levels were unchanged in males and pregnant females (23.6 ± 4.59 vs 26.9 ± 8.55 ng/mg tissue; 4.4 ± 1.49 vs 2.3 ± 0.31 ng/mg tissue, control vs TCDD). Plasma CORT levels were increased in TCDD treated males but unchanged in pregnant females (553 ± 24.7 vs 691 ± 35.4 ng/ml; 62.8 ± 15.70 vs 48.1 ± 13.43 ng/ml, control vs TCDD). Thus, it appears that in male, but not female rats, TCDD alters pituitary response to CRH which suggests that *in utero* exposure may have a direct affect on male fetus and its adult pituitary response in adulthood. (This abstract does not necessarily represent EPA policy.)

1036 METABOLISM AND DISPOSITION OF LUMINOL IN RATS.

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Luminol (LMN, 3-aminophthalhydrazide) chemiluminesses upon oxidation

and therefore is useful in assays for the detection of a variety of organic and inorganic substances. A major use of LMN is as a forensic tool for detecting biological fluids at crime scenes. Since there is considerable potential for human exposure, the present work has investigated the metabolism and disposition of [ring-^{14}C] LMN in male F344 rats. Results indicated that LMN was well absorbed from the gut following po administration of either 0.5, 5, or 50 mg/kg. The rate of elimination was proportional to dose with greater than 80% total dose excreted in urine within 12 hr post-dosing. Urine contained two radiolabeled metabolites, a glucuronide and a sulfate conjugate of parent LMN. No 3-aminophthalic acid was detected in urine. Little or no ^{14}C was present in tissues at 24 hr post-dosing. Similar results were obtained following iv administration of 0.5 mg/kg. Approximately 8% of an iv administered dose was excreted in bile within 6 hr post-dosing. LMN-derived radioactivity in bile consisted of the same glucuronide and sulfate conjugates isolated from urine. Transdermal absorption of LMN was negligible following application to the skin of 0.1 mg/kg in ethanol. Results of this study indicate that LMN has little apparent potential for bioaccumulation or toxicity at anticipated levels of human exposure.

1037 THE METABOLISM AND DISPOSITION OF 1-NITRONAPHTHALENE IN THE MALE SPRAGUE-DAWLEY RAT.

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1-Nitronaphthalene (1-NN) is a nitroaromatic mutagenic compound and environmental contaminant. 1-NN is mainly found in airborne particulates (0.7 mg 1-NN/kg of particulate) resulting from both light- and heavy-duty diesel engine emissions. 1-NN has been shown to produce both lung and liver toxicity. Even though these toxicities have been reported, the metabolism and disposition of 1-NN have not been elucidated. Studies were performed to characterize the disposition, metabolism, and excretion of 1-NN following i.p. and i.v. administration to male Sprague-Dawley rats. Ninety-six hr following i.p. (100 mg/kg) or i.v. (10 mg/kg) administration of [^{14}C]-1-NN, 55-60% of the dose was recovered in the urine, 32-36% in the feces, O% as expired [^{14}C]-CO_2 and [^{14}C]-organics, and 1% collectively in selected tissues, blood, and GI contents. Interestingly, 88% of the administered dose was excreted into the bile within 8 hr, suggesting extensive enterohepatic recirculation. The elimination rate constants of 1-NN were 1.26 (i.p.) and 0.84 hr^{-1} (i.v.) and elimination half-lives were 0.57 (i.p.) and 0.80 hr (i.v.). The systemic bioavailability of 1-NN following i.p. administration was 3%. Urinary metabolites were seperated by reverse-phase HPLC and identified by LC-MS/MS. The major metabolite following both routes of administration was identified as *N*-acetyl-*S*-(hydroxy-1-nitronaphthalene)-L-cysteine, which accounted for 26% (i.p.) and 18% (i.v.) of the dose. Hydroxylated (5-8%), dihydroxylated (1%), glucuronidated (1-2%), and sulfated (3-5%) metabolites were also identified in the urine. The presence of glucuronide and sulfate metabolites was confirmed by enzymatic hydrolysis. From these data, it appears that 1-NN undergoes extensive metabolism, enterohepatic circulation, and is rapidly eliminated. (Southwest Environmental Health Sciences Center P30-ES-06694.)

1038 INDUCTION OF RAT HEPATIC DRUG METABOLIZING ENZYMES BY DIMETHYLCYCLOSILOXANES.

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Low molecular weight dimethylcyclosiloxanes (DMCS) are important precursors in the synthesis of polydimethylsiloxane polymers widely used in industry and in personal care products. The objective of this study as to characterize the ability of the small DMCS, octamethylcyclosiloxane (D4) and decamethylcyclopentasiloxane (D5) to induce drug metabolizing enzymes in rats. Male Sprague Dawley rats were administered D4 or D5 in corn oil daily by gavage at different doses and for different times. Changes in the levels of activity and/or immunoreactivity of CYP6R2B1/2, CYP1A1/2, CYP3A2 and NADPH cytochrome P450 reductase in liver microsomes were determined. A dose response in CYP2B1/2 induction was observed with increasing concentrations of both D4 and D5. Significant increases in 7 pentoxyresorufin–O–depentylase (PROD) activity and CYP2B1 immunoreactive protein were detected after 4 days treatment with D4 and D5 doses 5 mg/kg. At higher doses, D4 induced PROD activity and CYP2B1 protein levels more efficiently than D5. Small but significant increases were also observed in 7–ethoxyresorufin–O–deethylase (EROD) activity at the same concentrations. Significant induction of reductase immunoreactive protein was observed D5 but not D4. To determine the time course of induction, rats were treated daily with 5 mg/kg D4 and sacrificed after 4, 8, 14 and 21 days. PROD activity was significantly increased after 4 days (2.4–fold) and 8 days (4.4–fold) and was slightly higher after 21 days (5.2–fold). Immunoreactive CYP2B1 levels increased to 8 days then remained relatively constant. In summary, D4 and D5 induced drug metabolizing enzymes in rat liver, especially YP2B, in manner similar to phenobarbital.

1039 REDUCTIVE BIOACTIVATION OF HCFC-141b TO FREE RADICAL INTERMEDIATES BY RAT LIVER MICROSOMAL CYTOCHROME P-450.

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The hydrochlorofluorocabon 1,1-dichloro-1-fluoroethane (HCFC-141b) is reductively metabolized to reactive species by rat liver microsomal cytochrome P-450 (P-450) resulting in the loss of the enzyme's activity and content, due to inactivation of its prosthetic heme group1. Since the addition of the spin trapping agent *N*-t-butyl—phenylnitrone (PBN) prevented P-450 loss, suggesting that free radical intermediates of HCFC-141b may be involved, we investigated whether free radical species a) are actually formed, by an electron spin resonance (ESR) technique, and b) are involved in the mechanism of the inactivation reaction, by measuring the level of conjugated dienes. Anaerobic incubations of liver microsomes from pyridine induced rats, at 37°C for 30 min, in presence of the substrate and of the spin trapping agent PBN resulted in the formation of an ESR spectrum compatible with that of a carbon centered radical intermediate. Similar incubations, but for the absence of PBN and in presence of increasing concentrations of substrate (0.1-10 mM), showed a dose-dependent formation of conjugated dienes as measured by the peak formed at λ=233 nm in the second derivative spectrum2. Conjugate dienes formation was inhibited by the addition of saturating concentrations of CO but not by 10 mM GSH or vit C. Our results indicate that HCFC-141b is reductively metabolized by P-450 2E1 to free radical intermediates leading to the modification of microsomal membrane lipids, a process which may contribute to the previously reported HCFC-141b-dependent inactivation of microsomal P-450.

1. Tolando et al (1996) *Xenobiotica*, 26: 425-435.
2. Corongiu & Milia (1983), *Chem Bio Interact*, 44: 289-297.

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1040 DETECTION OF THE DICHLOROACETATE RADICAL FROM THE REDUCTIVE DEHALOGENATION OF TRIHALOACETATES.

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The risk assessment of trichloroethylene (TRI) is currently being driven by the carcinogenic properties of its reported metabolite, dichloroacetate (DCA). The formation of DCA is hypothesized to proceed through the reductive dehalogenation of trichloroacetate (TCA), via a free radical intermediate. Although proof of this radical has been elusive, the detection of fully dechlorinated metabolites in the urine and the formation of lipid peroxidation by-products in microsomal incubations with TCA argue for its existence. We report here the trapping of the dichloroacetate radical with the spin trapping agent PBN, and its identification by GC/MS. By employing bromodichloroacetate (BDCA), a mono-brominated analog of TCA, to generate a high yield of the dichloroacetate radical, initial studies on its formation and on the subsequent characterization of the PBN adduct could be performed. The PBN/dichloroacetate radical adduct was found to undergo an unexpected intramolecular rearrangement during its extraction into organic solvent. A condensation reaction is hypothesized to eliminate an OH radical, thus rendering the PBN/adduct undetectable by ESR, the standard analytical tool for free radical detection. The dichloroacetate radical has been identified by GC/MS in both a chemical Fenton system and in murine microsomal incubations. (Supported by EPA grant R825954.)

1041 INDUCTION OF QUINONE REDUCTASE BY CRAMBENE, A NITRILE FROM CRUCIFEROUS VEGETABLE, IN HEPA 1c1c7 CELLS.

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Previously, we have shown that oral crambene (1-Cyano-2-hydroxy-3-butene) induces quinone reductase in the rat liver. Here we show that crambene is also active in a cell culture system. Mouse hepatoma cells, Hepa 1c1c7 cells, were cultured in 12 well plates with crambene in DMSO (0.1%), and β-naphthoflavone (2-5 μM) was used as positive control. Cells were analyzed for induction of quinone reductase and reported as specific activity per 10^6 cells. When cells were treated with β-naphthoflavone for 24 h at different times after plating (1 x 10^5 cells/well), they showed maximal induction if treated at approximately 3 x 10^5 cells per well, or 30% confluence. In all subsequent experiments, cells were treated when there were approximately 3 x 10^5 cells per well and then grown for 24 hours. Crambene (1 μM-1 mM) was incubated with Hepa 1c1c7 cells for 24 hours. This resulted in a typical dose-respond curve for quinone reductase induction. A higher concentration of crambene (10 mM) showed cytotoxicity, but only when DMSO was included. This indicates that DMSO (0.1%) increases the uptake of crambene into the cell. DMSO (0.1%) alone or with β-naphthoflavone caused no cytotoxicity (>95% viability). Crambene is present naturally in cruciferous vegetables and its ability to induce quinone reductase may be partly responsible for the effect of cruciferous vegetables in protecting against cancer. That induction occurs in cell culture as well as in the whole animal permits cell culture to be used in future mechanistic studies. (Supported by The Illinois Council on Food & Agricultural Research.)

1042 METABOLISM AND TOXICITY STUDIES ON NOVEL QUINOLINE QUINONES: QUINONE ACTIVATION BY NAD(P)H:QUINONE OXIDOREDUCTASE.

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We report on the effects of functional group changes on the metabolism of novel quinoline quinones by human recombinant NAD(P)H:quinone oxidoreductase (NQO1) and on the toxicity of these agents to non-small cell lung cancer (NSCLC) cells with either high NQO1 activity (H460) or with no detectable NQO1 activity (H596). Various substituents were added to the 2- and/or 6-positions of quinoline-5,8-dione. Overall, the quinoline quinones were much better substrates for NQO1 than analogous indolequinones. For example, the reduction rate for 5-methoxy-1,2-dimethylindole-4,7-dione, was 5.31 ± 0.93 μmol/min/mg whereas 6-methoxyquinoline-5,8-dione was reduced at a rate of 58.7 ± 18.5 μmol/min/mg. Substitution at the 2-position with phenyl, naphthyl or biphenyl yielded the lowest reduction rates for quinoline quinones, while heterocycles such as pyridyl were among the best substrates. The prototype quinoline quinone bioreductive agent, streptonigrin (SN), has a pyridyl group at the 2-position and is reduced by NQO1 at a rate of 47.4 ± 11.5 μmol/min/mg. The quinoline quinones were relativley non-toxic to both cell lines with IC_{50} values generally > 10 μM. The most selective compound, 6-(pyrrolidin-1-yl)quinoline-5,8-dione, was thirteen times more toxic to the high NQO1 H460 cells (IC_{50} = 3.72 μM) than to the NQO1-deficient H596 cells (IC_{50} > 50 μM). On the other hand, SN is over 90 times more toxic to the H460 cells (IC_{50} = 0.0143 μM) than to the H596 cells (IC_{50} = 1.36 μM). This is likely due to differences in the capacity of the quinoline quinones to form complexes with metals and DNA.

1043 POTENTIATION OF ACETAMINOPHEN-INDUCED HEPATOTOXICITY BY RETINOL IN THE BALB/C MOUSE.

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Previous results have shown that retinol pretreatment potentiates and attenuates various classical hepatotoxicants in male Swiss Webster mice. This study was designed to determine if retinol treatment would potentiate acetaminophen (APAP) hepatotoxicity in another mouse strain. Once this was established, we targeted the various metabolic pathways for acetaminophen metabolism to determine the mechanism of retinol's potentiation. 4 days of retinol (75 mg/kg/d) was required to potentiate APAP hepatotoxicity (ALT activity of 1954.1 ± 280 vs 4656 ± 951 for vehicle and retinol treatments, respectively, $p<0.05$). Hepatic microsomes prepared from all treatment groups in the 4 day retinol potentiation model were used to determine activity and polypeptide levels for cytochrome P450 enzymes. Results showed that Total hepatic P450 was not altered by 4 days of retinol treatment (1.04 ± 0.19 vs. 1.12 ± 0.08 nmol/mg for retinol and untreated respectively). Aniline hydroxylation and p-nitrophenol hydroxylation (p-NP) demonstrated that 4 days of retinol treatment did not alter P4502E1 catalytic activity compared to untreated or vehicle treated mice (aniline, 4.10 ± 0.74 vs 4.22 ± 0.41 nmol/mg/min and p-NP, 2.1 ± 0.19 vs 2.6 ± 0.49 nmol/mg/min for retinol and untreated respectively). Since glutathione (GSH) is the major detoxification route for APAP, the effect of retinol on GSH was also measured. The results showed that retinol treatment itself did not alter hepatic GSH. These results demonstrate that retinol treatment is not altering 2 of the metabolic pathways for APAP. However, we must determine if retinol alters APAP associated reductions in GSH via oxidative stress and we must determine if P450 catalytic activity is altered in APAP vs APAP + retinol treated groups. (Supported by a Laurenson Award from the Otago Medical Research Foundation.)

1044 CHEMICAL INDUCTION OF RAT UDP-GLUCURONOSYL TRANSFERASE (UGT) mRNAs RESPONSIBLE FOR THYROID HORMONE METABOLISM.

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Treatment of rats with the hepatic enzyme inducers phenobarbital (PB), pregnenolone-16α-carbonitrile (PCN), 3-methylcholanthrene (3-MC), and Aroclor 1254 (PCB) has been shown to decrease circulating levels of thyroid hormones as well as increase microsomal glucuronidation of thyroxine. Members of both the UGT1 and UGT2 families have been suggested to contribute to thyroid hormone glucuronidation. The purpose of this study, then, was to determine whether the aforementioned chemical inducers cause specific increases in the messenger RNAs that encode two UGT isoforms in rats, UGT1A1 and UGT2B2. Male Sprague-Dawley rats were placed on control diet or diet containing PB (800 ppm), PCN (1000 ppm), 3-MC (250 ppm), or PCB (100 ppm) for seven days, at which time livers were collected for RNA isolation. Analysis of thyroid hormones in serum samples revealed significant decreases in T_4 concentrations, with PCB treatment having the largest effect. No changes in T_3 were caused by any treatment. Increases in mRNA were examined by northern-blot analysis using a 100 bp synthetic probe for UGT1A1, and a specific full-length cDNA probe for UGT2B2. An increase in UGT1A1 mRNA of just over 3 fold was found in PCN-treated animals, as well as an increase in UGT2B2 mRNA of nearly 3 fold. Little or no increase in mRNA for UGT1A1 or UGT2B2 was found for animals treated with other inducers. These findings suggest that decreases in circulating T_4 following PCN treatment in rats may be due to increases in thyroid hormone glucuronidation by UGT1A1 and UGT2B2, but that induction of other UGT isoforms may be important for increases in glucuronidation caused by PB, 3-MC, and PCB. (Supported by NIH Grants ES-07079 and ES-008156, and EPA Grant R826297.)

1045 METABOLISM AND PHARMACOKINETICS OF IBUPROFEN IN THE CHIMPANZEE.

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Ibuprofen, a commonly used nonsteroidal antiinflammatory nonprescription drug is also an effective analgesic and antipyretic. Ibuprofen was included in a series of studies evaluating the chimpanzee as a surrogate human model for examining the metabolism and pharmacokinetics of xenobiotics. Three male and 3 female chimpanzees (10 to 14 years; 35 to 56 kg) were administered a single oral dose, 10 mg/kg, of ibuprofen. Whole blood was collected predose, 20, 40, 60 and 90 min, 2, 3, 4, 6, 8, and 24 h postdose. Plasma was harvested and analyzed by gas chromatography with mass spectrometric detection after the addition of a suitable internal standard. Plasma half-life ($t_{1/2}$; h) averaged 2.03 ± 0.16, quite similar to 2.0 ± 0.5 in humans. Clearance (Cl; L/kg•h) was 0.0307 ± 0.0073 in chimpanzees as compared to 0.0426 ± 0.0010 in humans. These data indicate that the chimpanzee may prove to be an excellent model for human pharmacokinetic studies.

1046 METABOLISM AND DISPOSITION OF ARSENITE (AsIII) AND ITS RELATIONSHIP TO HEME OXYGENASE (HO) INDUCTION IN MICE.

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HO is the rate limiting enzyme in heme degradation and it has a significant impact on intracellular heme pools. Rat studies indicate that HO induction is a sensitive, dose-dependent response to AsIII exposure in both liver and kidney. The objective of this study was to evaluate the relationship of HO induction and tissue levels of inorganic arsenic (iAs) and its metabolites in mice because human AsIII disposition and kinetics are more similar to mice compared to rats. Female B6C3F1 mice were given a single oral dose of 0, 1, 10, 30 or 100 μmol/kg AsIII. Levels of inorganic, mono- (MMA) and dimethylated arsenic (DMA) as well as HO activity were determined in liver and kidney 6 hr post dosing since HO induction peaks at 4 to 8 hrs after a single oral dose of 100 μmol/kg AsIII in mice. There was a significant dose-dependent trend of increasing HO induction in both liver and kidney with HO induction being ~two-fold higher in liver. Inorganic As was the predominant form of arsenic (As) in liver at all doses, whereas DMA was the predominant form of As in kidney at all doses. Three- to 4-fold higher levels of iAs were achieved in liver compared to kidney. MMA was the least abundant form of As in both liver and kidney, never exceeding more than 20% of the total As present. The concentration of iAs, rather than mono- or dimethyl As, demonstrated the strongest linear correlation with HO activity in both liver (r=0.9988) and kidney (r=0.9705). Results of this study suggest that HO induction is a biomarker of effect that is specific for tissue iAs since a maximally tolerated dose of DMA (5220 μmol/kg) was ineffective in inducing HO in mice. HO induction thus has potential for use as a biomarker of inorganic arsenic internal dose to target tissue and can be used as a tool to further the development of a biologically-based dose response model for As. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

1047 DIVERSITY OF HEPATIC ARSENITE METHYLTRANSFERASE AND ARSENATE REDUCTASE ACTIVITIES AMONG NEW AND OLD WORLD PRIMATES: AN IN VITRO STUDY.

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Common laboratory animals respond with a great degree of metabolic variation when challenged with inorganic arsenic exposure. Patterns of arsenic metabolic diversity are also beginning to emerge among human populations. This study investigated an evolutionary-related group of animals to determine whether distinctions could be made to explain the diversity in the metabolism of inorganic arsenic among mammals. A total of 30 animals representing 17 different species within the phylogenic order Primates were examined for hepatic cytosol arsenite methyltransferase and arsenate reductase activities. Three *Macaca sp.* (Rhesus, pig-tailed and long-tailed) and one of three *Saimiri sciureus* (squirrel monkey) animals demonstrated arsenite methyltransferase activity. The remaining 13 primate species were deficient of arsenite methyltransferase activity. The diversity of arsenite methyltransferase activity could not be related to dietary differences, geographical habitat or evolutionary relatedness among the primate species surveyed. All primate species examined showed arsenate reductase activity, with wide variation within and between species. There was no correlation between cytosolic sulfhydryl concentration and the amount of arsenate reductase activity in these cytosols. (Supported in part by the Superfund Basic Research Program NIEHS Grant ES-04940.)

1048 TERT-BUTYL HYDROPEROXIDE SUPPORTS LIPOXYGENASE-MEDIATED XENOBIOTIC OXIDATION.

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This study tests the hypothesis that organic hydroperoxides support xenobiotic oxidation mediated via the lipoxygenase pathway. Soybean lipoxygenase (SLO)-catalyzed oxidation of 3,3',5,5'-tetramethylphenylenediamine (TMPD), 3,3'5,5'-tetramethylbenzidine (TMBz), benzidine, guaiacol, and pyrogallol was evaluated in the presence of tert-Butyl hydroperoxide. Oxidation of TMPD to Wurster's Blue Free Radical was found to be proportional to the concentration of SLO, TMPD, and tert-Butyl hydroperoxide, and was linear with time. These results suggest the enzymatic nature of the reaction. TMPD exhibited the highest specific activity, while the lowest rate of oxidation was noted with guaiacol. The optimal conditions required to observe the maximal rate of oxidation of TMPD included: pH 8.5, 1.0 mM tert-butyl hydroperoxide, 1.25 mM TMPD, and 20 μg of SLO. Similar to tert-Butyl hydroperoxide, cumene hydroperoxide also supported oxidation of these xenobiotics. In contrast to the previously reported acidic pH optimum (pH 6.5) for hydrogen peroxide supported xenobiotic oxidation by SLO, both organic hydroperoxides displayed maximal xenobiotic oxidation rates under alkaline conditions (pH 8.5). The classical inhibitors of lipoxygenase viz. nordihydroguaiaretic acid (NDGA), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and gossypol, significantly inhibited the oxidation of TMPD, TMBz, benzidine, guaiacol, and pyrogallol. These results support the contention that SLO can metabolize xenobiotics in the presence of organic hydroperoxides.

1049 LIPOXYGENASE-MEDIATED HYDROGEN PEROXIDE-DEPENDENT N-DEMETHYLATION OF XENOBIOTICS.

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The hydrogen peroxide dependent N-demethylation capability of soybean lipoxygenase (SLO), a model enzyme, and human term placental lipoxygenase (HTPLO) was evaluated. Formaldehyde (HCHO), the product of N-demethylation, accumulated in the reaction media was estimated using Nash reagent. Model compounds, drugs, and pesticides, were tested as substrates. The reaction was dependent on the incubation time, pH of the medium, amount of enzyme, concentrations of the substrate and hydrogen peroxide. Under optimal assay conditions, the specific activity (nmoles HCHO/min/mg protein) of the SLO catalyzed N-demethylation of N, N-dimethylaniline was 200. Specific activities for phenothiazines tested ranged from 10 to 828, with promethazine displaying the highest activity. N-demethylation of a variety of pesticides yielded rates between 7 and 144, with aminocarb posting the highest value. Imipramine and related tricyclic antidepressants reacted at rates between 9 (Doxepin) and 210 (Triipramine). The SLO mediated reaction of aminopyrine showed a specific activity of 823, while the HTPLO supported aminopyrine oxidation showed a specific activity of 16. The aminopyrine cation radical, the presumed intermediate absorbing at 565 nm was detected in both systems. Under similar conditions, HTPLO catalyzed N, N-dimethylaniline N-demethylation at a rate of 33, while chlorpromazine exhibited a rate of 6. The results suggest that xenobiotics undergo N-demethylation via the lipoxygenase pathway, with significant substrate specificity.

1050 IN VITRO BIOTRANSFORMATION OF XANTHOHUMOL BY RAT LIVER MICROSOMES.

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Xanthohumol, a flavonoid from hops (*Humulus lupulus*) has been shown in various *in vitro* tests to have cancer chemoprevention properties. The flavonoid inhibits the growth of human breast, ovarian, and prostate cancer cells and also inhibits a number of carcinogen-activating human cytochrome P450s. We investigated the conversion of xanthohumol to various metabolites by rat liver microsomes. Two major metabolites were found with hepatic microsomes from untreated rats as detected by HPLC analysis. The reduced metabolite levels produced in incubations containing P450 inhibitors or cytochrome P450 reductase antibody suggest that cytochrome P450s were primarily responsible for the observed metabolites. Microsomes from phenobarbital-pretreated rats produced the same metabolites but changed their relative proportions. However, microsomes from β -naphthoflavone- and isosafrole-pretreated rats produced a new nonpolar metabolite in addition to the other two. As determined by LC-MS and ^{1}H-NMR analysis, two of the xanthohumol microsomal metabolites were tentatively identified as 5''-(2-hydroxyisopropyl)-[2'',3'':3',4']dihydrofurano-2',4dihydroxy-6'-methoxychalcone and a dehydrocycloxanthohumol derivative. The biological activity of these metabolites and their involvement in cancer chemoprevention remain to be established. (Supported by the Hop Research Council and NIEHS grants ES00210.)

1051 THE *IN VIVO* DISPOSITION AND METABOLISM OF METHYLEUGENOL IN THE FISCHER 344 RAT AND THE B6C3F$_1$ MOUSE.

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Methyleugenol is an alkenylbenzene compound found in many essential oils. It is added to cosmetics, soaps, fragrances, and herbal products as well as being found naturally in some foods. While human exposure to this compound is limited to low levels, its use in many products makes it likely that exposure is relatively constant. Therefore, the metabolic fate and possible activation of this compound *in vivo* is of interest. Studies were undertaken to examine the absorption, distribution, metabolism, and excretion of the compound following oral and intravenous (I.V.) administration to male Fischer 344 rats and oral administration to female B6C3F$_1$ mice. Following oral administration of [^{14}C]-methyleugenol (118 mg/kg, 50 uCi/kg) to rats, approximately 72% of the administered radioactivity was recovered in the urine and 13% in the feces within 72 hr. After I.V. administration (11.8 mg/kg, 120 uCi/kg) to rats, approximately 85% was recovered in urine and 8% in feces by 72 hr. In rats, following oral administration, no significant amount of [^{14}C]-equivalents could be detected in the blood. Following I.V. administration, methyleugenol and two possible metabolites were seen in the blood by HPLC; both metabolites were below the limit of detection within 50 min and were no more than 2.5% of the total dose at their peak in the blood at 8 min. Methyleugenol itself disappeared from the blood by 6 hr and was almost totally cleared from the blood by 1 hr. Similar to the rat, when methyleugenol (118 mg/kg, 50 uCi/kg) was administered orally to mice, 85% was recovered in urine and 6% in feces at 72 hr. Major urinary metabolites for both species were quantified by HPLC radioassay. Preliminary identification of metabolites by LC-MS/MS suggest the presence of sulfate and glucuronide conjugates of hydroxylated derivatives and a mercapturic acid. These results indicate that methyleugenol is almost totally metabolized following an oral dose in both species, and is quickly eliminated from the body. (This research was supported by NIEHS (NO1-ES-35367, P30-ES-06694 and T32-ES-07091-17).)

1052 THE DISPOSITION OF ISOEUGENOL IN THE MALE FISCHER-344 RAT.

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Isoeugenol is an oily liquid found in a number of plant oils including clove, bay leaves and pimento. Because of its clove-like fragrance, it is used in a number of consumable products including beverages, baked goods and chewing gums, as well as home and personal products such as soaps, detergents and perfumes. Although exposure to humans appears to be low but continual, no information is available on the disposition and metabolism of isoeugenol. Therefore the primary objective of these studies was to determine the absorption, distribution, metabolism and excretion of isoeugenol following oral and intravenous administration to male Fischer-344 rats. Following a single oral dose of [^{14}C]-isoeugenol (156 mg/kg, 50 μCi/kg), greater than 85% of the administered dose was excreted in the urine as sulfate or glucuronide metabolites by 72 hr. Approximately 10% was recovered in the feces, and less than 0.1% was recovered as CO_2 or expired organics. No parent isoeugenol was detected in the blood at any of the time-points analyzed. Following intravenous administration of a single dose of [^{14}C]-isoeugenol (15.6 mg/kg, 100 μCi/kg), isoeugenol was cleared from blood within one hr. Within 72 hr following intravenous administration, 82% of the total administered radioactivity was excreted as sulfate or glucuronide conjugates into the urine, 10% was recovered in the feces, and less than 0.1% was recovered as CO_2 and expired organics. The total amount of radioactivity remaining in selected tissues was less than 0.25% following either oral or intravenous administration. The results of these studies show that isoeugenol is rapidly metabolized and is excreted predominantly in the urine as phase II conjugates of the parent compound. (This research was supported by NIEHS (N01-ES-35367, and P30-ES-06694).)

1053 METABOLISM OF STRUCTUAL ANALOGS OF EUGENOL BY PULMONARY CYTOCHROME P450 ENZYMES.

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Eugenol, a food additive and dental anesthetic, has been shown to be a potent pulmonary toxicant. The toxicity of eugenol is dependent on cytochrome P450-mediated desaturation to a quinone methide reactive intermediate that can alkylate cellular macromolecules by a Michael-like addition reaction. The metabolism of structural analogs of eugenol, such as 4-ethyl-methoxyphenol (EMP), by rat liver microsomes, has been previously studied (Bolton, J.L., *et. al.*). A cytochrome P450-dependent quinone methide of EMP can be conjugated with glutathione at the exocyclic position resulting in chromatographically separable diastereomers. The present investigation utilizes goat lung microsomes as a metabolic model system, for the study of the reactive intermediates of the EMP. In addition to the previously characterized benzylic isomers of the glutathione conjugate of EMP, additional conjugates were formed. Incubations performed in the absence of glutathione produced a single metabolite that was depleted in the presence of gluathione. This metabolite has UV spectral characteristics that might be expected of a 3,4-substituted styrene. This metabolite may represent the product of a unique cytochrome P450-dependent terminal dehydrogenation of the alkyl substituent of EMP. Thus, pulmonary cytochrome P450 enzymes appear to uniquely catalyze the terminal dehydrogenation of EMP, a reaction that does not usually occur with hepatic cytochrome P450 enzymes. (Supported by USPHS Grant #HL13645.)

1054 AFLATOXIN B1 OXIDATION BY HUMAN CYTOCHROMES P450 CYP1A1, 2A6, 2C8, 2C9, 2C18 and 2C19.

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Several human cytochrome P450 (CYP) isoenzymes are known to metabolize the potent hepatocarcinogen aflatoxin B1 (AFB). However, the physiological relevance of individual CYPs is somewhat controversial. To clarify the situation, we examined the capacity of recombinant human CYPs 1A1, 2A6, 2C8, 2C9, 2C18 and 2C19 to metabolize AFB. All CYPs examined metabolized AFB to the genotoxic *exo*-aflatoxin-8,9-epoxide (AFBO) to varying degrees (2C18>1A1>2A6>2C19 ~2C8>2C9). In addition, CYP1A1 was very efficient in forming AFM1. The activities of CYPs 1A1 and 2C18 were sufficiently high to carry out kinetic analysis. The Kms for CYPs 1A1 and 2C18 with respect to AFBO formation were 55 ±33 (A.S.E.) and 298 ±33 μM and the corresponding Vmax values were 180 ±32 and 1144 ±66 pmol/min/nmol respectively. In addition, CYP1A1 possessed a Km and Vmax for AFM1 formation of 62 ±6 μM and 12,151 ±375 pmol/min/nmol respectively. Our data suggest that of the CYPs examined, only CYP1A1 is likely to metabolize AFB at low physiologically encountered concentrations. In contrast to liver, CYP1A1 is expressed at significant levels in lung, which maybe a target organ following AFB inhalation of contaminated grain dust. As CYP1A1 forms approximately 70-fold more of the detoxification product AFM1 relative to the genotoxic metabolite AFBO, it can be considered mainly a detoxification enzyme. (Supported by NIH ES 05780, ES 07033, GM32165 and 49054.)

1055 MICROSOMAL EPOXIDE HYDROLASE IS ASSOCIATED WITH, BUT NOT NECESSARY FOR, VITAMIN K$_1$ EPOXIDE REDUCTASE ACTIVITY.

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Microsomal epoxide hydrolase (mEH) has been identified as a major component of the partially purified Vitamin K$_1$ epoxide reductase (VKOR) multi-protein complex by criteria of co-elution of hydrolase and reductase activity, Western blotting, and N-terminal micro-sequencing of a predominant 50 kD protein isolated from the VKOR preparation. Km values of the partially purified VKOR complex for hydrolysis of styrene 7,8-oxide were similar to those for homogeneous mEH itself. A functional role for mEH in

VKOR activity was suggested by experiments showing that anti-mEH antiserum inhibited up to 80% of the VKOR activity of the partially purified reductase preparation. However, over-expression of mEH in V79 fibroblast cells did not produce a concomitant increase in VKOR activity. To directly assess the possible functional role of mEH in VKO reduction, we compared VKOR activity in hepatic microsomes isolated from wild-type and mEH-null ("mEH knockout") mice. Results of these experiments show that VKOR activity in mEH knockout microsomes is comparable to that present in wild type microsomes. Furthermore, anti-mEH antibody inhibits VKOR activities in both wild type and mEH knockout microsomes. We observe a protein in the knockout microsomes that is immunoreactive with anti-mEH antibody, and postulate that this protein may be required for VKOR activity.

1056 SELECTIVE HYDROLYSIS OF FATTY ACID MONOEPOXIDES INTO TOXIC *VICINAL* DIOLS BY SOLUBLE EPOXIDE HYDROLASE.

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The goal of this research was to test the hypothesis that soluble epoxide hydrolase (sEH) selectively hydrolyzes isomers of linoleate monoepoxides into toxic *vicinal* diols. By studying sEH metabolites of oxidized lipids, insight can be gained into the selective chemistry and the mechanism of action of this enzyme. The regioselectiviy of sEH for leukotoxin (LTX, 9,10-epoxy-12Z-octadecenoate) and isoleukotoxin (iLTX, 12,13-epoxy-9Z-octadecenoate) was determined. This was accomplished by developing and comparing analytical methods to separate epoxide and diols isomers, synthesizing standards for analysis, and optimizing the incubation assay and extraction method. It was found that both the mouse and human recombinant sEH have the same selectivity and that there is no preference by these enzymes for one regioisomer over the other. These data indicate that the mouse is a good animal model for humans in these studies. Also, the cytotoxicity of the linoleate diol regioisomers formed by sEH were measured *in situ*. In this assay, LTX and iLTX were added to cells expressing human sEH and cell death was measured after 24h. The LC_{50} for both regioisomers is similar, 156 uM (range of 155-157 uM) and 153 uM (range of 152-155 uM) for LTX and iLTX, respectively. LTX and iLTX were found to be non-toxic to cells expressing a control enzyme. Since the metabolism of LTX and iLTX by sEH appears to be non-preferential, and these metabolites have equivalent toxicity in this system, then a general sEH inhibitor could protect the system from diol toxicity.

1057 STRUCTURE-ACTIVITY RELATIONSHIPS OF EPOXY FATTY ACIDS.

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We have previously noted that leukotoxin (9,10-epoxy-12Z-enoic acid, methyl ester; ltx), an epoxy fatty acid correlated with adult respiratory distress syndrome, is toxic in various cell and mammalian systems (Moghaddam *et al. Nat. Med.* 3, 562-566 (1997)) when soluble epoxide hydrolase is expressed. The purpose of this research was to characterize structural elements necessary for toxicity. We hypothesized that: (1) altered alkyl chain length; (2) increased ester length; or, (3) a change in position of the epoxide would decrease toxicity. Conversely, we hypothesized that: (4) substitution of the carbonyl; or, (5) epoxide hydrolysis would increase toxicity. Eighty compounds were used to test these hypotheses. Compounds were either purchased or synthesized using standard oxidation and hydrolysis reactions, purified by flash chromatography and verified by TLC, GC-FID, GC-MS and NMR. Toxicity was assessed using a model system of eukaryotic cells expressing either transgenic human soluble epoxide hydrolase (hsEH) or β-galacatosidase (lacZ) as a control enzyme. We found that chain length, ester size, change in epoxide position, carbonyl substitution and epoxide hydrolysis all altered toxicity. Notably, we found: (1) significant differential toxicity, due to expressed enzyme, was only observed with 17-19 carbon compounds with internal epoxides; (2) increased ester length was strongly correlated with decreased toxicity; (3) hsEH protected cells exposed to compounds with terminal epoxides; (4) mixed results were observed with carbonyl substitution. In cases where the epoxide was toxic only to hsEH expressing cells, (5) the corresponding diol was toxic to cells expressing both enzymes, suggesting that these alkyl epoxides are pro-toxins. Overall these results strongly suggest that minor structural changes have major effects on the potency and mode of action of these compounds. This knowledge could lead to a means of reducing leukotoxin toxicity in humans.

1058 METABOLISM OF BUTADIENE MONOEPOXIDE BY FRESHLY ISOLATED HEPATOCYTES FROM MICE AND RATS.

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Butadiene monoepoxide (BMO) is the primary oxidative metabolites of the commonly-used petrochemical 1,3-butadiene (BD), and is thought to play a major role in BD-induced carcinogenicity. Studies of BMO metabolism in subcellular fractions have demonstrated formation of both bioactivation products, such as diepoxybutane (DEB), and detoxification products, such as 3-butene-1,2-diol (BDD) and GSH conjugates. The current study investigates metabolism of BMO in freshly isolated hepatocytes from B6C3F1 mice and Sprague-Dawley rats. Formation and elimination of BMO metabolites were examined over time (45 min) at low (5 μM), intermediate (25 μM), and high (250 μM) BMO concentrations. Hepatocytes from both species retained > 80% viability at these BMO concentrations throughout the experiments. To facilitate comparison, time course data were expressed as area-under-the-curve (AUC). In mouse hepatocytes, *meso*-DEB, *(±)*-DEB, and BDD were detected by GC/MS at all BMO concentrations. At the low BMO concentration, oxidation to DEB predominated over BDD formation, whereas at the high BMO concentration, hydrolysis to BDD became the major pathway. In rat hepatocytes, AUCs for DEB formation were 10 to 20-fold lower than in mice, and *meso*-DEB was detectable only at the high BMO concentration. The opposite was observed for BDD, with AUCs being much greater than seen in mouse hepatocytes. Elimination of *(±)*-DEB (10 μM) and BDD (30 μM) in both species could described empirically as pseudo-first order processes. Although methods to assess the contribution of GSH conjugation to BMO metabolism in hepatocytes are still under development, the results of our experiments demonstrate the ability of the isolated hepatocyte model to provide detailed information on coordinated metabolism of BMO. (Supported by NIEHS Grant R01 ES06841.)

1059 INTERRELATIONSHIPS AMONG FATTY ACID ETHYL AND METHYL ESTER SYNTHASES AND FATTY ACID ANILIDE SYNTHASE IN HEPG2 AND AR42J CELLS.

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We have previously demonstrated that fatty acid ethyl ester synthase (FAEES), purified from rat liver microsomes, is structurally and functionally similar to rat liver microsomal carboxylesterase (CE, pI 6.1) and suggested its role in conjugation of fatty acids with a variety of xenobiotic alcohols [Kaphalia et al., (1997) Chem. Res. Toxicol. 11, 211-218]. In the present studies, we further determined interrelatioship among various hepatic and pancreatic enzyme(s) involved in the biosynthesis of fatty acid ethyl esters (FAEEs), fatty acid methyl esters (FAMEs) and fatty acid anilides (FAAs) using HepG2 and AR42J cells. Incubation of HepG2 cells with tri-O-tolyl phosphate (TOTP), a specific inhibitor for hepatic S-esterase, resulted in a dose (0.1 - 5.0 FM)- and time (0.25-24 hr)- dependent inhibition of FAEE, FAME and FAA synthesizing activities in an identical manner. The optimum inhibition for FAEE, FAME and FAA synthesizing activities was found to be 86, 92 and 87%, respectively, in HepG2 cells following exposure to 2.5 FM TOTP for 24 hr. However, similar inhibition by TOTP was not observed in AR42J cells. A ~60 kD protein (subunit molecular weight) was detected in the postnuclear fractions of only HepG2 cells by Western blot analysis using antibodies raised against FAEES, purified from rat liver microsomes. Therefore, the conjugation of fatty acids with ethanol, methanol or aniline in the HepG2 cells appears to be catalyzed by the same enzyme(s). These studies also suggest that FAEE, FAME and FAA synthases present in HepG2 cells may be structurally and functionally similar to rat liver microsomal CE, however, different from that present in AR42J cells.

1060 IN VITRO RATES OF COUMARIN 3,4-EPOXIDATION ARE NOT PREDICTIVE OF SPECIES DIFFERENCES IN HEPATOTOXICITY.

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Coumarin, a perfume ingredient, is a rat liver and mouse Clara cell toxicant. Coumarin-mediated injury results from the formation of coumarin 3,4-epoxide (CE) and the subsequent quantitative rearrangement of CE to form a hepatotoxic aldehyde, *o*-hydroxyphenylacetaldehyde (*o*-HPA). Clear species

differences exist in the hepatotoxicity of coumarin, with injury being greatest in the rat> > mouse> human. To better understand the relationship between hepatic injury and CE formation, *o*-HPA production in F344 rat and B6C3F1 mouse hepatic microsomes was quantitated using a sensitive gas chromatographic method coupled with flame-ionization detection. At coumarin concentrations ranging from 10-2000 μM, *o*-HPA formation exhibited biphasic kinetics in rat and mouse liver microsomes. In both rat and mouse, a high-affinity CYP450 exhibited a K_m of 20 μM, with V_{max} values of 0.580 and 2.3 nmol/min/mg, respectively. At concentrations > 100 μM, the rat high capacity enzyme(s) exhibited a K_m of 1300 μM and V_{max} of 6 nmol *o*-HPA/min/mg. In contrast, mice are resistant to coumarin-mediated liver injury, yet the low affinity enzyme catalyzes *o*-HPA formation with a K_m of 462 μM and a V_{max} of 10 nmol/min/mg. Furthermore, the intrinsic clearance (V_{max}/K_m) of coumarin via epoxidation was 4-fold greater in mice than rats. These data indicate that, in liver, toxicity does not directly correlate with the rate of CE/*o*-HPA formation *in vitro*. Rather, the balance between bioactivation and detoxification likely dictates the susceptibility of a species to coumarin-mediated liver toxicity.

1061 EFFECTS OF XENOBIOTICS ON EXPRESSION OF HUMAN MICROSOMAL EPOXIDE HYDROLASE.

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Although microsomal epoxide hydrolase (mEH) is known to be readily inducible following exposure of laboratory animals to a variety of xenobiotics, little is known about the inducibility and regulation of human microsomal epoxide hydrolase. In the present study, we investigated the effects of several prototypic inducers of xenobiotic-metabolizing genes on human mEH mRNA, protein and enzyme activity levels in cultured HepG2 cells. Continuous treatment of cells for 12 to 72 hours resulted in a rapid and sustained 2- to 3-fold increase in mEH mRNA levels, as measured by Northern blotting. Induction of CYP1A1 mRNA was simultaneously determined as an internal control of cellular response to Ah receptor-mediated induction. Induction of mEH protein, determined by Western blot analysis, and enzyme activity, determined as [^{3}H] styrene oxide hydrolase activity, were preceded by mEH mRNA elevation. We conclude that modulation of human mEH is regulated, at least in part, at the transcriptional level. mEH mRNA expression was stimulated after 12 hours exposure to ?-naphthoflavone (BNF), a compound known to upregulate several xenobiotic metabolizing enzymes via both ARE (antioxidant responsive) and XRE (xenobiotic responsive) elements. mRNA expression was also stimulated by exposure to 2,3,7,8-tetrachlorodibenzodioxin (selective for XRE) and *tert*-butylhydroquinone (selective for ARE) alone, suggesting that both elements may play a role in regulation of mEH expression in human liver. Finally, we showed that concomitant exposure of cells to BNF and tumor necrosis factor α (TNFα) results in a decrease in mEH activity, suggesting a negative regulatory role for TNFα and, possibly, other cytokine substances.

1062 INHIBITION OF MICROSOMAL AND SOLUBLE EPOXIDE HYDROLASE BY METALS.

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Inhibition of xenobiotic-metabolizing enzymes by metals may represent an important mechanism in regulating enzyme activity. Fourteen cations were evaluated for inhibition of microsomal epoxide hydrolase (mEH) in mouse, rat and human liver microsomes and soluble epoxide hydrolase (sEH) in mouse, rat and human liver cytosol. The IC_{50} values determined for inhibition of mEH and sEH in all three species were remarkably similar. Of the metals tested, Hg^{2+} was the most potent inhibitor (IC_{50} = 1 μM); Zn^{2+}, Cd^{2+} and Cu^{2+} were also strong inhibitors (IC_{50} = 10 μM, 10 μM, 20 μM respectively). Nickel (divalent) and Pb^{2+} were moderate inhibitors (IC_{50} = 500 μM), but Al^{2+}, Ba^{2+}, Ca^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+}, Mg^{2+} and Mn^{2+} inhibited epoxide hydrolase activity less than 50 % at 1 mM metal concentration (IC_{50} < 1 mM). Inhibition by all metals except Hg^{2+} could be reversed with the chelators EDTA, EGTA, 1,10-phenanthroline and dipicolinic acid. Additionally, mouse liver microsomes and cytosol were incubated with these chelators. Following incubation at 4° C, samples were dialyzed to remove chelator. Both mEH and sEH activity recovered was greater in samples treated with chelator than in control incubations. Similar treatment with the protease inhibitor Nα-*p*-tosyl-L-lysine chloromethyl ketone (TLCK) did not affect enzyme activity recovered. During systemic inflammation, hepatic metallothionien is induced, and liver metal concentrations increase while serum metal concentrations are decreased. The inhibition of microsomal and soluble epoxide hydrolase by metals may represent a mechanism of downregulation of enzyme activity during inflammation.

1063 BIOTRANSFORMATION AND KINETICS OF EXCRETION OF METHYL TERT.-BUTYL ETHER IN RATS AND HUMANS.

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Methyl tert.-butyl ether (MTBE) is widely used as an additive to gasoline to increase oxygen content and reduce tail pipe emmission of pollutants. Therefore, widespread human exposure may occur. To contribute to the characterisation of potential adverse effects of MTBE, its biotransformation was compared in humans and rats after inhalation exposure. Human volunteers (3 males and 3 females) and rats (5 males and 5 females) were exposed to 4 (4.5 ± 0.4) and 40 (38.7 ± 3.2) ppm MTBE for 4 hours in a dynamic exposure system. Urine samples were collected for 72 h in 6 h intervals and blood samples were taken in regular intervals for 48 h in humans. In urine, the MTBE-metabolites *tert*.-butanol, 2-methyl-1,2-propane diol and 2-hydroxyisobutyric acid were quantified, MTBE and tert.-butanol were determined in blood samples. After the end of the exposure period, blood concentrations of MTBE were similar after 40 (6.7 ± 1.6 μM in humans and 5.9 ± 1.8 μM in rats) and 4 ppm MTBE (1.9 ± 0.4 μM in humans and 2.3 ± 1.0 in rats). MTBE was rapidly cleared from blood with a half life of 2.6 ± 0.94 h in humans and 0.5 ± 0.2 h in rats. The blood concentrations of tert.-butanol were 21.8 ± 3.7 in humans and 36.7 ± 10.8 in rats after 40 ppm MTBE and 2.7 ± 0.3 in humans and 1.9 ± 0.5 in rats after 4 ppm MTBE. Tert.-butanol was also rapidly cleared from blood with half lifes of 5.3 ± 2.1 h in humans and 0.4 ± 0.1 h in rats. In urine of both species, 2-hydroxyisobutyrate was recovered as major excretory product in urine. Both tert.-butanol and 2-methyl-1,2-propane diol were minor metabolites. All metabolites of MTBE excreted with urine were also rapidly eliminated in both species. Elimination half lives for the different urinary metabolites of MTBE were between 10 and 17 h in humans and 3.5 and 4.5 h in rats. The obtained data indicate that MTBE-biotransformation and excretion is similar in rats and humans and MTBE and its metabolites are rapidly excreted in both species. Approximately 50 % of the calculated amount of MTBE retained after the end of the exposure was recovered as metabolites in urine of both humans and rats. (Supported by the Health Effects Institute, agreement No. 96-3.)

1064 TOXICOKINETICS AND URINARY METABOLITES IN HUMANS AFTER EXPOSURE TO ^{13}C-LABELED METHYL *t*-BUTYL ETHER.

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Methyl *t*-butyl ether (MTBE) is widely used as an oxygenate in gasoline for a complete combustion. Following inhalation exposure to MTBE, *t*-butyl alcohol (TBA) has been identified in human urine with a low recovery (<1%) (*Nihlén et al. TAP 1998;148:274*). The present study was conducted to characterize additional metabolites in urine of MTBE exposed humans and to evaluate the toxicokinetics of MTBE and TBA. Four healthy male volunteers were exposed to 50 ppm [1,2-$^{13}C_2$]-MTBE vapor (mouth only) for 2 hr during light physical work load (50 W). Blood, urine, and expired air were collected up to 22 hr post exposure. MTBE, TBA and acetone were analyzed by gas chromatography. In addition, characterization of urinary metabolites were accomplished by ^{1}H-decoupled ^{13}C nuclear magnetic resonance (NMR) spectroscopy. Low net uptake (39%), postexposure exhalation (17%), and recovery of TBA in urine (<1%) were observed. Toxicokinetics of MTBE and TBA in this mouth only exposure did not differ from a previous whole-body chamber exposure. Chemical shifts for the ^{13}C-labeled portion of the urinary metabolites were consistent with the shifts obtained for spiked standards of α-hydroxybutyric acid (HBA) and 2-methyl-1,2-propanediol (MPD). NMR signals were not present for labeled MTBE, TBA, or possible MTBE-derived glucuronide conjugates. HBA and MPD occur at significantly higher levels in the urine (detected by NMR) than MTBE and TBA (detected by GC). To our knowledge this is the first characterization of MTBE metabolites, other than TBA, in humans. These data are important for assessing human health risks associated with MTBE exposure. (Funded, in part, by PREEM Environment Foundation, Sweden and dues payments of CIIT member companies.)

1065 ACTIVATION OF RAT LIVER MICROSOMAL FLAVIN-CONTAINING MONOOXYGENASE BY DEPRENYL, AN INHIBITOR OF MONOAMINE OXIDASE B.

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Deprenyl, a well known inhibitor of monoamine oxidase (MAO) B, inhibits the conversion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to 1-methyl-4-phenyl pyridinium (MPP) which causes parkinsonism in experimental animals. Deprenyl inhibits MAO B by binding to the flavin moiety contained in MAO. Flavin-containing monooxygenase (FMO) also possesses the flavin moiety but is known to metabolize MPTP to a non-toxic MPTP N-oxide. As the FMO contains flavin moiety, we thought that deprenyl may also inhibit FMO activity in liver microsomes and limit the production of MPTP N-oxide. In an effort to determine whether deprenyl inhibits liver microsomal FMO activity, thiobenzamide S-oxidation (a standard FMO assay method) was conducted in the presence of varying concentrations of deprenyl . With liver microsomes obtained from mouse, rat, rabbit and human, deprenyl (0.1 to 1.0 mM) was found to activate, instead of the expected inhibition, the FMO activity up to 2.9 fold. When compared with the ability of n-octylamine to activate the liver microsomal FMO activity, deprenyl was found to be more potent. Clogyline, a well known inhibitor of MAO A activity, also activated the liver microsomal FMO activity, but was not as potent as deprenyl. Thus, by activating the liver microsomal FMO activity, deprenyl may enhance the metabolism of MPTP toward its non-toxic N-oxide producing pathway. This together with inhibition of MAO B, deprenyl may serve as an effective antidote against MPTP inducible parkinsonism.

1066 N-DEMETHYLATION OF CAFFEINE BY RECOMBINANT HUMAN FLAVIN-CONTAINING MONOOXYGENASES.

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Caffeine (CA), a trimethylxanthine contained in coffee, is known to be N-demethylated to theobromine (TB), paraxanthine (PX) and theophylline (TP) and also to trimethethylurate by various cytochrome P-450 (CYP) isoforms. Recently, using human liver microsomes and specific inhibitors of CYP and flavin-containing monooxygenase (FMO), we reported that majority of TB and TP, but minor portion of PX produced from CA are catalyzed by FMO [BBRC 235: 685-688 (1997); Chem-Biol. Interact. 113: 1-14 (1998)]. The aim of this study is to confirm and compare the role of recombinant human FMOs and CYP1A2 expressed in E. coli and purified in N-demethylating CA. CA was oxidized with various recombinant FMO isozymes or CYP1A2 and the produced metabolites were analyzed using an HPLC system. With recombinant human FMO1, FMO2 and FMO5, TB and TP, but no PX were produced. TB was produced at 0.023, 0.021 and 0.018 pmol/min/pmol FMO and TP at 0.011, 0.020, and 0.017 pmol/min/pmol FMO, respectively by FMO1, FMO2 and FMO5. With FMO3, the major form in human liver, 2.12 pmol TB/min/pmol FMO (nearly 100 fold faster than other FMO isozymes) and 23.69 pmol PX/min/pmol FMO were produced. With recombinant human CYP1A2 expressed together with P-450 reductase in E. coli, 11.22 pmol TB, 65.64 pmol PX and 1.3 pmol TP were produced per min per pmol of CYP. In the FMO catalyzed CA oxidation, formaldehyde was generated along with the formation of N-demethylated metabolites of CA. This suggested that FMOs could produce N-demethylated CA metabolites instead of the expected N-oxide metabolites. In conclusion, both TB and PX are produced from CA by FMO3 and CYP1A2 and supported the observation made in our previous studies. (Supported by an intramural grant from Inha University for 1998).

1067 IDENTIFICATION OF A HUMAN LUNG FMO2 VARIANT.

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Flavin-containing monooxygenase (FMO) is a phase I biotransformation enzyme which catalyzes oxygenation of N, S and P soft nucleophiles. FMO2 is a lung-specific isoform in rabbit and monkey, in which FMO2 levels are high compared with other monooxygenases and might play a significant role in xenobiotic metabolism. FMO2 has not been well characterized in humans. Human FMO2 cDNA has a premature stop codon and produces a truncated protein. In this study, we examined FMO2 DNA and protein from human lung specimens. Among samples we examined, results of genotyping by PCR showed that some individuals were heterozygous for full-length FMO and expressed a full size protein observed by western blotting; other samples were homozygous for the truncated gene and no measurable protein was detected by western blotting. Genotyping results to date indicate that the expression of the full-length FMO2 variant is seen only in African Americans. Enzyme activity measured by N,N-dimethylaniline (DMA) assay revealed that none of the samples had detectable activity. mRNA levels were quantitated by both RT-PCR and Northern blotting. Compared with monkey, various amounts of FMO2 transcript are expressed in human lungs (0% to 100% of monkey) which indicates that protein levels might be determined by postranscriptional stability. The results from heterologous expression of monkey FMO2 indicated that full-length FMO2 N-oxygenates both DMA and nicotine, whereas, no activity was detectable with the truncated protein. In summary, human FMO2 might not play the same role in drug metabolism as it does in monkey or rabbit. (Supported by NIH grant HL38650.)

1068 DIETARY INDOLE-3-CARBINOL (I3C) AND 3',3-DIINDOLYLMETHANE (DIM) INHIBIT FMO-MEDIATED TAMOXIFEN (TAM) N-OXIDATION IN THE RAT.

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Our laboratory has previously shown that indole-3-carbinol administered in the diet to male Fischer 344 rats has the very unusual property of inducing a number of cytochromes P450 (CYPs), especially CYP1A1, while markedly inhibiting the levels of FMO1 protein and its catalytic activity [Larsen-Su, S. and Williams, D.E., *Drug Metabol. Disp.* 24:927-931, 1996]. We hypothesized that liver microsomes from rats fed I3C or one of its major acid condensation products formed *in vivo*, DIM, should exhibit a marked shift in the metabolic profile of drugs which are substrates for both monooxygenase systems. Male rats were fed AIN76A powdered diets containing 0, 1000 or 2500 ppm I3C or DIM for 4 weeks. Liver microsomal FMO1 levels were determined by western blotting and CYP (N-demethylation and 4-hydroxylation)- and FMO (N-oxide)-mediated TAM metabolism by TLC. Both I3C and DIM reduced FMO1 protein levels (8% (I3C) and 84% (DIM) at 1000 ppm, and 90% (I3C) and 97% (DIM) at 2500 ppm. In liver microsomes from rats fed I3C, the ratio of CYP to FMO metabolites of TAM increased from 1.3 in controls to 1.9 and 4.1 in the 1000 and 2500 ppm groups, respectively, mostly due to a reduction in N-oxidation. Likewise, DIM diets increased the ratio to 3.0 and 3.1 at levels of 1000 ppm and 2500 ppm, respectively. These results agree with a previous preliminary study which also found that covalent binding of ^{14}C-TAM to liver microsomal protein was increased about 2-fold in rats fed I3C. The observation that we could not completely inhibit TAM N-oxidation (maximum of 55-65% at doses with decreased FMO by >95%) suggests a role for CYP in this reaction. (Supported by PHS grants HL38650 (DEW) and ES00834 (DK) from NIH.)

1069 DEVELOPMENTAL AND BIOCHEMICAL CHARACTERIZATION OF HEPATIC AND SERUM A-ESTERASES IN THE RAT.

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Juveniles are more susceptible than are adults to the acute toxicity of organophosphorus (OP) insecticides. Juvenile detoxication capabilities are significantly lower than those of adults. The development of liver and serum A-esterase (AEST) isozymes was studied in male and female rats at postnatal days (PND) 3, 12, 21, 33, and 80 by direct (production of 3,5,6-trichloro-2-pyridinol) and indirect (inhibition of exogenous cholinesterase by low concentrations of non-hydrolyzed substrate) quantitation. All activities increased substantially with age in both tissues. Male hepatic microsomal activity measured directly had comparable activities at PND33 and 80, while activity measured indirectly was different at each age. Female hepatic activity (direct) declined at PND80, whereas activity measured indirectly was similar at PND33 and 80. Sex differences were found at all ages except PND21 (male>female at PND12, 33, and 80) (direct), and at PND21, 33, and 80 (female>male at PND21 and 33) (indirect). Male serum activity measured

directly had similar activities at PND33 and 80, while activity measured indirectly was similar at PND21 and 33. Female serum activity had its highest activity at PND80 (direct), and was different at each age (indirect). Sex differences were apparent only at PND12 and 80 (male>female at PND12, reversed at PND80) (direct), and at PND33 and 80 (female>male activity) (indirect). These pattern differences may reflect differences in the rates of development of AEST isozymes. To further characterize the biochemical role of AEST in age-related differences in OP toxicity, liver AEST were purified through the application of detergent solubilized microsomes to anion exchange and gel filtration chromatography columns. Native and SDS gel electrophoresis were used to analyze AEST activity in the native and denatured states, respectively. By observing the development of AEST isozymes and determining their role in the detoxication of OP insecticides, the reasons for age differences in OP toxicity may be further understood. (Supported by NIH RO1 ES04394 and F31 ES05752.)

1070 PON1-KNOCKOUT MICE ARE HIGHLY SENSITIVE TO CHLORPYRIFOS-OXON AND DIAZOXON TOXICITY.

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Paraoxonase (PON1) is an esterase that can hydrolyze the toxic metabolites of several organophosphate (OP) insecticides, such as parathion, chlorpyrifos and diazinon. Increasing PON1 levels by injection of purified enzyme protects animals from OP poisoning. In the present study, we used PON1-knockout (*PON1*$^{-/-}$) mice to further examine the *in vivo* function of PON1 in detoxifying OP insecticides. *PON1*$^{-/-}$ mice were generated by gene targeting and had no detectable paraoxonase activity in serum and liver. Mice were challenged with chlorpyrifos (CPS), chlorpyrifos-oxon (CPO), diazinon (DZS) and diazoxon (DZO) by dermal exposure. Compared to wild-type mice (*PON1*$^{+/+}$), *PON1*$^{-/-}$ mice were extremely sensitive to CPO. At the lowest dose (1.5 mg/kg), CPO did not affect AChE activity in *PON1*$^{+/+}$ mice but reduced it by 80% and 74% in brain and diaphragm, respectively, in *PON1*$^{-/-}$ mice. *PON1*$^{-/-}$ mice that received 6 mg/kg of CPO died within 2 hours, while *PON1*$^{+/+}$ mice that received the same dose of CPO survived without any symptoms of OP poisoning. *PON1*$^{-/-}$ mice also showed a much higher sensitivity to another PON1 substrate, DZO. The lowest dose of DZO (1 mg/kg) resulted in 80% inhibition of brain AChE in *PON1*$^{-/-}$ mice but had no effect on *PON1*$^{+/+}$ mice. The higher doses (2 and 4 mg/kg) were lethal to *PON1*$^{-/-}$ mice but caused no inhibition of brain AChE in *PON1*$^{+/+}$ mice. Hemizygous (*PON1*$^{+/-}$) mice showed an intermediate sensitivity to DZO. Furthermore, when animals were exposed to the parent compounds, CPS and DZS, *PON1*$^{-/-}$ mice also showed a higher sensitivity to both chemicals compared with *PON1*$^{+/+}$ mice. However, there was no observable difference in DZS toxicity between *PON1*$^{+/-}$ and *PON1*$^{-/+}$ mice. These results clearly demonstrate the significant role of PON1 in OP detoxication. (Supp. in part by NIEHS Grant ES-07033, ES-09601, and NIH Grant PO1HL30568.)

1071 EFFECTS OF TISSUE HOMOGENATE CONCENTRATION ON ORGANOPHOSPHATE OXON INHIBITION AND IC50 VALUES OF HEPATIC ALIESTERASES AND BRAIN ACETYLCHOLINESTERASE IN ADULT RATS.

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The organophosphate (OP) compounds [chlorpyrifos-oxon (C=O), paraoxon (P=O) and methyl paraoxon (MP=O)] are the active oxygen analogs of the parent insecticides, chlorpyrifos, parathion and methyl parathion, respectively. Aliesterases (Aliest) [carboxylesterase (EC. 3.1.1.1)] and acetylcholinesterase (AChE) (EC. 3.1.1.7) are B-esterases which are inhibited by OPs. Each enzyme can be persistently and stoichiometrically phosphorylated by these oxons. Inhibition of Aliest (non-target enzyme) will reduce the amount of oxon that reaches the target, AChE. Dilute tissue homogenates are usually used to conduct enzyme studies to investigate Aliest detoxication pathways and the potencies of inhibitors for AChE, and the results are used to predict activities in intact tissues. It is not known if these predictions could be affected by tissue homogenate concentration. This study was designed to determine if OP oxon inhibition and IC50 values of liver Aliest could be affected by tissue homogenate concentration. Also studied were the *in vitro* sensitivities of AChE to inhibition by these oxons. Aliest and AChE activities increased as the tissue homogenate concentration increased for each sex. The IC50 values remained constant over a range of lower tissue concentrations, but increased at higher concentrations. There were no significant differences in percent inhibition and in the IC50 values between male and female rats. There were no significant differences in IC50 values obtained at various dilutions of purified Aliest (porcine liver) or AChE (electric eel), suggesting that tissue components, other than the enzymes, may be responsible for interacting with the oxons, effectively lowering their concentrations and therefore reducing the IC50 values *in vitro* at higher tissue concentrations. These results indicate that tissue homogenate concentration is a very important factor to be considered when conducting *in vitro* Aliest or AChE activity, inhibition and IC50 assays.

1072 KINETICS AND INDIVIDUAL VARIATION IN GSH CONJUGATION OF TRICHLOROETHYLENE (TRI) IN HUMAN LIVER AND KIDNEY.

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Isolated human hepatocytes exhibited time-, Tri concentration-, and cell concentration-dependent formation of S-(1,2-dichlorovinyl)glutathione (DCVG) in incubations in sealed flasks with 25 to 10,000 ppm Tri in the headspace (= 0.011 to 4.4 mM in hepatocytes). Maximal formation of DCVG (22.5 ± 8.3 nmol/ 20 min per 106 cells) occurred with 500 ppm Tri. Time-, protein concentration- and both Tri and GSH concentration-dependent formation of DCVG were observed in human liver and kidney subcellular fractions. Two kinetically distinct systems were observed in both cytosol and microsomes from pooled liver samples whereas only one system was observed in subcellular fractions from pooled kidney samples: Liver cytosol exhibited K_m values (μM Tri) of 333 and 22.7 and V_{max} values (nmol DCVG formed/min per mg protein) of 8.77 and 4.27; liver microsomes exhibited K_m values of 250 and 29.4 and V_{max} values of 3. 10 and 1.42; kidney cytosol and microsomes exhibited K_m values of 26.3 and 167, respectively, and V_{max} values of 0.81 and 6.29, respectively. DCVG formation in liver samples from 20 individual donors exhibited 8.5-fold variation in microsomes but only 3.4-fold variation in cytosol. In co-incubations of pooled liver cytosol and microsomes, addition of an NADPH-regenerating system produced marked inhibition of DCVG formation but addition of GSH had no effect on cytochrome P-450-catalyzed formation of chloral hydrate. These results indicate that both human kidney and liver have significant capacity to catalyze DCVG formation, indicating that the initial step of the GSH-dependent pathway is not limiting in the formation of nephrotoxic and nephrocarcinogenic metabolites.

1073 GLUTATHIONE-DEPENDENT METABOLISM OF 3-(7*H*-PURIN-6-YLTHIO) ACRYLIC ACID (PTA) TO THE CHEMOTHERAPEUTIC AGENT 6-MERCAPTOPURINE (6-MP).

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The elevated levels of glutathione (GSH) and glutathione-S-transferases (GST) observed in many multi-drug resistant tumor cells, suggest that anticancer agents might be selectively targeted to these cells upon metabolism of carefully designed GST substrates (prodrugs). The reported antimicrobial activity of PTA, a 6-MP derivative, led us to hypothesize that the biological activity of PTA was due to its GST mediated metabolism to 6-MP, making it a potential prodrug targeting drug-resistant cells. Thus, we examined the *in vivo* and *in vitro* metabolism of PTA in rats. HPLC analyses of urine of rats treated i.p. with PTA (100 mg/kg) showed that 6-MP was formed *in vivo* and excreted in urine (mostly within 6 h) without toxicity as indicated by liver and kidney function tests. *In vitro* assays revealed that two metabolites of PTA, 6-MP and the major metabolite, *S*-(6-purinyl) glutathione (PG), were formed in a time- and GSH-dependent manner. Formation of 6-MP occurred non-enzymatically in the presence of GSH, but was enhanced 2- and 7- fold by the addition of liver and kidney homogenates, respectively. Purified rat liver GSTs also mediated the formation of 6-MP from PTA. However, PG formation was not enhanced by the addition of liver homogenate or purified GSTs compared to buffer incubations, suggesting two different pathways by which PG and 6-MP were formed in liver. No time-dependent accumulation of PG was observed in kidney homogenate incubations. This result was due to further metabolism of PG by γ-glutamyltranspeptidase, dipeptidase and β-

lyase, as indicated by the use of the inhibitors acivicin and aminooxyacetic acid. Collectively, the results of this study show that PTA is metabolized to 6-MP both *in vivo* and *in vitro*. Therefore, PTA may have antitumor properties against tumor cells having high levels of GSH and GST.

1074 THE COUPLED REDUCTION OF *p*-NITROSOPHENOL WITH ETHANOL OXIDATION CATALYZED BY HORSE LIVER ALCOHOL DEHYDROGENASE: TOXICOLOGICAL IMPLICATIONS OF THE RECYCLING OF NAD+/NADH.

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The kinetics of the coupled reaction of C-nitrosoarene reduction and ethanol oxidation catalyzed by horse liver alcohol dehydrogenase (ADH), in which the ADH-bound coenzyme undergoes oxidation/reduction cycling, has been studied and compared with the kinetics of the corresponding single reactions. The experiments were performed using steady-state kinetic techniques and p-nitrosophenol (pNSP) as an aldehyde-substrate analogue. Analysis of the kinetic data obtained with either unlabeled or deuterium-labeled ethyl alcohol suggests that the coupled reaction can be described by an apparent ping-pong bi bi mechanism with competitive substrate (pNSP) inhibition. This is evidenced by the double-reciprocal plots of the bisubstrate reaction, which generate a series of hyperbolic concave up-lines and a primary deuterium isotope effect, which is observed for only one of the two half-reactions. The pH-dependence of the kinetic constants, the primary deuterium isotope effect and the products distribution indicate that the mechanism of pNSP reduction in the coupled reaction system differs from that observed for the individual single reaction. The reduction of pNSP in the direct single reaction, which appears to proceed in two consecutive steps via two alternative mechanism results predominantly in formation, of pAP. When pNSP is coupled to ethanol oxidation the primary product of reduction is benzoquinoneimine (BQI), which is not reduced to pAP, but subsequently is converted to an arylnitrenium ion. The arylnitrenium ion can undergo nonenzymatic hydrolysis to yield 1,4-benzoquinone, also a strong electrophile. (Supported by NIAAA grant AA06758 to GWW.)

1075 IN VIVO MICRODIALYSIS IN A COMPARATIVE STUDY OF HEPATIC METABOLISM OF PHENOL IN THREE FISH SPECIES.

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Physiological-based toxicokinetic (PB-TK) models are valuable tools used to describe the relationship between xenobiotic toxicity at the cellular and organ level to that observed in the whole animal. The ability to obtain metabolic rate constants in several species for chemicals that undergo biotransformation is important to the development of accurate PB-TK models. Numerous *in vitro* techniques have been employed to mimic the *in vivo* system however, data from direct comparisons is limited. In this study, we continue our investigation of *in vivo* phase I hepatic metabolism of phenol using microdialysis as a tool for assessing Michaels-Menton kinetics in various fish species to provide 1) validation of rate and capacity inputs for PB-TK models, and 2) a comparison of rate constants for different species. A microdiaylsis probe was surgically implanted into the liver of adult rainbow, lake and brook trout which were subsequently confined to respirometer-metabolism chambers. Phenol (1-300mM) was delivered directly to the liver in Cortland's saline at a flow rate of 1 µl/min which consistently resulted in 80-85% delivery of phenol. Hydroquinone (HQ) and catechol (CA) production in all three species increased with increasing phenol dose. Maximum production was observed between 200 and 260mM delivered phenol. Relative K_m and V_{max} were comparable between the three species for both HQ and CA. The data obtained through microdialysis may be useful in resolving uncertainties which are central to fish PB-TK modeling namely, the role of metabolism in chemical disposition predicted by the models.

1076 COMPARISON OF THE METABOLISM OF [^{14}C]BENZENE AND [^{14}C]PHENOL IN THE ISOLATED PERFUSED MOUSE LIVER.

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Hepatic metabolism of benzene (BZ) is a necessary first step before BZ can exert its hematotoxic effects (Sammett et al., J. Toxicol. Env. Hlth. 5:785,1979). The major metabolites of BZ both *in vivo* and *in vitro* are phenol (P) and its conjugates (Snyder and Hedli, Env. Hlth. Perspect. 104(Suppl. 6), 1165,1996). Administration of BZ, but not P, to mice results in bone marrow depression (Snyder et al., Env. Hlth. Perspect. 100:293,1993). It was, therefore, of interest to determine whether hepatic BZ metabolism produced potentially hematotoxic metabolites which were not produced during P metabolism. Mouse livers were perfused with a solution of either [^{14}C]BZ or [^{14}C]P in the orthograde (portal vein to central vein) direction or retrograde (central vein to hepatic vein) direction to compare the metabolites formed from each substrate and to investigate the role of hepatic zonation of metabolic enzymes involved in BZ and P hydroxylation and subsequent conjugation. Perfusate samples were collected, metabolites were separated and identified by HPLC, and quantified by the levels of radioactivity found in each peak. Regardless of the substrate or the direction of perfusion the major compounds released from the liver were P, phenylgucuronide, phenylsulfate, hydroquinone (HQ), and HQ glucuronide. Comparison of the orthograde perfusion studies of BZ and P revealed that a larger percentage of the radioactivity released from the liver was identified as the unconjugated, hematotoxic metabolite HQ after BZ perfusion than after P perfusion. A comparison of the results of perfusing BZ or P in orthograde versus retrograde direction showed that the enzymes involved in hydroxylation of BZ or P are generally closer to the central vein than those involved in conjugation of the hydroxylated metabolites. In addition, the amount of radioactivity covalently bound to liver macromolecules was measured after each perfusion and determined to be proportional to the amount of HQ and HQG detected in the perfusate samples.

1077 IN VITRO SPECIES COMPARISON OF THE METABOLISM OF THE ANTITUMOR AGENT NSC 652287.

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NSC 652287 is one of a family of thiophene containing compounds that have shown a highly selective pattern of differential growth inhibition and cytotoxic activity in the National Cancer Institute Anticancer Drug Screen. The compound has notable activity against renal carcinoma cell lines with GI50's of 10 - 30 nM. This is of particular interest since there are few therapeutic options with reliable activity against metastatic renal cell carcinomas. However, the compound has shown pulmonary toxicity in dogs and monkeys, renal damage in rats, whereas mice are a relatively resistant species. The target organ toxicity may be a consequence of differential metabolism among species. We have compared the metabolism of NSC 652287 in liver S9 fractions from dogs, monkeys, rats, and mice. The rate of metabolism was found to be faster in the monkey than in the other species tested. HPLC analysis showed that all species produced two metabolites with elution times of 5 and 9.5 min. Dog S9 fractions generated two additional metabolites with elution times of 3 and 7 min. In addition, NSC 652287 was found to be cytotoxic to primary human hepatocytes at concentrations of 10 mM (as indicated by MTT reduction and LDH assay), and a metabolite eluting at 5 min was generated. Metabolism by recombinant microsomes from human lymphoblastoid cells expresssing a specific CYP450, identified the cytochrome P450 1A2 as the major isozyme metabolizing NSC 652287. Current studies are in progress to further determine the potential role of CYP450 in the toxicity of NSC 652287 and to compare the metabolism of NSC 652287 in pulmonary and renal tissues from different species.

1078 THE MUTAGENIC DNA REPLICATION MACHINERY CONTRIBUTES TO THE GENOMIC INSTABILITY OF CANCER CELLS.

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The development of the malignant cellular phenotype is accompanied by an accumulation of multiple genetic alterations. These alterations are due in part to a reduction in the fidelity of the DNA synthetic process, and potentially a specific reduction in the efficiency and fidelity with which replication-associated DNA mismatch repair proteins function. Using a novel forward mutagenesis assay we have determined that the DNA replication process carried out by the intact cellular DNA replication apparatus, (i.e., the DNA synthesome), derived from malignant breast cells is significantly error-prone when compared to that carried out by the non-malignant breast cell synthesome. In order to evaluate whether mismatch repair proteins contribute significantly to the observed mutation frequency of the DNA synthe-

some of cancer cells, we performed co-localization and co-immunoprecipitation experiments followed by Western blotting analyses to determine whether the mismatch repair proteins are physically associated with the DNA synthesome. We present here, the for the first time, evidence indicating that most of the known DNA mismatch repair proteins are components of the DNA synthesome. Furthermore, our results demonstrate that the *in vitro* DNA synthesome model system can be used to examine the contributions made by both the DNA replication and the mismatch repair proteins to the processes which give rise to the observed genomic instability exhibited by many types of malignancies. (This work was supported in part by an individual U.S. Army D.O.D. Breast Cancer Research Program pre-doctoral fellowship and an NIEHS post-doctoral award to JWS, NIH awards (#CA57350 and CA73060) to LHM, and an NIH award (#CA74904) to RJH.)

1079 DELAYED GENOMIC INSTABILITY INDUCED BY GAMMA IRRADIATION IN YEAST, MAMMALIAN CELLS AND IN VIVO IN THE MOUSE.

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Multiple genetic changes are required for the development of a malignant tumor cell and many environmentally-induced cancers show a delayed onset of more than 20 years following exposure. In fact, the frequency of genetic changes in cancer cells is higher than can be explained by random mutation. It was thus proposed that a sub-population of cells develop a mutator phenotype. A common characteristic of cancer cells, widely thought to be a major contributor to the progressive, multistage development of malignant disease, is an unstable, "hypermutable" genome. Cultured mammalian cells which survive exposure to ionizing radiation and other DNA damaging agents often develop a persistent genomic instability reminiscent of that observed in tumor cells. These delayed effects cannot be explained by the carry-over of DNA damage, nor can they be due to mutations at specific loci. We have studied the development of similar phenotypes in the yeast Saccharomyces cerevisiae, in human cells and in vivo in mice. Following exposure to gamma radiation, alkylating agents and oxidative mutagens a high proportion of yeast colonies derived from surviving cells displayed elevated frequencies of genetic deletions up to 50 generations post-irradiation. Other phenotypes observed include elevated frequencies of forward mutation and a lowered plating efficiency. Irradiation of male mice also caused persistent effects on genetic deletions in the offspring after mating with unirradiated females. Irradiated mammalian cell cultures have shown persistent oxidative stress, changes in ploidy, and elevated p53 and p21 levels. Elucidating the mechanism by which these effects arise may shed light on the development of persistent genomic instability in human cancers.

1080 POLYCYCLIC AROMATIC HYDROCARBONS WITH BAY-LIKE REGIONS INHIBITED GAP JUNCTIONAL INTERCELLULAR COMMUNICATION AND INDUCED THE ACTIVATION OF MAPK.

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Many polycyclic aromatic hydrocarbons (PAHs) are known carcinogens. Considerable research has been devoted to predicting the genotoxic-tumor initiating potential of PAHs based on chemical structure. However, structure/function relationships to the nongenotoxic-epigenetic events of tumor promotion are sparse. PAHs containing a bay or bay-like region were shown to be potent inhibitors of gap junctional intercellular communication (GJIC), an epigenetic event involved in the release of an initiated cell from growth suppression. We tested the epigenetic toxicity of PAHs containing bay-like regions by comparing the effects of methylated vs. chlorinated isomers of anthracene on the temporal activation of mitogen activated protein kinase (MAPK) and the down-regulation of GJIC. Specifically, we used anthracene, 1-methylanthracene, 2-methylanthracene, 9-methylanthracene, 1-chloroanthracene, 2-chloroanthracene, 9-chloroanthracene and determined the effects of these compounds on GJIC and the activation of extracellular receptor kinase (ERK 1 and 2), a MAPK, in F344 rat liver epithelial cells. Results showed that bay or bay-like regions formed by either a chlorine or methyl group reversibly inhibited GJIC and activated MAPK, whereas the linear-planar isomers had no effect on GJIC or MAPK. MAPK activation occurred 10-20 min after the down-regulation of GJIC which indicates that MAPK is not involved in the initial regulation of GJIC. Conversely, inhibition of GJIC may somehow trigger activation of MAPK. This study revealed that structural determinants of PAHs affect epigenetic events involved in tumor promotion, namely, the reduction of GJIC followed by the activation of intracellular mitogenic events. (Research supported by grants from USAFOSR (F49620-97-1-022) and NIEHS (P42 ES04911-09).)

1081 CHARACTERIZATION OF SIGNAL CROSS-TALK BETWEEN THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR ALPHA AND SPHINGOMYELINASE PATHWAYS.

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Kupffer cell activation and release of cytokines have been linked to cell proliferation and suppression of apoptosis induced by peroxisome proliferators (PP) in hepatocytes. Sphingolipids are second messengers for several cytokine receptors. We hypothesized that one or more sphingolipids could activate the peroxisome proliferator-activated receptor alpha (PPARα) and be responsible in part for PP-induced cell proliferation and inhibition of apoptosis. Sphingomyelinase (SMase) is the enzyme responsible for sphingolipid release from membranes. In transactivation assays, we tested the ability of SMase and several cell-permeable sphingolipid analogs to activate a PPAR-responsive luciferase reporter gene in HepG2 Cells. We also examined the ability of these sphingolipid analogs to activate β-oxidation and pro- and antiapoptotic protein expression and induce cell proliferation in mouse primary hepatocytes. SMase treatment resulted in a concentration-dependent activation of the PPAR-responsive luciferase reporter gene in the transactivation assay. Treatment of mouse primary hepatocytes with sphingosine resulted in induction of PP-responsive proteins involved in β-oxidation of fatty acids. The effects of both SMase and sphingosine treatments were additive to the effects of the potent PP Wy-14,643 in the transactivation assay and the induction of β-oxidation enzymes. This additivity suggests that both SMase and sphingosine treatments activate PPARα. However, none of the sphingolipid analogues activated PPARα in a transactivation assay, suggesting that sphingolipid effects on PPARα activation is indirect. Ceramide induced cell proliferation in primary mouse hepatocytes and suppressed proapoptotic proteins, whereas sphingosine induced proapoptotic proteins. Our results suggest sphingolipids may be key lipid mediators involved in PP-induced cell proliferation and suppression of apoptosis via activation of PPARα. Thus there appears to be cross-talk between cytokine and nuclear receptor signal transduction pathways that regulate hepatocyte growth.

1082 IMPACT OF PEROXISOME PROLIFERATORS ON LIVER PHOSPHOLIPID BIOSYNTHESIS.

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Sponsor: J M Frazier.

Peroxisome proliferators (PPs) are a structurally diverse class of commercial/industrial chemicals and therapeutic drugs that cause hepatocarcinogenesis in rodents. We are studying the effects of these compounds on liver metabolism using nuclear magnetic resonance (NMR) spectroscopy in an effort to understand the biochemical mechanism of action. Previous data have suggested that PPs influence the pathways for biosynthesis of liver phosphatidylcholine (PtdC). These metabolic pathways are important in processes of tumorigenesis. PtdC can be derived from choline (*de novo* pathway) or through methylation of phosphatidylethanolamine (PtdE methyltransferase pathway, PEMT). We examined the effects of various PPs on these pathways utilizing ^{13}C–NMR. Rats were treated with either perfluorooctanoic acid (PFOA), perfluorodecanoic acid (PFDA), di(2-ethylhexyl) phthalate (DEHP), Wy–14,643 (Wy), or vehicle solution. Wy and PFOA are strong tumor promoters, whereas DEHP is much weaker and PFDA is non-tumorigenic. At various times postreatment (specific for each PP) rats were infused (iv) with [1–^{13}C]choline + [2–^{13}C]ethanolamine for 1 h. Livers extracts were prepared and analyzed by NMR. Fluxes were derived for the *de novo* synthesis of PtdC (F1), *de novo* synthesis of PtdE (F2), and the synthesis of PtdC via PEMT (F3). For all groups, F3 was positively correlated with F2 ($n=50$, $r^2=0.83$). The percentage of PtdC derived via the *de novo* vs. PEMT pathways was greatest for Wy (95% vs. 5%) and DEHP (87% vs. 13%), and least for PFDA (39% vs. 61%) ($p<0.02$). These data indicate that Wy and DEHP activate *de novo* synthesis of PtdC and inhibit the PEMT pathway. The increase in PEMT–derived PtdC with PFDA treatment is due to a significant increase in the *de novo* synthesis of PtdE ($p<0.01$). Our data corroborate other studies suggesting that tumorigenesis requires *de novo* synthesis of PtdC, and that the activity of PEMT may regulate these processes.

1083 TISSUE SPECIFIC EFFECTS OF ACRYLAMIDE (AMD) ON DNA SYNTHESIS IN MALE RATS.

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Chronic exposure of rats to AMD leads to the formation of tumors in the thyroid, adrenal medulla, and mesothelium of the testes in male rats through apparently nongenotoxic mechanisms. A number of potential mechanisms for nongenotoxic activity have been proposed including increased cell proliferation, induction of oxidative stress, blockage of gap junctional communication (GJIC). In addition, based on the tissue pattern of the tumor response, AMD may cause a disturbance in the hypothalamus-pituitary-endocrine organ axis, which may result in uncontrolled regulation of hormones that affect these organs. The present studies examined DNA synthesis, plasma hormone levels, GJIC, and oxidative stress in the target organs in male rats treated for 7 to 28 days with AMD (2 or 15 mg/kg/day; gavage). DNA synthesis was determined by immunochemical staining for both PCNA and BrdU. Increases in labeling index were observed in adrenal medulla and thyroid consistent with the observed occurrence of AMD-induced tumors. No changes in GJIC or oxidative damage (MDA and OH8dG) were seen in target tissues at the AMD doses examined. Analysis of plasma hormones showed no significant change in prolactin, testosterone, or TSH. A small decrease in LH and FSH was seen in the animals treated at the highest dose of AMD studied (15 mg/kg/day). Overall these results demonstrated that of the nongenotoxic mechanisms examined, AMD only produced a significant increase in cell proliferation in two of the target organs in which tumors were observed.

1084 A POTENTIAL MECHANISM FOR 2-BUTOXYETHANOL (2-BE) INDUCED MOUSE LIVER NEOPLASIA.

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Results from a recently reported NTP bioassay showed that 2-BE induced liver tumors in B6C3F1 mice but not in F344 rats. 2-BE exposure also resulted in an increase in hemolysis with an apparent iron loading in the liver. Iron accumulation has been linked to the induction of oxidative stress and liver neoplasia. Our hypothesis is that 2-BE induces cancer in the mouse liver through iron loading and subsequent oxidative stress selectively in the mouse liver. Previous studies showed that the mouse liver has a lower antioxidant capacity compared to the rat, potentially making the mouse liver more susceptible to oxidative damage. The present study was undertaken to determine if 2-BE, its major metabolite (BAA), or iron (Fe^{2+}) produced oxidative stress in mouse and rat hepatocytes. Oxidative stress endpoints examined were 8-hydroxydeoxyguonosine (OH8dG), malondialdehyde (MDA) and Vitamin E. Hydrogen peroxide (1 mM) treatment served as a positive control. 2-BE and BAA at sublethal concentrations, failed to induce oxidative stress in either rat or mouse hepatocytes. Treatment with Fe^{2+} resulted in a dose-dependent increase in OH8dG and MDA in hepatocytes from both species. Similarly, vitamin E levels decreased in a dose-dependent manner in hepatocytes from both species following Fe^{2+} treatment. The effects of Fe^{2+} in mouse hepatocytes were significantly greater than those seen in the rat. These results showed that treatment with Fe^{2+} and not 2-BE or BAA, induced oxidative stress in rodent hepatocytes and suggest that in vivo, the liver effects of 2-BE may be related to oxidative stress induced by iron overload.

1085 STRONG, TOXICITY ASSOCIATED, PROMOTION OF RAT URINARY BLADDER CARCINOGENESIS BY PHENETHYL AND BENZYL ISOTHIOCYANATES.

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Modification effects of phenethyl isothiocyanate (PEITC) and benzyl isothiocyanate (BITC), which are components of cruciferous vegetables generally regarded as potent chemopreventive agents, were examined with regard to rat urinary bladder and liver carcinogenesis. Male F344 rats were given a single i.p. injection of diethylnitrosamine (DEN) and then treated with N-butyl-N-(4-hydroxybuthyl)nitrosamine (BBN) for 4 weeks. Starting 3 days after completion of this initiation, groups of 21 rats each were placed on diets containing PEITC or BITC at a dose of 0.1% each, or basal diet alone for 32 weeks. Further groups of 6 animals each received PEITC, BITC or basal diet alone without initiation. Histopathological examination revealed the incidences of transitional cell carcinoma to be significantly increased in the PEITC (100%) and BITC (100%) groups as compared with the basal diet group value (24%). In addition, bladder tumors were induced in PEITC and BITC groups without initiation. Although the incidences of liver tumors were not significantly altered, multiplicity of foci was increased in the PEITC group. Sequential observation of the bladder epithelium for up to 7 days in male and female rats fed 0.1% PEITC or BITC showed that the initial effect was toxicity as evidenced by degeneration, inflammation and erosion followed by marked papillomatosis. These changes were stronger in the BITC than the PEITC case and in males than in females. These results indicate that PEITC and BITC are strong bladder promoters and further suggest that they can act as bladder carcinogens. Toxicity and cell proliferation appear responsible for the induction of the lesions. Influence on the liver appears weak, if promotion present.

1086 EFFECTS OF DIMETHYLARSENIC ACID (DMA) ON URINARY PARAMETERS AND BLADDER EPITHELIUM IN FEMALE F344 RATS.

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DMA caused urothelial tumors in F344 rats when fed in a chronic long-term study; the female rat was more sensitive than the male rat. Dietary treatment with DMA was not tumorigenic in the mouse. Current evidence indicates that DMA is not genotoxic. We examined the effects of feeding DMA to female F344 rats for 10 weeks at levels of 0, 2, 10, 40, and 100 ppm on urinary parameters and on the urothelium. Reversibility of the effects was evaluated in a high dose recovery group (100 ppm DMA for 10 weeks followed by 0 ppm DMA for 10 weeks). Urine chemistries were similar in the control and treated groups except for a dose responsive increase in calcium levels despite an increase in urine dilution. Examination of the urine by scanning electron microscopy showed no abnormal urinary solids. Urothelial toxicity and regenerative hyperplasia of the bladder were present at the two highest doses by light and scanning electron microscopy with an increased bromodeoxyuridine labeling index. The urothelial effects were reversible when treatment was withdrawn.

1087 COMPARISON OF THE URINARY AND UROTHELIAL EFFECTS OF o-PHENYLPHENOL (OPP) AND SODIUM OPP (Na-OPP) FED TO MALE F344 RATS.

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OPP and Na-OPP induce bladder tumors in rats, with Na-OPP significantly more potent than OPP. To evaluate the short term effects of OPP and Na-OPP in groups of 10 male F344 rats, the chemicals were fed in Purina diet at the equimolar doses of 1.25 and 2.0%, respectively, for 10 wks. Urinary pH was 6.6 and 7.4, respectively, compared to 6.8 for controls. No urinary precipitate or microcrystalluria was detected following OPP feeding, but calcium phosphate-containing precipitate was present after Na-OPP feeding. Urothelial hyperplasia was detected by light and scanning electron microscopy along with superficial urothelial necrosis; the changes were greatest following Na-OPP. The labeling index following an one hour pulse of bromodeoxyuridine was 2.31, 1.98 and 0.09 for OPP, Na-OPP and controls. These data show that OPP and Na-OPP produce toxicity and regenerative hyperplasia when fed to male rats. The effect with Na-OPP was generally greater compared to OPP and was associated with the appearance of urinary calcium phosphate-containing precipitate, similar to that seen with other sodium salts fed to male rats. (Supported in part by NCI grant CA32513.)

1088 NONADDITIVE DNA-DAMAGING EFFECTS OF GENOTOXINS IN MIXTURE: 1. MUTAGENICITY IN SALMONELLA.

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Most animal studies on genotoxins focus on exposures to single chemicals, however, humans are usually exposed to mixtures of genotoxins. Cancer and developmental toxicity risks of gentoxins in mixture are generally estimated by assuming additivity of the components. Two or more genotoxins acting

sequentially or simultaneously may present a greater or lesser risk than could be predicited assuming additivity. We studied the effect of one genotoxin on the binding of a second genotoxin to DNA in an in vitro system; binding of the two toxins was not additive (Said and Shank,1991,1995). In the study reported here the effect on one genotoxin on the mutagenicity of a second genotoxin was evaluated in the Salmonella assay for several pairs of compounds. Pretreatment of frameshift strain TA98 with UV-activated aflatoxin B1 (AFB1) (0.4 ug/plate) or synthetic AFB1-8,9-epoxide (0.17 ug/plate) enhanced the mutagenicity of N-acetoxy-acetylaminofluorene (N-Aco-AAF) 2-3 times above theoretical additive effects. Pretreatment of base-substitution strain TA100 with N-Aco-AAF (0.1 ug/plate) inhibited the mutagenicity of AFB1, N-methyl-N-nitrosourea (MNU) and benzo(a)pyrene by 3,10,and 1.5 times below theoretical additive effect; resp. When strain TA102 containing an excision repair system, was pretreated with N-Aco-AAF for 2 hr, inhibition of MNU mutagenicity was partially released. Dose-response relationships for these enhancing or inhibitory effects were demonstrated. These results show nonadditive effects for mixtures of genotoxins in a bacterial mutagenicity assay.

1089 THE EFFECTS OF IRON ON NUCLEAR CALCIUM AND DNA FRAGMENTATION IN ISOLATED RAT HEPATOCYTES.

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Although it is well known that iron overdose can produce hepatotoxicity, the exact mechanism of cell death has yet to be determined. Free radical generation, lipid peroxidation and changes in cytosolic calcium concentrations, have been demonstrated in iron toxicity, but not definitively established as the underlying cause of cytotoxicity. This study examined the possibility that the mechanism of iron toxicity may involve disruptions in nuclear calcium regulation, which could result in DNA fragmentation. The study was performed using hepatocytes isolated from male Sprague-Dawley rats. Incubation of hepatocyte cultures with ferrous sulfate (0.5 – 10 μ M) for 18 hours produced dose-dependent decreases in cell viability, as measured by Lactate Dehydrogenase (LDH) release. These same concentrations of ferrous sulfate also produced dose-dependent increases in nuclear calcium concentrations, as measured by o-cresolphthalein complexation. Decreases in cell viability were found to correlate well with increases in nuclear calcium concentration, suggesting that a disruption in nuclear calcium regulation may be involved in, or at least an indicator of cytotoxicity. Ferrous sulfate concentrations (3-10 μ M) which were shown to significantly decrease cell viability and increase nuclear calcium, were also shown to cause DNA fragmentation in the hepatocytes as measured by agarose gel electrophoresis. These results suggest that iron-induced hepatotoxicity may be mediated by increases in nuclear calcium, perhaps through the activation of calcium-dependent endonucleases resulting in DNA fragmentation and ultimately cell death. Further research will examine the time course of cell damage, as well as possible protective mechanisms against iron toxicity.

1090 NONADDITIVE DNA-DAMAGING EFFECTS OF GENOTOXINS IN MIXTURE: 2. COVALENT BINDING TO DNA.

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Mutual interactions of pre-existing DNA adducts with a second genotoxin were examined at the level of genomic DNA (calf thymus DNA, ctDNA) and on a sequence-specific level (oligonucleotides). Pretreatment of ctDNA with N-acetoxy-acetylaminofluorene (N-Aco-AAF) (0-1.8% nucleotides modified) reduced levels of Gua-N7-aflatoxin B1 (AFB1) adducts formed after subsequent treatment with AFB1-8,9-epoxide (~2x reduction). No binding modulations following N-Aco-AAF treatment of ctDNA previously modified with AFB1-8,9-epoxide were observed. Oligodeoxynucleotides containing either Gua-C8-AAF or Gua-C8-aminofluorene adducts and a neighboring unadducted guanine (G) (target G), located 1,2 or 4 nucleotides from the adduct, were reacted, as single or double-stranded substrates, with dimethylsulfate (DMS) or AFB1-8,9-epoxide. A modified Maxam-Gilbert technique showed that the presence of the AAF adduct lowerved the extent to which AFB1-8,9-epoxide, but not DMS, reacted with the target G. Binding of AFB1-8,9-epoxide to the target G was attenuated(5-fold) when located immediately adjacent to the AAF, but not an aminofluorene adduct, in ds-DNA. Reaction with AFB1-8,9-epoxide increased when the target G was located 2 or 4 nucleotides from the AAF adduct. An optical titration technique was used to measure non-covalent binding of AFB1 and ethidium to DNA. An enhanced binding affinity of both compounds to DNA was observed in spite of the reduced covalent adduct yields suggesting that the ease by which AFB1-8,9-epoxide forms an intercalative intermediate within DNA does not dictate its overall reaction kinetics. The steady-state presence of specific adducts (e.g. Gua-AAF) in certain sequence contexts may mask favorable binding sites of selected genotoxins (e.g. AFB1-8,9-epoxide) thereby reducing productive adduct yields. Such a mechanism may account for non-additive mutagenic responses observed in Salmonella typhimurium.

1091 MOLECULAR DOSIMETRY OF N2,3-ETHENOGUANINE IN CONTROL AND VINYL CHLORIDE-EXPOSED RATS.

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Vinyl chloride (VC) monomer is an important industrial chemical and a widespread environmental contaminant that is a known human and animal carcinogen. VC is metabolically converted to chloroethylene oxide, which alkylates DNA to form exocyclic base adducts, including the promutagenic N^2,3-ethenoguanine (N^2,3-εGua). Here we present the first report of a VC dose-response based on DNA adduct formation. 12 week-old male Sprague-Dawley rats were exposed to 0, 10, 100, or 1100 ppm VC for 1 or 4 wk (6 hr/d, 5 d/wk). Mixed liver cell DNA was digested to deoxynucleosides, and N^2,3-εdGuo was purified with immunoaffinity chromatography, hydrolyzed to N^2,3-εGua, and quantitated with gas chromatography-high resolution mass spectrometry. Endogenous N^2,3-εGua was measured at 0.08± 0.04 and 0.11± 0.05 per 10^6 unmodified dGuo in 1 and 4 wk controls, respectively. Exposure to 10, 100, and 1100 ppm VC for 1 wk increased N^2,3-εGua to 0.20± 0.05, 0.68± 0.09, and 1.25± 0.20 per 10^6 dGuo, respectively. After 4 wk exposure, the corresponding amounts were 0.53± 0.11, 2.28± 0.18, and 3.78± 0.55 N^2,3-εGua per 10^6 dGuo. A statistically significant, 3-fold accumulation of N^2,3-εGua between 1 and 4 wk was observed at all exposures. The dose-response relationship between VC exposure and N^2,3-εGua in the liver was supralinear, with a steep slope between 0 and 100 ppm and relatively little increase at 1100 ppm. This dose-response is consistent with tumor incidences observed in long term cancer bioassays. Relatively small (2.6- and 4.8-fold) increases in N^2,3-εGua over endogenous levels were observed at 10 ppm VC, an exposure only one order of magnitude greater than the occupational TLV. VC appears to be a good model chemical for reevaluation under the EPA Proposed Guidelines for Carcinogen Risk Assessment (1996), which encourages the incorporation of non-tumor data when sufficient evidence exists to support such an alternate approach. (This work was supported by NIH grants ES07126 and ES05948.)

1092 QUANTITATIVE ANALYSIS OF DNA ADDUCTS AND ABASIC SITES INDUCED BY PENTACHLOROPHENOL-DERIVED QUINONE AND HYDROQUINONE IN CALF-THYMUS DNA.

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Pentachlorophenol (PCP) has been shown to be carcinogenic in mice. The mechanism of PCP carcinogenesis is unknown, but may involve PCP-derived quinoid metabolites, i.e., tetrachlorohydroquinone (Cl_4HQ) and tetrachloro-1,4-benzoquinone (Cl_4-1,4-BQ), and their associated oxidative stress and/or direct DNA adducts. We hypothesized that PCP-derived quinone/hydroquinone are capable of inducing DNA adducts which rapidly form abasic (AP) sites. In this study, we investigated the differential induction of direct DNA adducts and AP sites in calf-thymus DNA treated with Cl_4-1,4-BQ and Cl_4HQ. DNA adducts derived from PCP quinone/hydroquinone were analyzed by ^{32}P-postlabeling after nuclease P1 enrichment and were quantified by relative adduct level. The number of AP sites was measured by a slot-blot technique. Three major and several minor adducts were detected in CT-DNA treated with a high dose of Cl_4-1,4-BQ (5 mM), with total adduct numbers of ~13 adducts per 10^6 total nucleotides. Parallel, treatment-related increases in the number of AP sites were detected with ~ 6 AP sites per 10^6 total nucleotides in the presence of metal ion. In contrast, we detected 250 AP sites per 10^6 nucleotides in CT-DNA treated with Cl_4HQ (0.3 mM). At a low dose of Cl_4HQ (1 μ M) we were able to detect a 3-fold increase in the number of AP sites over control. Formation of stable DNA adducts was also detected in Cl_4HQ (0.3 mM)-treated DNA at 1 adduct per 10^6 total nucleotides. Overall, these results suggest that PCP-derived quinone/hydroquinone were capable of inducing specific types of DNA damage and AP sites. The AP sites induced by PCP quinoids are likely to be the result of oxidative stress and represent a possible mechanism for clasto-

genicity and carcinogenicity of PCP. (This work was supported by the National Institute of Environmental Health Sciences through grant P42ES05948 and T32ES07126.)

1093 EKER RAT MODEL CHARACTERIZATION: THE RESPONSE TO GENOTOXIC CARCINOGENS FOLLOWING FOUR MONTHS OF DOSING.

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The Eker rat carries a germline mutation in the Tsc-2 tumor suppressor gene which predisposes these rats to the development of renal cell carcinoma (RCC). This study was an initial effort to characterize the responses of the Eker rat to non-renal carcinogens. Two genotoxic rat liver carcinogens, 2,4-diaminotoluene (2,4-DAT) and 2-nitropropane (2-NP), were administered by oral gavage to male Long Evans rats (wild type or bearing the Eker mutation) for 4 months. Dosages were: 2,4-DAT, 6.5 mg/kg, 7 times per week; 2-NP, 89 mg/kg, 3 times per week. Vehicles were 0.01 M Hcl and 10% Emulphor, respectively. Renal proliferative lesions were counted in two microscopic sections of kidney (one longitudinal and one cross section) from each Eker rat. The incidence of tumors was determined histopathologically in all tissues in all animals. The incidence of renal hyperplastic tubules was increased in 2,4-DAT treated Eker rats compared to vehicle controls. There were increases in renal cell carcinomas in 2,4-DAT treated Eker rats and in the incidence of overall neoplasia in 2-NP treated Eker rats relative to vehicle controls. No increase in renal proliferative lesions or tumors were seen in wild-type rats which lacked the susceptibility mutation. CONCLUSIONS: 1) Eker rats have increased sensitivity to genotoxic carcinogens, and 2) carcinogen exposure increases tumor number and/or decreases tumor latency in this model. (Sponsored by TAP Holdings Inc.)

1094 BINUCLEATED OCTOPLOID HEPATOCYTES DISPLAY INCREASED SUSCEPTIBILITY TO DNA SYNTHESIS INDUCED BY NONGENOTOXIC CARCINOGENS.

S C Hasmall and R A Roberts. *Zeneca Central Toxicology Laboratory, Macclesfield, UK.* Sponsor I Kimber.

Induction of increased hepatic DNA synthesis by nongenotoxic chemicals such as the peroxisome proliferator, diethylhexylphthalate (DEHP) is frequently associated with subsequent hepatocarcinogenesis. However, 1,4-dichlorobenzene (DCB), a noncarcinogen in male rat liver, is a potent hepatomitogen that induces a similar profile of S-phase labelling index (LI) to that of DEHP. Since hepatic polyploidisation is a normal process, we examined the hypothesis that variations in the ploidy class in which DNA synthesis is induced may be indicative of hepatocarcinogenic potential. In a previous study, male Fischer 344 rats were dosed for 7 days with DCB or DEHP and the hepatocytes isolated for flow cytometry and immunocytochemical detection of bromodeoxyuridine (BrdU). DCB, unlike DEHP, reduced the proportion of tetraploid cells (4N plus 2× 2N) and increased the proportion of mononucleated octoploid cells (8N). Also, DEHP induced a higher LI in binucleated octoploid (2× 4N) hepatocytes than did DCB. These 7 day exposure data raised two questions: since the BrdU label could disseminate into other populations, what constituted the target populations for induction of DNA synthesis and, secondly, is the LI profile induced by DEHP common to other nongenotoxic hepatocarcinogens? To identify the target population for S-phase induction, in the present study we used a 1hr BrdU pulse label during the peak of S-phase (29-30hr). Again, DCB and DEHP increased LI in all hepatocyte sub-populations except the 2× 2N population. LI in the 2× 4N population was increased significantly by DEHP, (2.9±0.2% DEHP; 0.2±0.1% control) but not by DCB (1.0±0.2%). In addition, a second nongenotoxic hepatocarcinogen and peroxisome proliferator, methylclofenapate (MCP), induced a similar profile of S-phase to DEHP since the LI in the 2× 4N population was 3-fold the average hepatic LI. Thus, 2× 2N hepatocytes cells may represent a population where induction of S-phase poses a more substantial carcinogenic risk.

1095 THE PEROXISOME PROLIFERATOR RESPONSE ELEMENT (PPRE) UPSTREAM OF THE HUMAN ACYL COA GENE IS INACTIVE IN MOST INDIVIDUALS: SIGNIFICANCE FOR SPECIES DIFFERENCES IN RESPONSE TO PEROXISOME PROLIFERATORS.

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Peroxisome proliferators (PP) cause rodent hepatocarcinogenesis but humans appear to be nonresponsive to the adverse effects of this class of nongenotoxic carcinogens such as hepatocyte growth perturbation, peroxisome proliferation and tumours. The peroxisome proliferator activated receptor alpha (PPARα) mediates the effects of PPs in rodents via a peroxisome proliferator response element (PPRE) upstream of PP responsive such as acyl coenzyme A oxidase (ACO) and CYP4A1. Surprisingly, when the human ACO promoter was cloned, it was found to be active and contained a consensus PPRE (3' TGACCA T TGACCT 3') at 1918 to 1906 base pairs (bp) upstream of the transcription start site. To confirm and extend these findings, we isolated a 2 Kb genomic clone of the 5' region of the human ACO gene. However, in transient transfection reporter gene assays using either Hepa1c1c7 or NIH3T3 cells, this promoter fragment was inactive unlike the rat ACO promoter reporter plasmid. The PPRE within our cloned fragment of the human ACO promoter differed at two positions (3' TGACAG T TGACCT 5') from the previously published active human ACO promoter. Having demonstrated that the PPRE we had cloned from one individual was inactive, we studied the frequency of occurrence of the inactive human PPRE in the human gene pool. Using a polymerase chain reaction (PCR) strategy, we isolated genomic DNA from 22 unrelated human individuals of mixed racial origin. In addition, we isolated genomic DNA from the human hepatoma cell line HepG2, since these cells are used to model the lack of human response to PPs *in vitro*. In each case, the PPRE contained the inactive sequence (3' TGACAG T TGACCT 5'). These data suggest that the human ACO gene promoter found in a representative human population is inactive, providing a molecular basis for lack of human response to the adverse effects of the PP class of nongenotoxic hepatocarcinogens.

1096 A STRATEGY FOR ASSOCIATING PEROXISOME PROLIFERATOR-INDUCED HEPATOCELLULAR CHANGES WITH PATTERNS OF GENE EXPRESSION.

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Peroxisome proliferators are a class of structurally diverse hepatocarcinogens that are thought to induce liver tumors in rodents by promoting the growth of pre-existing initiated cells, however the mechanism of action is still largely unknown. High-density cDNA arrays can be used as a powerful tool to determine how xenobiotics perturb homeostasis. To further understand mechanism(s) of peroxisome proliferator action, we are developing a strategy for identifying characteristic patterns of gene expression and their association with specific tissue responses resulting from peroxisome proliferator treatment using high-density arrays. Preliminary experiments using the Atlas Mouse cDNA expression array (Clontech) show changes in gene expression of several cell cycle and apoptosis genes in mice treated with the peroxisome proliferator Wy14, 643. An array with peroxisome proliferator-responsive genes will also be used to help identify genes that are characteristic of treatment. Use of these different arrays will allow an association of specific gene expression patterns across dose and treatment with changes in specific endpoints related to both peroxisome proliferation and carcinogenesis (i.e. cell proliferation/apoptosis). Characterization of such changes will become a useful screening tool for not only identifying specific modes of action of chemicals or chemical classes but also providing insights into mechanism(s) of action of different classes of toxicologically and pharmacologically relevant compounds.

1097 SEXUAL DIMORPHISM IN HEXACHLOROBENZENE (HCB)-INDUCED MODULATION OF INTERCELLULAR COMMUNICATION IN THE LIVER.

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HCB causes liver tumors in female rats whereas only a small percentage of males are responsive. We showed that the administration of 5 consecutive daily doses of HCB promoted, with greater efficiency in female than male rats, liver tumor formation induced by diethylnitrosamine (DEN) injected 95

days later. Reduction of intercellular communication has been postulated to play a role in the mode of action of epigenetic carcinogens such as HCB. Gap junctions are formed by integral proteins, termed connexins. This study aimed to assess the effects of HCB on liver connexins. Male and female rats (N= 6 per group) were given HCB (100 mg/kg, po in corn oil) for 5 consecutive days and sampled on day 50. Hepatic RNA was isolated and subjected to Northern blot analysis for three connexins (Cx26, Cx32 and Cx43). cDNA probes for each connexin were used and gel loading corrected with an 18S rRNA probe. Autoradiograms were quantified by densitometry. mRNA levels for Cx26 and Cx32 were significantly ($p<0.05$) lower in livers from HCB-treated female rats compared to controls, whereas Cx43 mRNA level was 1.5 times lower ($p>0.05$) in treated rats as compared to controls. For male rats no significant differences ($p>0.05$) were observed for any of the three connexins. Together these data suggest that HCB reduces gap junction formation in female rat liver. This female-specific response correlates with the sexual dimorphism for tumor formation, thereby suggesting that the mechanism for the HCB effect involves reduced intercellular communication.

1098 REVERSIBILITY OF PHENOBARBITAL AND DEHP-INDUCED INHIBITION OF GAP JUNCTION INTERCELLULAR COMMUNICATION (GJIC) AND INDUCTION OF DNA SYNTHESIS IN THE RAT.

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While the exact mechanism(s) by which nongenotoxic hepatocarcinogens function has not been completely defined, down regulation of GJIC and increased DNA synthesis appear to be important in the carcinogenesis process. In addition, many nongenotoxic carcinogens appear to function at the tumor promotion stage. A hallmark of tumor promotion is the reversibility of the tumor promoting effect following removal of the promoter. In this study, the reversibility of the effects of phenobarbital (PB) and di-2-ethylhexyl phthalate (DEHP), two rodent hepatic nongenotoxic carcinogens, on GJIC and DNA synthesis was examined. DEHP (20,000 ppm) and PB (500 ppm) were fed to groups of male F344 rats. DEHP produced an increase in DNA synthesis and decrease in GJIC after 2 weeks of treatment. Similar changes were observed with PB. Following removal of PB or DEHP and replacement with control NIH-07 diet, GJIC and DNA synthesis returned to control levels after 2 and 4 weeks. In DEHP treated rats, peroxisomal beta oxidation (PBOx) was significantly elevated (13-fold over control) after 2 weeks of treatment and PBOx returned to control levels after removal of DEHP. Liver analysis of DEHP and metabolites revealed measurable levels of monoester (MEHP) after treatment which were not detectable in liver after removal of DEHP from the diet. This study showed that the modulation of GJIC and DNA synthesis observed after exposure to nongenotoxic hepatic carcinogens is reversible and dependent upon continuous tumor promoter treatment.

1099 DOSE-RESPONSE AND TIME COURSE STUDIES OF DEHP ON HEPATIC PEROXISOMAL β -OXIDATION (PBOx), GAP JUNCTION INTERCELLULAR COMMUNICATION (GJIC) AND DNA SYNTHESIS IN THE RAT.

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In nongenotoxic hepatic carcinogenesis induced by peroxisomal proliferating compounds, several important hepatic processes have been reported to be disrupted and may contribute to the mechanism of carcinogenesis. In the present study, the hepatic effects of DEHP (di-2-ethylhexyl phthalate) on PBOx, GJIC and DNA synthesis were examined in F344 rats following exposure to nontumorigenic (1000 ppm) and tumorigenic (6000, 12000, 20000 ppm) doses of DEHP. Also, DEHP and major metabolites were measured. In the dose-response study, DEHP was administered for 2 weeks at doses of 1000, 6000, 12000 and 20000 ppm. At tumorigenic doses (> 1000 ppm), a dose-dependent increase in the induction of PBOx and DNA synthesis was observed. In addition, inhibition of GJIC at doses above 1000 ppm was seen. Coupled with these cellular changes, analysis of DEHP and metabolites revealed that MEHP was increased in the rat liver in a dose-dependent manner. In the time-course study, effects of DEHP (6000 and 20000 ppm) were examined following 1, 2, 4, and 6 weeks of treatment and compared to the effects of 6000 ppm DEHP for 18 and 24 months. DEHP produced an increase in PBOx (4 to 6 fold) at 6000 ppm at all times and (9 to 20 fold) at 20000 ppm over the 6 weeks of exposure. Similarly, inhibition of GJIC ranged from 25-40% with all DEHP exposures. An increase in DNA synthesis was seen after 2 or 4 weeks (60000 and 20000 ppm DEHP respectively) which returned to control values by 6 weeks. Liver MEHP levels exhibited a dose and time dependent increase. Collectively, these data demonstrate that modulation of the endpoints noted above appear to correlate with the observed tumorigenicity of DEHP and may be predictive indicators for nongenotoxic carcinogenicity.

1100 SPECIES SPECIFICITY FOR HEPATIC EFFECTS OF DEHP ON GAP JUNCTION INTERCELLULAR COMMUNICATION (GJIC), DNA SYNTHESIS AND PEROXISOMAL BETA OXIDATION (PBOx).

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DEHP (di-2-ethylhexyl phthalate) was examined for its effect on PBOx, DNA synthesis and GJIC in male F344 rats, B6C3F1 mice and SG hamsters to determine if modulation of these parameters correlated with the observed hepatic tumorigenicity of DEHP. DEHP was administered in the diet for 2 and 4 weeks, at tumorigenic and nontumorigenic doses. Tumorigenic doses were 6000, 12000, and 20000 ppm in rats and 6000 ppm in mice. Nontumorigenic doses were 1000 ppm in rats and 500 ppm in mice. Doses of 1000 and 6000 ppm were used in hamsters, which are refractory to DEHP-induced tumorigenicity. In the rat, treatment with 6000-20000 ppm DEHP produced an increase in hepatic DNA synthesis at 2 weeks whereas the 1000 ppm had no effect. In the mouse, increased DNA synthesis was observed at 6000 ppm. No change in DNA synthesis was observed in the hamster. DEHP effects on PBOx were similar, producing a dose dependent increase after both 2 and 4 weeks. In the mouse PBOx was increased whereas in the hamster, a slight increase (2-fold) was observed at the highest dose studied. GJIC was decreased after 2 and 4 weeks of treatment (25-50%) at the tumorigenic doses in the rat and mouse. GJIC was not inhibited in hamster liver at either dose or time studied. Analysis of DEHP and major metabolites in liver showed that the monoester metabolite (MEHP) was greater in mice than in rats or hamsters following DEHP exposure. Comparable levels of MEHP were observed in rats and hamsters. These data show that inhibition of GJIC and increased DNA synthesis in the liver correlated with the observed dose and species specific tumorigenicity of DEHP seen after chronic treatment. This lends additional support to a mechanistic role for blockage of GJIC and increased DNA synthesis in the hepatic carcinogenicity of peroxisome proliferators.

1101 THE HEPATIC EFFECTS OF DI-ISONONYL PHTHALATE (DINP) AND RELATED ANALOGS IN RATS AND MICE.

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The short-term hepatic effects of DINP in rats and mice at tumorigenic and non-tumorigenic doses were evaluated for comparison with chronic studies and with data for other structurally similar dialkyl phthalate esters ranging from C7 to C11. Groups of male F344 rats were fed diets with DINP at concentrations of 0, 1000, or 12000 ppm and male B6C3F1 mice at 0, 500, or 6000 ppm DINP. After 2 or 4 weeks of treatment, changes in liver weight, replicative DNA synthesis, peroxisomal β -oxidation (PBOx) and inhibition of gap junction intercellular communication (GJIC) were examined. In addition, hepatic concentrations of the parent compound and major metabolites were determined. Relative to controls in both species, increased liver weight and PBOx at the high dose were consistent with peroxisomal proliferation. Hepatic GJIC was inhibited and DNA synthesis was increased at the high dose, which is also consistent with the tumorigenic response in rats and mice at these doses. These hepatic effects were not observed at the low doses. Similarly, no liver tumors were observed in rats and mice treated with low doses in chronic studies. The monoester metabolite (MINP) was detected in the liver at greater levels in mice than rats. This result is also consistent with the dose-response observations in rat and mouse chronic studies. Similar responses were observed for DEHP and other C7-C11 dialkyl phthalate esters. Collectively, these data indicate that in rats and mice, DINP and other C7-C11 phthalates exhibit a threshold for inducing cellular events which correlated with the tumorigenic response of these compounds in chronic feeding studies.

1102 ABSENCE OF LIVER EFFECTS IN CYNOMOLGUS MONKEYS TREATED WITH PEROXISOMAL PROLIFERATORS.

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To evaluate the human relevance of liver effects observed in rats and mice, high doses of peroxisome proliferators were given by gavage in 0.5% methyl cellulose (10 ml/kg) to groups of adult male monkeys (n=4) for 14 days. In comparison to vehicle control, di-isononyl phthalate (DINP, 500 mg/kg/d), di-2-ethylhexyl P (DEHP, 500 mg/kg/d), and clofibrate (C, 250 mg/kg/d) produced no statistically significant changes in body weight, organ weights, urinalysis, hematology, clinical chemistry, nor other signs of toxicity. In contrast, related studies show that lower daily doses of these chemicals produce changes in the livers of rats and mice within this time frame. C produced some emesis, small increases in serum triglyceride, decreased calcium, and decreased weights of testes/epididymides and thyroid/parathyroid. In the monkeys that were evaluated by H&E staining, no signs of inflammation or necrosis were observed in the liver, kidney or testes. Treatment with DINP, DEHP, and C produced no change in hepatic peroxisomal β -oxidation or replicative DNA synthesis. DINP, DEHP and C did not inhibit hepatic gap junction intercellular communication (GJIC) in fresh liver slices from treated monkeys using incision loading dye transfer and direct measurement of dye flow (Lucifer yellow). In monkeys, liver analyses indicate that phthalate parent and monoester metabolites were present at much lower levels than in rodents. This study indicates that the hepatic effects produced by high doses of phthalates in rats and mice do not occur in monkeys. These data, along with results from *in vitro* hepatocyte studies, indicate that rodents are not good animal models for predicting the hepatic effects of phthalates in primates, including humans.

1103 INTERACTION OF CO-PLANAR AND NON-PLANAR PCBS IN PROMOTION OF ALTERED HEPATIC FOCI.

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Synergistic tumor promoting ability has been reported in mixtures of co-planar and non-planar PCBs. This study was designed to evaluate interactive liver cancer promoting effects of two environmentally relevant PCBs, the co-planar PCB 126 (3,4,5,3',5'-pentachlorobiphenyl); and non-planar PCB 153 (2,4,5,2',3',5'-hexachlorobiphenyl). Promotion of altered hepatic foci was evaluated with an 8 week medium term bioassay using rats treated with diethylnitrosamine as an initiator, followed by gavage treatment with PCBs in corn oil and partial hepatectomy. PCBs were administered individually and in combination three times per week by oral gavage; PCB 126 at 0.1-10μ g/kg body weight and PCB 153 at 10-10,000μ g/kg body weight. Treatment with PCB 126 or 153 alone resulted in a dose dependent increase in glutathione-S-transferase placental form (GST-P) positive preneoplastic foci compared with controls. Treatment with the binary PCB mixture resulted in less than additive GST-P positive focus formation. These findings differ from reported studies that demonstrated synergism for preneoplastic liver foci formation between these congeners under a different protocol. Transforming growth factor beta may play a role in altering hepatocellular proliferation and apoptosis rates after PCB exposures. Studies to evaluate this hypothesis are underway. (This work is supported by grants NIEHS 1KO8ES00314 and NIEHS Superfund Basic Research Program P42ES05949.)

1104 METOLACHLOR, A NONGENOTOXIC RAT HEPATOCARCINOGEN, INDUCES CYTOCHROME P450 2B1/2B2 AND ALTERS THYROID HOMEOSTASIS.

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Metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide], the 2nd most popular corn herbicide in the U.S., is a significant contaminant of surface waters. Metolachlor is a weak rat hepatocarcinogen; however, it is nongenotoxic, which suggests a tumor promoter mode of action. Many liver tumor promoters have been shown to induce a panel of xenobiotic metabolizing enzymes, which include cytochrome P450s and Phase II conjugating enzymes. Increased UDP-glucuronyl transferase, a Phase II enzyme, can have an adverse effect on thyroid homeostasis. Phenobarbital, the prototypical rat liver tumor promoter, induces several cytochrome P450s in the rat, particularly CYP2B1/2B2, and decreases serum thyroid hormone levels through an extrathyroidal mechanism. These two parameters were investigated in rats treated with metolachlor. Liver microsomal proteins from male Sprague-Dawley rats treated with a nonhepatotoxic dose of metolachlor (150 mg/kg/day, ip; 1, 4, 9 & 28 days) were probed by immunoblotting with anti-CYP2B1/2B2. We observed a strong induction of CYP2B1/2B2 with metolachlor treatment at all time points. A modest decrease in serum thyroxine was observed at 9 days, with a return to control levels at 28 days. Further, histological examination of H&E and PCNA stained thyroid gland revealed mild hyperplasia and hypertrophy in 9-day metolachlor-treated rats. These data support a phenobarbital-like mode of action for metolachlor hepatocarcinogenicity and further suggest common alterations in thyroid homeostasis. (NIH ES00044, ES07046, and North Carolina ARS)

1105 MECHANISTIC STUDIES OF KOJIC ACID ON THYROID TUMOR PROMOTION ON ENHANCEMENT OF THYROID CARCINOGENESIS.

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In order to elucidate the mechanisms for reduction of serum thyroid hormones caused by continuous administration of kojic acid (KA) and its thyroid tumor promotion effect, male F344 rats were given pulverized basal diet containing 0.008%, 0.03%, 0.125%, 0.5% and 2% KA for 4 weeks. The thyroid ^{125}I uptake was significantly decreased in the groups received 0.03% KA or more. Significant reduction of organic formation ratio of iodine was only observed in the 2% KA group. The serum T3 and T4 levels in the 2% KA group were significantly decreased as compared to those of the control. Pronounced elevation of the serum TSH levels were observed in the 2% KA group. Both absolute and relative thyroid weights were significantly increased in the groups receiving 0.5% of KA or more. Histopathologically, both decreased colloid in the thyroid follicles and follicular cell hypertrophy in the thyroid were apparent at high incidences in the groups given 0.03% of KA or more. In addition, thyroid capsular fibrosis was evident in all rats in the 2% KA group. The results suggest that KA-alteration of thyroid-related hormone levels are due to inhibition of iodide uptake and iodine organification in the thyroid, with promoting effects on development of thyroid proliferative lesions probably secondary to prolonged serum TSH stimulation resulting from negative feedback through the pituitary-thyroid axis.

1106 PROMOTION ACTIVITY OF KOJIC ACID ON RAT THYROID CARCINOGENESIS AND ITS EFFECT ON THYROID HORMONS.

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Kojic acid(KA) is produced by many species of *Aspergillus* , *Acetobactor* and *Penicillium*.. The *Aspergillus flavus* group has traditionally been used in Japan for the production of fermented foodstuffs such as "miso", soy sauce and "sake". Thyroid tumors were observed in mouse caricinogenecity study given diet containing 1.5 and 3% KA. To examine whether KA exerts promoting effect on thyroid carcinogenesis, male F344 rats were initiated with N-bis(2-hydroxypropyl)nitrosamine (BHP: 2800 mg/kg body weight, single s.c. injection), and starting one week later, received pulverized basal diet containing 2% or 0% KA for 12 weeks. Untreated control rats were given basal diet for 13 weeks. Animals were killed at weeks 4 and 12. The serum T3 and T4 levels were significantly decreased (1/2 to 1/3 of values of the BHP alone group) and serum TSH was markedly increased (13 to 19 times higher than the values of the BHP alone group) in the BHP+KA group at weeks 4 and 12. Thyroid weights were significantly increased in the BHP+KA and KA alone groups. Focal thyroid follicular hyperplasias and adenomas were observed in 4/5 rats and 3/5 rats in the BHP+KA group at week 4, respectively. At weeks 12, these lesions were observed in all rats in the BHP+KA group. No changes in thyroid-related hormone levels or thyroid histopathological lesions were observed in either the BHP alone or the

untreated control groups. Measurement of liver T4-uridine diphosphate glucuronosyltransferase (UDP-GT) activity at week 4 revealed no significant intergroup differences. These results suggest that thyroid proliferative lesions were induced by KA administration due to continuous serum TSH stimulation through the negative feedback mechanism of the pituitary-thyroid axis, with decreases of T3 and T4 caused by a mechanism independent of T4-UDP-GT activity.

1107 COMPARATIVE 30-WEEK DERMAL TUMOR PROMOTION EVALUATION OF CIGARETTE SMOKE CONDENSATE FROM A REFERENCE CIGARETTE AND AN ECLIPSE PROTOTYPE (7026A) TEST CIGARETTE IN FEMALE SENCAR MICE.

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A 30-week dermal tumor promotion assay was conducted to compare the potential dermal tumor promotion activity of mainstream cigarette smoke condensate (CSC) collected from an ECLIPSE prototype cigarette (7026A), which primarily heats tobacco, and a 1R4F (University of Kentucky) reference cigarette. Mainstream CSC's were collected by cold trap from smoke generators using the Federal Trade Commission puffing regimen. Female SENCAR mice were "initiated" with a single 75 ug application of DMBA to the shaved dorsal skin. The CSC's were then applied to the skin three times per week for 29 weeks. Each CSC was administered at 10, 20, or 40 mg tar/application to groups of 40 animals. End-points included body weights, clinical observations, organ weights, dermal tumor development data and histopathology. The numbers of dermal tumors and the numbers of dermal tumor-bearing animals for the 1R4F reference CSC were statistically different from the "DMBA-initiated" and "acetone-promoted" control groups, and increased with increasing exposure. The number of dermal tumor-bearing animals for the high-dose 7026A prototype CSC was statistically significantly different from the control groups. The numbers of dermal tumors for the mid- and high- dose 7026A prototype CSC were statistically different from the control groups and increased with increasing exposure. When corresponding dose levels of reference and 7026A CSC were compared to each other, the mid- and high-dose 7026A CSC groups had statistically fewer dermal tumors and tumor-bearing animals. In this assay, the dermal tumor-promotion potential of the 7026A CSC was, therefore, substantially reduced when compared to the 1R4F reference CSC.

1108 THE EFFECT OF CIGARETTE SMOKE ON THE METABOLISM OF THE TOBACCO-SPECIFIC NITROSAMINE 4-(METHYLNITROSAMINO)-1-(3-PYRIDYL)-1-BUTANONE (NNK) IN THE A/J MOUSE.

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The effect of short-term exposure to whole cigarette smoke on the metabolism of the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) was studied in the A/J mouse. Mice (n=12) were exposed to either HEPA-filtered room air (control group) or 1R4F cigarette smoke for 2 hrs at a concentration of 0.6 mg particulate matter per liter of air. Immediately following nose-only inhalation, all animals received an intraperitoneal (i.p.) administration of 7.5 µmole/mouse of NNK containing 10 µCi of [^{3}H]NNK and then placed in metabolic cages for 24 hr urine collections. Collected urine was used to quantify the stable end products of the α -hydroxylation metabolic pathway, which activates NNK, and stable end products of NNK detoxification. The metabolites resulting from NNK activation by α -hydroxylation (hydroxy acid and keto acid) were significantly ($p<0.05$) reduced to 85% and 58% of control, respectively. Moreover, detoxification to the NNAL and NNAL-glucuronide metabolites increased significantly ($p<0.05$) to 108% and 116% of control, respectively. These results indicate that co-administration of cigarette smoke shifts the metabolism of NNK towards detoxification pathways compared to when NNK is administered alone. These results are consistent with previous studies demonstrating that cigarette smoke co-administration markedly reduces the formation of O^6MeG DNA adducts following NNK administration.

1109 TUMOR PROMOTING ACTIVITIES OF XYLAZINE (XZ) AND ITS METABOLITE, 2,6-DIMETHYLANILINE (DMA), IN A TWO-STAGE NASAL CARCINOGENICITY MODEL IN RATS INITIATED WITH N-BIS(2-HYDROXYPROPYL)NITROSAMINE (DHPN).

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XZ is an a_2-adrenergic agonist that is used in food-producing animals as a sedative agent. It has been reported that DMA, a metabolite of XZ, induced nasal cavity tumors in rat 2-year carcinogenicity study, but a carcinogenicity study of XZ has not been performed. To examine the tumor promoting effects of these chemicals, five-week old F344 male rats were given a single subcutaneous injection of DHPN (2400 mg/kg). Starting one week after the injection, the animals were given diet containing DMA (3000 ppm), XZ (1000 ppm, a maximum tolerated dose), or basal diet for 52 weeks. Histopathologically, the total incidences of the nasal tumors including papillomas, adenomas or carcinomas in the DMA, XZ and basal diet groups were 16/30 ($p<0.01$), 6/20 and 4/20, respectively. Dysplastic cell foci in the DMA, XZ and basal diet groups were observed at the incidences of 10/30 ($p<0.01$), 2/20 and 1/20, respectively. In the second experiment, rats were given diet containing DMA (3000 ppm) or XZ (1000 ppm) for 28 days, and plasma levels of DMA and/or XZ were measured. Plasma levels of DMA in the DMA group at days 7 and 28 were 0.36 and 0.20 µg/ml, respectively, but those of DMA and XZ in the XZ group were below the detection limit (0.02 µg/ml). These results strongly indicate that DMA has a tumor promoting effect in the rat nasal cavity, and suggest that XZ is not carcinogenic to the rat nasal cavity, because the amounts of XZ and DMA in plasma were too small to exert promotion activity in the XZ group.

1110 MIREX SKIN TUMOR PROMOTION IN MALE MICE IS REGULATED BY ESTRADIOL.

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Mirex, an organochlorine pesticide, is a potent tumor promoter in female mouse skin. Previous studies in our laboratory have shown that intact female mice are 3 to 4 times more sensitive to mirex promotion than ovariectomized (OVX) female mice, and that 17β -estradiol (E2) implants in OVX female mice can partially restore the intact female response. In addition, female mice are 3 to 4 times more sensitive to mirex promotion than male mice, further supporting a role for E2 in mirex promotion. Since male mice are relatively resistant to mirex promotion, we wanted to determine if chronic systemic E2 elevation could increase the mirex tumor response of male mice to that of females. Intact male mice were initiated with 7,12-dimethylbenz[*a*]anthracene and two weeks later were divided into groups and subjected to the following treatments; castration and blank implants, castration and E2 implants or sham castration and blank implants. Castrated male mice developed 3 fold fewer tumors than intact mice, and E2 implants were able to completely reverse the effects of castration on mirex promotion, but could not increase the male tumor response to that of intact female mice. We found that serum E2 in castrated male mice with E2 implants was 15.5 ± 5.7 pg/ml, which is at the low end of the intact cycling female range of 5.8 to 79.6 pg/ml. This difference could account for the inability of male mice to acquire the female mirex tumor response. Surprisingly, the serum E2 levels in intact and castrated male mice with blank implants were 10.1 ± 3.6 pg/ml and 9.1 ± 3.1 pg/ml, respectively. To address the disparity between serum E2 levels and mirex promotion sensitivity in intact and castrate mice, we assayed E2 in skin and found that skin E2 levels are at least ten times greater than serum E2 levels in intact male mice, and we are currently examining skin E2 levels in castrate mice. These data suggest that E2 is important in regulating mirex promotion in male mice and that cutaneous E2 levels and factors regulating E2 levels and/or responsiveness are as important as systemic serum E2. (NEIHS Grant ES08127)

1111 SIMULATION MODELING OF A MULTIDOSE, MULTITIME POINT STUDY TO TEST CLONAL GROWTH MODELS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) PROMOTION OF ALTERED HEPATIC FOCI IN THE RAT.

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Stochastic clonal growth models utilizing mutation, apoptosis, and cell division rates to describe the growth of single cell clones into altered hepatic foci (AHF) have been used successfully to simulate the growth of AHF under the influence of carcinogens such as TCDD and polychlorinated biphenyls. Three hypotheses involving the mechanism of TCDD mediated promotion were used to guide parameterization of the two-stage clonal growth model: (1) a short-term burst of cell division in clonal cells; induction of a mitoinhibitory environment, from which clonal cells escape through either (2) increased cell division or (3) reduced apoptosis. Seven models of TCDD action predicated on these hypotheses were tested against data on the number and volume fraction of GST-P positive AHF from the study. Models based on a single responsive clone type were not capable of describing the data. A model consisting of two types of clones provided the best fit to the data. One type is unresponsive to TCDD, and one responds to TCDD with a burst of cell division that declines with time. These and other observations highlight the usefulness of clonal growth models for exploring the behavior of preneoplastic clones under the influence of promoting agents.

1112 CORN OIL RAPIDLY ACTIVATES NUCLEAR FACTOR kB (NF-kB) IN KUPFFER CELLS IN VIVO.

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N-6 polyunsaturated fatty acids (*N*-6 PUFA), major constituents of corn oil, are tumor promoters and increase the rate of growth of established tumors. Both *N*-6 PUFAs and peroxisome proliferators (PPs; *e.g.*, WY-14,643) activate the peroxisome proliferator activated receptor α. Furthermore, it has been proposed that PPs cause an increase in hepatocyte proliferation by mechanisms involving activation of NF-κ B and production of mitogenic levels of TNFα by Kupffer cells; however, whether *N*-6 PUFAs are involved in increased cell proliferation in liver is not well understood. Here, it was hypothesized that *N*-6 PUFAs would produce mitogens leading to increased cell proliferation in liver. It was found that a single dose of corn oil (2 ml/kg, *i.g.*), but not olive oil or medium-chain triglycerides (saturated fat), caused ~3-fold increase in hepatocyte proliferation. Similarly, when activity of NF-κ B in whole rat liver and isolated hepatocytes or Kupffer cells was measured at various time intervals for up to 36 hours, only corn oil activated NF-κ B. Corn oil increased NF-κ B activity about 3-fold 1 to 2 hours after treatment exclusively in the Kupffer cell fraction. In contrast, increases were small and only occurred after about 8 hours in hepatocytes. The activation of NF-κ B and increase in cell proliferation due to corn oil were prevented completely when rats were pretreated for 4 days with either dietary glycine (5% w/w), an agent that inactivates Kupffer cells, or the NADPH oxidase inhibitor diphenyleneiodonium (1 mg/kg). Furthermore, arachidonic acid (100 μ M), the major metabolite of linoleic acid, activated superoxide production ~4-fold when added to isolated Kupffer cells *in vitro*. It is concluded that corn oil rapidly activates NF-κ B in Kupffer cells via oxidant-dependent mechanisms. This can trigger production of low levels of mitogens in liver and promote proliferation of hepatocytes. (ES04325)

1113 DEFICIENCY OF METALLOTHIONEIN PROMOTES SKIN CARCINOGENESIS CAUSED BY 7, 12 - DIMETHYLBENZ[A]ANTHRACENE.

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Metallothionein (MT) has been proposed to play an antioxidative role. We studied possible involvement of MT in the process of chemical carcinogenesis using MT-null transgenic mice since chemical carcinogenesis can be suppressed by free radical scavengers and antioxidant supplementation. As a cancer initiator, 7,12-dimethylbenz[a]anthracene (DMBA), a well-studied skin complete carcinogen, was topically applied to the MT-null mice as well as their wild-type control mice. In the first experiment without using promoters, DMBA was applied as an initiator at a single dose of 100, 250, 500 or 1000 g DMBA on the dorsal skin. After 14 weeks of DMBA treatment the skin tumor occurred in a dose-dependent manner in MT-null mice only, while no change was observed in wild-type control mice. In another experiment based upon two-stage carcinogenesis, DMBA was applied at a dose of 50 g, followed by TPA, a phorbol ester, twice a week for 20 weeks, which shows that MT-null mice is considerably prone to skin cancer. Histological examination revealed that skin tumors were squamous cell papillomas. The promotion by TPA gave rise to larger numbers of papilloma than the first DMBA experiment. The tumor cells showed proliferative activity as shown by PCNA staining. The present results demonstrate that deficiency of MT enhances the promotion of DMBA-induced skin carcinogenesis.

1114 THE REGULATORY ROLE OF C-MYC ON TELOMERASE ACTIVITY.

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The c-myc proto-oncogen is commonly overexpressed and amplified in human breast tumors. The c-myc gene encodes a sequence-specific DNA binding protein involved in cell proliferation and oncogenesis. However, the precise mechanisms by which c-myc promotes oncogenesis are not clear. In the present study, the regulatory role of the proto-oncogen, c-myc, was investigated. The results revealed that overexpression of c-myc following exposure of human breast cancer cells (MCF-7) to 17ß-estradiol mediated a profound increase in cell proliferation without any effect on telomerase activity in cells treated with c-myc sense oligomers. On the contrary, all cells treated with antisense c-myc oligonucleotides showed a profound decrease in cell proliferation as well as telomerase activity.

1115 PHENOLPHTHALEIN METABOLITE INHIBITS CATECHOL-O-METHYLTRANSFERASE (COMT) MEDIATED METABOLISM OF CATECHOL ESTROGENS.

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Phenolphthalein (PT), formerly used in over-the-counter laxatives, was recently identified as a multisite carcinogen in rodents, but the molecular species responsible for the carcinogenicity is not known. A novel catechol metabolite of PT, hydroxyphenolphthalein (PT-CAT), was identified and may be the molecular species responsible for at least part of the toxicity/carcinogenicity of PT. We hypothesize that PT-CAT inhibits the enzyme catechol-O-methyltransferase (COMT) and potentiates genotoxicity by either PT-CAT itself or the endogenous catechol estrogens (CEs) in susceptible tissues. The present studies were conducted to determine the effects of PT treatment and PT-CAT itself on the COMT-mediated catabolism of 4- and 2-hydroxyestradiol both in vitro and in vivo. Female mice were treated with PT (50 mg/kg/d) for 21 days and then sacrificed. PT-CAT concentration in urine plateaus by 7 days of exposure. In vitro experiments demonstrated that PT treatment resulted in an increase in free CEs, which are normally cleared by COMT and a concurrent decrease in the capacity of hepatic catechol clearance by COMT. In vitro, PT-CAT was a substrate of COMT, with kinetic properties within the range measured with endogenous substrates. PT-CAT was a potent mixed-type inhibitor of the O-methylation of catechol estrogens, with 90-300 nM IC50s. The above data, when taken together, suggest that chronic administration of PT may enhance metabolic redox cycling of both PT-CAT and the catechol estrogens and this, in turn, may contribute to PT-induced tumorigenesis.

1116 UPREGULATION OF THE AH RECEPTOR IN HUMAN BREAST CARCINOMA CELL LINES IN DIRECT PROPORTION TO THEIR MALIGNANCY.

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The Ah receptor (AhR), a ligand-activated helix loop helix transcription factor, binds environmental polyaromatic hydrocarbons, e.g., dioxin, and mediates their toxic effects. This study was designed to investigate the expression of AhR and its possible role in breast carcinogenesis, using MDA-MB231 and T47D series of breast carcinoma cell lines and Sager's 21T series which are closely matched pairs of mammary carcinoma cell lines characterized by exhibiting gradient order of malignancy (21MT> 21NT> 21PT). The MT lines showed the highest expression of AhR (~10 fold higher than PT), and NT was medium between MT and PT which expressed >20 fold higher than

normal mammary tissues. MDA-MB231 cell lines expressed higher AhR levels than the less malignant T47D cell series, irrespective of their estrogen receptor (ER) status. Although TCDD treatment in MDA-MB231 and T47D series have resulted in substantially lowering the AhR levels, the 21T series were less responsive to that effect of TCDD. TCDD treatment resulted in ~5-fold induction of urokinase plasminogen activator (u-PA) in MB231 while dramatically lowering its level in ER-transfected S30 variant of MB231. The plasminogen activator inhibitor-2 (PAI-2) was induced by TCDD in MB231 and ER-negative T47D with the opposite effect in the ER-positive cell lines. While PAI-2 levels in 21T series were inherently high in all three lines and less sensitive to TCDD treatment, the levels of uPA receptor expressed in these cells were proportional to their malignancy in direct correlation to the AhR levels. In conclusion, these data identify the AhR as a key regulator in breast cancer progression, and establish a positive correlation between the AhR expression and components of uPA system. While the AhR activation by TCDD is associated with induction of some components of u-PA system, the lack of this response in the 21T series suggests that the receptor over-expression and its PAH-dependent activation are probably different pathways. The mechanisms underlying these pathways are currently under investigation. (This work was supported by US Army grant DMAD 17-94-J14054.)

1117 EFFECTS OF BROMOETHANE (BE) ON THE ESTROGEN RECEPTOR (ER) AND PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) LABELING IN HUMAN MCF7 CELLS.

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BE is a haloethane used primarily as an intermediate in organic synthesis, and in gasoline ethylation. In a 2-year NTP bioassay, BE induced endometrial neoplasms in the uterus of B6C3F1 mice, but decreased the incidence of mammary neoplasms in female F344/N rats. The mechanisms of these effects are unclear; however, we have shown that BE does not have a direct estrogenic effect on the uterus of ovariectomized B6C3F1 mice, but may regulate ER expression. In the present study, we treated estrogen-responsive MCF7 human breast cancer cells with BE at 100, 250, 500, or 1000 mg/L every 24 hours for 3 consecutive days. Cells were harvested on days 2 and 4, and the expression of ER was evaluated using Western blotting and immunohistochemistry. Immunoexpression of PCNA was done to determine the effects of BE treatment on cell proliferation. On day 2, control and treated cells expressed a 66 kDa protein consistent with the ER. This protein was faintly expressed on day 4 by cells treated with 500 or 1000 mg/L of BE. Immunoexpression of ER was observed in control and BE-treated groups on day 2; however, by day 4 cells exposed to 500 or 1000 mg/L did not express ER. The percentage of ER-positive nuclei in cells exposed to 500 or 1000 mg/L of BE was significantly ($p<0.001$) lower than that of the controls on day 2. On day 4, the PCNA labeling index of cells incubated with 1000 mg/L was significantly ($p<0.01$) lower than that of controls. These data suggest that continuous exposure of MCF7 cells to BE at high concentrations reduces ER expression and PCNA labeling. These observations may be important in understanding the mechanism of decreased mammary gland neoplasia observed *in vivo*.

1118 NEU ACTIVATION BY ORGANOCHLORINES (OCS) IS CORRELATED TO THEIR ESTROGENIC ACTIONS IN MCF-7 CELLS.

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The question why some OCs which show very low potency as direct agonists to the estrogen receptor act in an estrogenic manner both *in vitro* and *in vivo* has not been adequately answered yet. In the current study, we examined the effects of selected OCs on activation of Neu, a receptor tyrosine kinase known to play an important role in breast cancer. Following the initial finding made in this laboratory, we studied Neu in a cell free system, in intact cells and 14 day foci formation using human breast cancer MCF-7 cells. The OCs most active in all these tests were alpha-, beta-, and gamma-HCH, o,p'-DDT, o,p'-DDT, 2,2'-dichlorobiphenyl and 2,4,5-T. In all cases the order of potencies during these and less active OCs was essentially identical, indicating the closeness in responses. To ascertain that the long term foci formation is also triggered by the initial activation of Neu kinase, we have tested the inhibitory action of a specific antibody against the ligand binding domain of Neu. The result clearly indicated that the OCs induced foci formation which is known to be promoted by estrogen, could be specifically suppressed by Neu antibody, but significantly by antibody against EGF receptor. Neu is known to act in a synergistic manner with estrogen in general, and therefore, our current hypothesis is that the activation of these OCs to activate this tyrosine kinase is causally related to their estrogenic actions on cell proliferation activities in estrogen target tissues. (Supported by NIH R01-ES/CA 07284, ES05233 and ES05707.)

1119 ESTRADIOL METABOLISM IN RAT STRAINS DIFFERING IN SUSCEPTIBILITY TO MAMMARY CARCINOGENESIS.

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Rat strains are known to differ markedly in their susceptibility to estrogen-induced mammary tumors. Specifically, the ACI strain is extremely sensitive to estrogens, whereas the Sprague-Dawley (SD) strain is quite resistant. We are investigating the basis of this strain difference by comparing the metabolism of estradiol (E2) by liver microsomes from the two strains. Both strains exhibit hydroxysteroid dehydrogenase activity, with estrone (E1) being the major product at E2 concentrations above 1 μ M. 2-Hydroxyestradiol (2-OH-E2) also is formed. As the E2 concentration is decreased, however, cytochrome P450-dependent hydroxylation becomes a more dominant pathway for both strains. At the lower concentrations the SD preparations still yield E1 as the major product. ACI liver, however, produces primarily hydroxylated-E2 at these concentrations. Of particular interest is the production of 4-Hydroxyestradiol (4-OH-E2) as a major product by ACI microsomes. This difference is most apparent at E2 concentrations below 100nM. This profound difference in the disposition of E2, and that this difference is most pronounced at E2 concentrations nearing the physiological range, suggests that this difference may contribute to the relative sensitivity of these strains to E2 carcinogenicity. (Supported by DAMD 17-97-1-7155 and ES-07079.)

1120 INDUCTION OF QUINONE REDUCTASE (QR) AND GLUTATHIONE *S*-TRANSFERASE (GST) BY GREEN TEA EPIGALLOCATECHIN GALLATE AND QUERCETIN IN MCF-7 HUMAN BREAST CANCER CELLS.

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The polyphenolic compounds quercetin and green tea epigallocatechin gallate (EGCG) are flavonoids which have potent chemopreventive activity in a variety of animal models of cancer. One proposed mechanism for the inhibition of chemical carcinogenesis by these agents is induction of phase-II detoxification enzymes. In this investigation, the effects of quercetin, commercial green tea extract, and EGCG on GST and QR activities were evaluated in MCF-7 human breast cancer cells. Following 24 hr treatment with flavonoids, a maximum 2-fold increase in GST and QR enzyme activities were demonstrated in this cell model. Quercetin caused a significant increase in QR protein expression, as measured by western blot analysis. These results demonstrate that major dietary polyphenols, such as quercetin, green tea EGCG and whole green tea extract are capable of inducing phase-II enzymes in MCF-7 cells. The induction of these enzymes *in vivo* would result in the elimination of reactive metabolites of chemical carcinogens. This suggests a mechanism for chemoprevention of mammary cancer by flavonoids.

1121 PSP94 EXPRESSION IN PROSTATE CANCER PERSISTS AFTER ANDROGEN DEPRIVATION THERAPY.

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PSP94 is one of the three major proteins secreted by prostate gland. In contrast to PSA and PAP, PSP94 was found to be androgen-independent in vitro. This prompted us to study the effect of hormonal therapy on PSP94 expression in prostate cancer. 87 radical prostatectomies from untreated patients (Group 1) and 74 from patients with neoadjuvant hormonal therapy (Group 2) were studied. Formalin-fixed, paraffin-embedded tissues were stained by ABC technique using a primary rabbit antibody against human PSP94 purified from semen. Tumor foci on each section were identified and were graded according to the scheme of Gleason. The intensity of the staining was graded on a scale of 0 to 2+, and the extent of the staining was classified as

0, 1-25, 25-50, 50-75, 75-100% on each focus. The tumor grade, pathological stage and pretreatment serum PSA levels were correlated with PSP94 expression in both groups. In Group 1, PSP94 positive staining was seen in 100% of low grade tumors and 12.5% in high grade tumors. In Group 2, a decreased PSP94 expression was observed in low grade tumors while a significant higher PSP94 expression was noted in high grade tumors (36%). No significant correlation between PSP94 expression levels and the other variables was noted in both groups.PSP94 expression in untreated prostate cancer has an inverse correlation with the tumor grade ($p<0.001$),while in neoadjuvant hormonal therapy group, an increased PSP94 expression was observed in high grade tumors . This discrepant expression pattern suggests that the regulation of PSP94 may be altered under androgen deprivation conditions.

1122 PROSTATE CANCER RISK FACTORS IN AN AREA OF COAL, IRON, AND STEEL INDUSTRIES.

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Epidemiological and toxicological data point to an impact of cadmium and polycyclic aromatic hydrocarbons on prostate cancer. Aim of the study was to identify possible risk occupations in an industrial center of the German coal, iron and steel industries. Occupations performed for more than 6 months and life time smoking habits of 238 cases with histologically proven prostate cancer and of 414 controls with benign prostatic hyperplasia were examined with a questionnaire. Confounder-adjusted odds ratios were estimated by Fisher's exact test and logistic regression analysis. No differences in the smoking habits described by pack years could be found between cases and controls. Confounder-adjusted odds ratios for both age (OR 2.56) and duration of employment (OR 2.22) were elevated in a subgroup of underground hard coal miners. An elevated prostate cancer risk was also observed for painters/varnishers (OR 2.84 adjusted for age, OR 2.91 adjusted for duration of employment). Steelworkers showed no increased risk (OR 0.93 adjusted for age, OR 0.94 adjusted for duration of employment). Businessmen showed a remarkably low prostate cancer risk (OR 0.38 adjusted for age, OR 0.37 adjusted for duration of employment). Coal dust components and dietary factors due to high energy expenditure must be discussed as possible risk factors for prostate cancer in hard coal miners.

1123 OCCUPATIONAL AND NON-OCCUPATIONAL RISK FACTORS IN BLADDER CANCER PATIENTS IN AN INDUSTRIALIZED AREA IN EASTERN GERMANY.

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Several occupational bladder carcinogens are metabolized by polymorphic enzymes. Therefore, the distribution of the polymorphic enzymes N-acetyltransferase 2 (NAT2; substrates: e.g., aromatic amines), glutathione transferase M1 (GSTM1; substrates: e.g., reactive metabolites of polycyclic aromatic hydrocarbons), and glutathione transferase T1 (GSTT1; substrates: small molecules with 1-2 carbon atoms) were investigated in bladder cancer patients in the former center of the chemical industry in Halle-Wittenberg in the former German Democratic Republic. Occupations performed for more than 6 months by 136 patients of the urological clinic in Wittenberg were examined with a questionnaire. All patients had a histologically approved transitional cell cancer of the urinary bladder. In addition, several occupational and non-occupational risk factors were considered. The genotypes of NAT2, GSTM1, and GSTT1 were determined from leucocyte DNA by PCR. Compared to the normal population in Central Europe, in the entire group the percentage of GSTT1 negative persons (22.1 %) was ordinary, the percentages of slow acetylators (59.8 %) and of GSTM1 negative persons (58.8 %) were slightly elevated. Shifts in the distribution of the genotypes were observed in subgroups who had been exposed to asbestos (6/6 GSTM1 negative, 5/6 slow acetylators), chlorinated solvents (9/15 GSTM1 negative) or who did work in rubber manufacturing (8/10 GSTM1 negative). The overrepresentation of GSTM1 negative bladder cancer patients in the industrialized area, which was more pronounced in several occupationally exposed subgroups, points to chemoprotection by the enzyme GSTM1 in bladder carcinogenesis.

1124 DECREASE OF GLIAL FIBRILLARY ACIDIC PROTEIN IN RAT FRONTAL CORTEX FOLLOWING ALUMINUM TREATMENT.

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Aluminum lactate was either intraperitoneally administered or stereotactically injected bilaterally into rat cerebral ventricles. Rats were sacrificed at various times after aluminum treatment and frontal cortex, hippocampus and striatum were dissected out. A microtiter plate based sandwich ELISA was used to measure glial fibrillary acidic protein (GFAP) concentration. GFAP levels were significantly decreased in frontal cortex seven days after lateral ventricular injection or fourteen days post systemic treatment. In contrast, neither hippocampus nor striatum had any significant changes in astrocytic intermediate filament protein. These results suggest a selective and progressive diminution of astrocytic responsivity in frontal cortex following either systemic or intraventricular aluminum dosing. An increased level of GFAP is considered a marker of reactive astrogliosis and has been reported in aluminum exposed rabbits. The depression of GFAP levels reported here in a species not forming neurofibrillary tangles (NFT) may reflect extended impairment of astrocytic function and suggests that these cells may be the primary targets of aluminum neurotoxicity.

1125 TRIMETHYLTIN ACTIVATES GLIA TO RELEASE TNF-α THROUGH REACTIVE OXYGEN SPECIES PRODUCTION.

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The trisubstituted organotin compound trimethyltin (TMT) is highly neurotoxic after *in vivo* exposure eliciting both neuronal and glial responses in the central nervous system. Recently we demonstrated that *in vitro* TMT-induced neural cell death in the presence of glial cells is reduced by a TNF-α antibody, suggesting that glial cells support neural cell death through TNF-α release. Thus, in this study we evaluated the ability of TMT to activate glia to release TNF-α and other cytokines, and the molecular mechanisms involved. Exposure of a mixed population of glial cells (astrocytes + microglia) to TMT is followed by a dramatic change in morphology and a dose dependent TNF-α release, significative from 1 μM TMT. Differently, no release of IL-6 is detectable. TMT induces TNF-α release from both highly enriched culture of astrocytes and highly enriched culture of microglia, suggesting that both cell populations are sensitive to the compound and collaborate to TNF-α production. It has been demonstrated that TNF-α release often occurs after reactive oxygen species (ROS) production in dependence of NF-κB activation. TMT 0.5 - 2.5 μM induces a dose dependent ROS production in glial cells that precedes TNF-α release. Both ROS production and the release of this cytokine are completely prevented by the use of ETYA 50 μM (a non-metabolizing analogue of arachidonic acid) and indometacine 10 μM. These results suggest that TMT-induced release of TNF-α from glial cells is dependent by the production of ROS as a consequence of the metabolism of arachidonic acid through the activation of ciclooxygenase. (Acknowledgments: this work was supported by CNR grant n° 97.04658.CT13.)

1126 CYTOKINE AND GROWTH FACTOR GENE EXPRESSION IN RESPONSE TO TRIMETHYL TIN (TMT)-INDUCED NEUROTOXICITY IN THE ADULT RAT HIPPOCAMPUS.

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Cytokines and growth factors are proposed to play a role in the homeostasis of the central nervous system (CNS) and its response to injury and disease. A role for these pleiotropic mediators in the early activation of microgial and astrocytic reactions to neural injury constitutes a dominant theme in contemporary neuroscience literature, despite the fact that most data are derived from *in vitro* preparations. Understanding the earliest events associated with toxicant-induced brain injury is necessary to detect and modify CNS damage following injury or disease. Here we examined the *in vivo* expression of interleukin-1alpha (IL-1alpha), interleukin-6 (IL-6), transforming growth factor-beta (TGF-beta), brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF) using the TMT model of neuronal injury (8mg/kg i.p.) using semi-quantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR) at 24 hours post TMT. This timepoint was chosen based on reports in the literature that mRNA for TGF-beta and IL-6 are up-regulated at this time with other types of nerve injury. No significant changes for

BDNF, NGF, or IL-1alpha were seen. mRNA for TGF-beta and IL-6 were increased slightly less than 2 fold, which is a very small increase, but is consistent with published reports. These results suggest that these neuroimmune/neurotrophic mediators do not play a crucial role in the activation of microglia and astrocytes following toxicant-induced injury to the CNS. Additional toxicants need to be examined in order to determine the generality of these observations to the neurotoxic condition.

1127 ORGAN DISTRIBUTION OF TIN AND TRACE ELEMENTS IN MICE GIVEN TRIMETHYLTIN (TMT) – RELATION TO THE TMT TOXICITY.

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Trimethyltin has been recognized as a potent neurotoxic substance. There is no information available concerning its possible interaction with trace elements in the body. In the experiments performed in male mice (CD-1, Charles River) TMT was injected ip in the form of chloride at the dose of 2.5 $mg.kg^{-1}$. The concentration of Sn and various trace elements was determined in the liver, kidneys and in the brain of intoxicated mice divided into 4 subgroups sacrificed at various time intervals (24th –168th h) of the experiment using AAS. The results were compared with the values received in corresponding control group. The concentration of Sn in the liver decreased during the experiment to 10.8%, in the kidneys to 10.6% and in the brain to 12.5% of the value at 24th h. The significant increase of the level of zinc, copper and manganese in the liver at 24th h and 48th h was noticed. Only negligible changes of the trace element levels in the brain – the main target organ of TMT toxicity - were demonstrated. A potent metal binding chelator sodium *N*-benzyl-4-*O*-(β -D-galactopyranosyl)-D-glucamine-*N*-carbodithioate (BLDTC) injected simultaneously sc with TMT decreased the concentration of tin in brain (by 5.1%). The changes of trace element levels in the liver were also corrected. The results suggest the liver might play a role in the acute toxicity of TMT. This hypothesis is supported by the fact that TMT increases the level of lipid peroxidation (measured as malondialdehyde production) not only in the brain but also in the liver as has been demonstrated in the parallel experiment in mice done in our laboratories.

1128 MANGANESE (Mn) EXPOSURE PROMOTES CELLULAR OVERLOAD OF IRON (Fe) IN CULTURED NEURONAL AND NEUROGLIAL CELLS IN VITRO.

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Our recent studies indicate that Mn exposure potently inhibits aconitase, an enzyme possessing a [4Fe-4S] cubane cluster and regulating the expression of some critical proteins involved in cellular Fe regulation (Zheng, et al., Brain Res. 799:334, 1998). This study was performed to test the hypothesis that disruption in Fe regulatory processes by Mn may lead to an altered cellular Fe status in 3 major types of brain cells. The cultured astrocytes, oligodendrocytes or PC12 cells were exposed to Mn as $MnCl_2$ in culture medium. Cellular Fe uptake was determined by quantitation of residual ^{59}Fe radioactivity in cells following addition of 1.5-2.0 μM ^{59}Fe as $FeCl_3$ in culture medium. In astrocytes, Mn exposure greatly increased cellular ^{59}Fe uptake (5.3 fold at 100 μM, $p<0.0001$) as compared to the controls. The effect was dependent upon Mn concentrations within the range of 10-100 μM. While incubation of astrocytes with 50-250 μM MPP^+ or 10-100 μM Hg did not affect cellular Fe uptake, the treatment with 100-250 μM MPTP showed a significant decrease (-26-69%, $p<0.05$) in Fe uptake compared to controls. When the oligodendrocytes and PC12 cells were incubated with 100 μM Mn, the treatment raised cellular Fe by 3.2 fold ($p<0.0001$) and 2.7 fold ($p<0.026$) in oligodendrocytes and PC12 cells, respectively. Our results demonstrate that Mn, but not MPTP or MPP^+, promotes the cellular overload of Fe. The deleterious effect of Mn on cellular Fe status appears to occur in major cell types of the CNS, but the astrocytes seem to be more sensitive to Mn than other cell types tested. (Supported by Calderone Foundation and other private funds.)

1129 MANGANESE (Mn)-INDUCED NEUROTOXICITY: IN VIVO INTERACTION WITH IRON (Fe).

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Our in vitro studies have shown that Mn exposure promotes cellular Fe overload in cultured neuronal and neuroglial cells (Zhao and Zheng). The current study was designed to investigate whether Mn alters Fe homeostasis in blood, CSF and brain. Groups of 8-10 rats received saline, Mn (36 mg/kg/wk, ip), Fe (250 mg/kg/wk, sc), or Mn (ip) plus Fe (sc) for 4 wks. Mn and Fe concentrations in tissues and body fluids were determined by AAS. While Mn injection alone caused a 32% decrease in plasma Fe ($p<0.01$) and no changes in plasma total iron binding capacity (TIBC), it increased CSF Fe by 3 fold ($p<0.01$) as compared to the controls. In rats treated with either Fe alone or Fe combined with Mn, both Fe and Mn levels in plasma and CSF were significantly increased. Combined exposure of Mn with Fe in rats greatly increased Mn concentration in striatum (4.5 fold), substantia nigra (6.2 fold), hippocampus (2.7 fold), frontal cortex (3.3 fold), and choroid plexus (8.3 fold); however, the same treatment raised Fe content selectively only in striatum (1.5 fold, $p<0.05$) and choroid plexus (2 fold, $p<0.05$). Northern blot analyses showed that none of the above dose regimens altered the expression of brain MT-I. In addition, we found that chronic Mn exposure appeared to increase the expression of glutamine synthetase by 34% ($p<0.05$) compared to controls. Our results indicate that Mn exposure seems likely to facilitate Fe transport from blood to CSF and increase Fe levels in selected brain regions. (Supported in part by NIEHS RO1 ES08146 and P20 ES06831.)

1130 MANGANESE TOXICITY IN CATECHOLAMINERGIC CELLS.

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Manganese (Mn) is an essential nutrient that, at excessive levels, produces extrapyramidal symptoms resembling those observed in patients with Parkinson's disease. Mn toxicity was tested in human neuroblastoma cells (SK-N-SH) and in a mouse catecholaminergic cell line (CATH.a). Mn was demonstrated to be more toxic (EC_{50} = 33 μM) towards the catecholamine producing CATH.a cells when compared to the non-catecholaminergic SK-N-SH cells (EC_{50} = 300 μM). This observation implicates catecholamines in the toxicant response. The Mn toxicity in the CATH.a cell line was augmented (EC_{50} = 1 μM) by pretreatment of the cells (36 hours) with an inhibitor of glutathione synthesis (1 mM L-BSO). Protection from Mn toxicity was observed with vitamin C (250 μM). The studies also evaluated the effect of Mn on tyrosine hydroxylase (TH), the rate limiting enzyme in catecholamine production. Exposure of cells to Mn produced an increase in the activity of TH (as normalized per cell). This effect was not mediated by the ability of Mn^{+2} to substitute for Fe^{+2} in the active site of TH due to the fact that addition of Mn^{+2} directly to the metal deficient enzyme did not restore activity. These results suggest that while toxic to catecholamine cells, Mn^{+2} has the potential to simultaneously upregulate biogenic amine biosynthesis.

1131 METHYLMERCURY ALTERS GLUTAMINE SYNTHETASE PROTEIN LEVELS IN NEONATAL RAT CORTICAL ASTROCYTE CULTURES.

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Glutamine synthetase (GS, EC 6.3.1.2) is a glial specific enzyme localized largely in astrocytes. GS is responsible for detoxification of NH_4^+ in the brain via its ligation to glutamate, forming the non-excitotoxic amino acid glutamine. Inhibition of GS activity produces neurodegeneration likely due to increased levels of NH_4^+ and glutamate, decreased glutamine levels, or both. Previous in vitro studies suggest mercurials inhibit GS activity. Since methylmercury (MeHg) preferentially accumulates in astrocytes, we examined its effect on GS, as a potential mechanism for MeHg-induced neurotoxicity. Primary neonatal rat cortical astrocyte cultures (20-24 days in vitro) were treated with 0.5, 1, or 2 μM MeHg for 0 to 48 hours. Total cellular proteins were collected and immunoreactive GS protein levels were determined by Western analysis. MeHg initially decreased GS protein levels with the largest decrease (~ 50%) occurring at 12 hrs. By 24 hrs, protein levels had

nearly returned to baseline, and by 48 hrs GS protein levels had increased to ~ 50% above baseline. The initial decrease in GS protein levels may be related to MeHg-induced oxidative damage that renders it more susceptible to proteolytic degradation. The ability of astrocytes to increase GS protein levels at 48 hrs despite continued exposure to MeHg might be due to their capacity to increase intracellular buffering systems for MeHg or reactive oxygen species, or both. (Supported by ES07331.)

1132 TRANSFECTION OF ASTROCYTES WITH METALLOTHIONEIN-I INCREASES THEIR RESISTANCE TO METHYLMERCURY-INDUCED CYTOTOXICITY.

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Metallothionein-I (MT-I) was expressed in neonatal rat primary astrocyte cultures and an astrocytoma cell line by pGFAP-MT-I plasmid transfection under the control of the astrocyte-specific glial fibrillary acidic protein (GFAP) promoter. Following transient transfection of the pGFAP-MT-I plasmid, MT-I mRNA and MT-I protein levels were determined by northern blot and immunoprecipitation analyses, respectively. The ability of cells overexpressing MT-I to withstand acute methylmercury (MeHg) treatment was measured by the release of preloaded $Na_2{}^{51}CrO_4$, an indicator of membrane integrity. Transfection with the pGFAP-MT-I plasmid led to increased mRNA (2.5 fold in astrocytes and 7.4 fold in astrocytomas) and MT-I protein (2.4 fold in astrocytes and 4.0 fold in astrocytomas) levels compared with their respective controls. Increased expression of MT-I was associated with attenuated release of $Na_2{}^{51}CrO_4$ upon MeHg (5 μM) treatment. These results demonstrate that MT-I can be highly expressed both in primary astrocyte cultures and astrocytomas by pGFAP-MT-I plasmid transfection, and lend credence to the hypothesis that increased expression of MT-I affords protection against the cytotoxic effects of MeHg. Taken together, the data suggest that MT offer effective cellular adaptation to MeHg cytotoxicity.

1133 MEHG-INDUCED CHANGES IN MITOCHONDRIAL TRANSMEMBRANE POTENTIAL AND MITOCHONDRIAL MASS IN RAT EMBRYONIC CNS CELLS.

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Human and animal evidence suggests that the developing central nervous system is more sensitive to methylmercury (MeHg) exposure compared to adults. In previous studies we have shown MeHg (0-4 M, 0-24 hr) mediated gene induction of P21, Gadd 53 and mitochondrial cytochrome c oxidase subunit I (COX I) genes and cell cycle inhibition in rat embryonic CNS cells. Mitochondria are known to be a target for MeHg, and exposure can decrease both mitochondrial transmembrane potential ($\Delta\psi$) and intracellular ATP. Using confocal laser scanning microscopy, we examine changes in mitochondrial transmembrane potential and mitochondrial mass using the mitochodria-specific fluorochromes CMX-rosamine and MitoTracker Green, respectively, in MeHg exposed primary rat embryonic CNS cells. Cells were exposed to MeHg (0-2 μM) for either 8 hr or 24 hr. MeHg caused a dose-related decrease in $\Delta\psi$ over time and a dose-dependent increase in the mitochondrial mass over time. While the activity of COX did not change following MeHg exposure in this study, the expression of COX I is known to increase from previous studies. ND1, another mitochondrial gene was induced by exposure to MeHg for 24 hr, but no change was observed at 8 hr. Results suggest that MeHg decrease mitochondrial transmembrane potential and increase mitochondrial mass, which may be due to the increased mitochondrial number or swelling of mitochondria or both. (This research supported by US EPA grant R825358.)

1134 PROTECTIVE EFFECT OF CYCLOSPORIN A ON METHYLMERCURY (MeHg) -INDUCED CELL DEATH IN CEREBELLAR GRANULE CELLS.

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Cerebellar granule cells are particularly sensitive to degeneration following exposure to MeHg. MeHg alters Ca^{2+} homeostasis in granule cells resulting in increases of intracellular calcium ($[Ca^{2+}]_i$); MeHg-induced increases in $[Ca^{2+}]_i$ appear to contribute to granule cell mortality. Intracellular stores including mitochondria contribute to this increase in $[Ca^{2+}]_i$. Recent studies have shown that Cyclosporin A (CsA), an immunosuppressive cyclic endecapeptide which prevents the mitochondrial permeability transition, can delay the increase of $[Ca^{2+}]_i$ induced by MeHg in granule cells in culture. In the present study, we examined if CsA, at concentrations which prevent MeHg-induced elevations in $[Ca^{2+}]_i$ will protect cerebellar granule cells from MeHg-induced cell death. Granule cells were isolated from 7 day neonatal rats and cell viability was determined after 6-8 days *in vitro* using the Live/Dead Viability/Cytotoxicity Assay. Cells were exposed to MeHg concentrations of 0.2 μ M for 76 min, 0.5 μ M for 45 min, 1 μ M for 38 min and 2 μ M for 30 min. These MeHg exposure conditions induce characteristic $[Ca^{2+}]_i$ elevations in granule cells. At 30 min after MeHg exposure, cell viability remained unchanged; however, by 24 hrs after cessation of MeHg exposure, viability decreased to 78% in 0.2 μ M MeHg, 49% in 0.5 μ M MeHg, 16% in 1 μ M MeHg, and 9% in 2 μ M MeHg. In contrast, 10 min preincubation with 5.0 μ M CsA followed by MeHg exposure, protected granule cells from MeHg-induced cell mortality at 24 hr post-exposure at concentrations of 0.2 μ M and 0.5 μ M MeHg. Simultaneous incubation with CsA and MeHg produced the same protective effect. Control cells exposed to CsA for 10 min showed no immediate change in viability, but at 24 hr exhibited a decrease in cell viability at concentrations of 1.0 μ M and higher, suggesting that CsA itself can be cytotoxic. These results suggest that CsA may protect granule cells from MeHg-induced cell death by inhibiting the mitochondrial transition. (Supported by NIH grant ES03299.)

1135 EVIDENCE FOR INDUCTION OF THE MITOCHONDRIAL PERMABILITY TRANSITION PORE BY METHYLMERCURY (MeHg) IN RAT CEREBELLAR GRANULE CELLS.

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The mitochondria permeability transition pore (MTP) is an inducible pore located on the inner mitochondrial membrane, which forms and opens under conditions of high matrix Ca^{2+} and mitochondrial membrane depolarization. MeHg causes release of intracellular Ca^{2+} stores followed by influx of extracellular Ca^{2+}, and mitochondrial depolarization reportedly results from MeHg exposure. Previous experiments demonstrated that the potent MTP inhibitor cyclosporin A (CsA) significantly delays the time-to-onset of both intracellular and extracellular Ca^{2+} phases of MeHg-induced increases in $[Ca^{2+}]_i$. Thus, we hypothesized that MeHg induces the MTP. We used single cell microfluorimetry to monitor changes in mitochondrial membrane potential (Ψ_m) of neonatal rat cerebellar granule cells in culture exposed to MeHg. Fluorescence from the potentiometric dye TMRE was measured continuously during a 10 min pretreatment with 5 μM CsA and a 45 min treatment with 0.5 μM MeHg. MeHg alone caused a significant, irreversible decrease in TMRE (tetramethylrhodamine ethyl ester) fluorescence, from 100% to 8± 2% in 45 min. Pretreatment with CsA reduced the amount of depolarization (to 43± 8% in 45 min) and delayed its time-to-onset. Use of an EGTA-containing buffer provided similar results for both the MeHg-alone and CsA-pretreated cells, indicating that the loss of mitochondrial membrane potential is not dependent on $Ca^{2+}{}_e$. These results suggest that mitochondria undergo a permeability transition in response to MeHg exposure, which is delayed but not prevented by exposure to CsA. (Supported by NIH grant ES03299.)

1136 EFFECTS OF METHYLMERCURY (MeHg) ON REGULATION OF INTRACELLULAR CALCIUM IN RAT CEREBELLAR NERVE TERMINALS.

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MeHg is a prominent environmental neurotoxicant with pronounced effects in the cerebellum, especially on granule cells. The effects of MeHg likely result from a variety of cellular actions; one mechanism may involve MeHg -induced increases of intracellular calcium concentration ($[Ca^{2+}]_i$) leading to Ca^{2+} -mediated cell death. The purpose of the present study was to examine the sensitivity of isolated nerve terminals (synaptosomes) of rat cerebellum to changes in $[Ca^{2+}]_i$ induced by MeHg. Cerebellar synaptosomes were isolated, loaded with the Ca^{2+} fluorophore fura-2, and fluorescence was monitored continuously using alternating excitation at 340 and 380 nm. The ratio of emitted fluorescence at 510 nm was calculated before and during exposure to MeHg (5-50μ M). MeHg caused a biphasic increase in fluorescence ratio- indicative of an elevation of $[Ca^{2+}]_i$. The first phase occurred within 10 sec of MeHg application and the second after 1 min. Removal of Ca^{2+} from the medium didn't affect the first phase, but completely ablated the second phase, indicating that the second phase arose from entry of extracellular Ca^{2+}. A significant increase in fluorescence ratio occurred at concentrations of MeHg $\geq$ 6.5μ M for both phases. In contrast, previous experiments using

cortical terminals showed that MeHg did not significantly affect $[Ca^{2+}]_i$ at concentrations < 25 μ M at 1 min. Thus, cerebellar nerve terminals appear to be more sensitive to the action of MeHg to disrupt Ca^{2+} regulation than are cortical terminals. Pretreatment with ω -conotoxin MVIIC (0.5 μ M) and ω -Aga toxin IVA (10 and 100nM), peptide toxins which block Q- and P-type Ca^{2+} channels respectively, caused decreases in both phases, suggesting that MeHg may enter the cell, in part through P- and/or Q- type Ca^{2+} channels. (Supported by NIH Grant ES03299.)

1137 EVALUATION OF SENSORY EVOKED POTENTIALS IN LONG EVANS RATS GESTATIONALLY EXPOSED TO MERCURY (Hg^0) VAPOR.

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This study assessed sensory function following gestational exposure to mercury (Hg^0) vapor. Pregnant Long Evans dams were restrained and nose-only exposed to 0 (n = 12) or 4 mg/m³ Hg^0 vapor (n = 10) for 2 hr/day over gestational days 6 – 15. One male and one female offspring from each dam were allocated for electrophysiological evaluation of sensory function. At 19 - 23 weeks of age, the animals were surgically implanted with epidural screw electrodes. The following endpoints were quantified about 1 week after surgery: flash and pattern visual evoked potentials (0.08, 0.16, 0.32 cpd), brainstem auditory evoked potentials (rarefaction click, 4, 16, 64 kHz), cortical and cerebellar somatosensory evoked potentials, and ventral caudal tail nerve compound nerve action potentials and nerve conduction velocity. Three intensities were used for each stimulus condition. No significant treatment-related effects were indicated on the animal's body weight, colonic temperature, tail nerve temperature, nerve conduction velocity, cortical or cerebellar somatosensory potentials, flash evoked potentials, pattern evoked potentials, or brainstem auditory evoked responses. Although not highly statistically significant, there was an apparent slight decrease in amplitudes and increase in latencies of peaks of compound nerve action potentials in male animals after Hg^0 exposure. The data indicate that gestational exposure to 4 mg/m³ Hg^0 produced minimal changes in adult sensory electrophysiology. However, a small change in peripheral nerve function, of unknown biological significance, cannot be excluded.

1138 EFFECTS OF METHYLMERCURY CHLORIDE AND MERCURIC CHLORIDE ON NEURITE OUTGROWTH AND CELL VIABILITY IN PC-12 CELLS.

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Rat pheochromocytoma (PC-12) cells differentiate to resemble sympathetic neurons upon exposure to nerve growth factor (NGF) making them a popular model for the study of neuronal development. We examined the effects CH_3HgCl or $HgCl_2$ on neurite outgrowth and cell viability using undifferentiated (unprimed) and differentiated (primed) PC-12 cells. When unprimed PC-12 cells are incubated with 50 ng/ml of NGF for 7 days (priming period), neurite growth was observed as early as 48 hours after exposure and increased throughout the priming period. Once primed, PC-12 cells can be replated and neurite outgrowth can be assessed relatively acutely (6-24 hours). At 24 hours, the EC_{50} for inhibition of neurite outgrowth in primed PC-12 cells in the presence of NGF for CH_3HgCl was approximately 0.02-0.03 μM, and approximately 0.1-0.3 μM for $HgCl_2$. In the absence of NGF, neurite outgrowth is low in controls, with little effect of CH_3HgCl or $HgCl_2$ noted. The mercurial compounds had opposite effects on neurite outgrowth in unprimed PC-12 cells as compared to primed PC-12 cells. At low concentrations (0.01-0.1 μM), both mercurial compounds appear to initiate neurite outgrowth in the presence or absence of NGF. In addition to neurite outgrowth, cell viability was assessed in parallel cultures. The EC_{50} for cytotoxicity of CH_3HgCl in both primed and unprimed PC-12 cells was approximately 1-3 uM. In the primed and unprimed PC-12 cells, the EC50 $HgCl_2$ was approximately 2-3 μM. These results show that cytotoxicity occurred at concentrations that were 10 fold higher than the EC_{50} for inhibition of neurite outgrowth, suggesting that the mercurial compounds may act specifically on the process of differentiation. (DKP was supported by NIEHS Training Grant T32ES07126.)

1139 LEAD ENHANCES NGF-INDUCED NEURITE OUTGROWTH IN PC12 CELLS VIA ENHANCED MAP KINASE ACTIVATION.

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Rat pheochromocytoma (PC12) cells were cultured for four to six days under the following conditions: in the presence of no toxins; various concentrations of lead acetate; nerve growth factor (NGF); and in the presence of lead acetate and NGF. Lead acetate enhanced NGF-induced neurite outgrowth in PC12 cells in a concentration dependent manner and promoted the formation of multiple neurites per cell. However, lead acetate alone did not cause neurite outgrowth in PC12 cells. Further studies showed a correlation between the effect of lead acetate on enhanced NGF-induced neurite outgrowth and enhanced NGF-induced mitogen activated protein (MAP) kinase activation. Lead acetate in the presence of NGF caused a significant concentration dependent increase in MAP kinase activation at 24 and 48 hour lead exposure times. The identity of the kinase being activated was confirmed to be MAP kinase by Western blot analysis. Thus, lead acetate can play a role in MAP kinase intracellular signaling initiated by NGF, and therefore cause amplified neurite outgrowth in PC12 cells. (This work was supported by The Department of Veterans Affairs and NIEHS Grant #5K14 ES00286-02.)

1140 LEAD ACETATE MODULATES THE *IN VITRO* PRODUCTION OF NITRIC OXIDE BY C6 GLIAL CELLS.

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The CNS neurotoxic effects of lead (Pb) are well documented, however, its molecular toxicity targets have not been clearly delineated. The present investigations were conducted to test the hypothesis that Pb exposure may perturb signal transduction systems leading to nitric oxide (NO) production by astroglial cells. C6 glioma cells were cultured in DMEM/no phenol red/2.5% fetal calf serum and stimulated with TNF-α (10 ng/ml) in combination with IL-1β (1 ng/ml), IFN-γ (100 U/ml) or LPS (1μg/ml). Generation of NO was quantified as the stable nitrite endproduct by the Griess reaction. C6 cells generated 12.3-13.8 nmol nitrite/10^6cells/24hr. Pretreatment with the nitric oxide synthase inhibitor Nω-nitro-L-arginine methyl ester (1-100μM) concentration-dependently inhibited NO production. Cells were then exposed to added lead acetate (100 nM-5 μM) for 3 hr and stimulated with the cytokine pairs for 24 hr. Pb concentration-dependently inhibited generation of NO up to 90% while no inhibition was noted in sodium acetate treated controls. In repeated experiments, calculated lead acetate IC_{50} (the concentration which inhibited NO generation by 50%) ranges were: 1.95-2.81 μM for TNFα + IL-1β, 3.55-4.6 μM for TNFα + IFN-γ and 1.64-2.41 μM for TNFα + LPS. The effects of Pb were not due to loss of cell viability or decreases in cell number. The central focus of ongoing investigations is identification of the molecular toxicity target(s) in these signal transduction pathways. Glial cells provide critical supportive and nutritive functions for neurons. Glial cells are a source of NO which is now recognized as a major messenger molecule in the CNS where it may function in neurotransmission, regulation of cerebral blood flow, and modulation of synaptic plasticity and sensory signaling. Perturbation of the NO generating pathways in the CNS may contribute to neurophysiologic changes noted following occupational or environmental exposure to Pb. (This research was supported in part by National Institutes of Health Grants RR08111 and RRO3020.)

1141 UPREGULATION OF TH MRNA EXPRESSION BY INORGANIC LEAD IN PC12 CELLS AND RAT LOCUS COERULEUS.

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We have previously reported that inorganic lead (Pb^{2+}) causes an increase of tyrosine hydroxylase (TH) activity in the hippocampus of rat in vivo and in PC12 cells in vitro. In the present study we investigated the effects of Pb on TH mRNA expression in rat locus coeruleus and in PC12 cells as a model system. The results of competitive RT-PCR showed that TH mRNA level was increased by 152% in locus coeruleus of rat exposed to 0.2% lead acetate from gestational day 16 through postnatal day 21. Exposure of PC12 cells to 0.5 μ M Pb^{2+} for 2 hr increased TH mRNA level by 28%. Furthermore, in PC12 cells, the same Pb treatment resulted in 90% enhance-

ment of TH gene transcription rate and 180% increase of the expression of chloramphenicol acetyltransferase (CAT) reporter gene construct with the 5' flanking region of the TH promoter (bases –272 to +27). These results indicate that Pb^{2+}-induced increase in TH mRNA expression reflects, at least in part, the increase of gene transcriptional rate. The enhancement of the TH gene transcriptional rate may involve lead responsive element(s) in the proximal promoter region of 272 bases of TH gene. (This work was supported by NIEHS grants ES09516 and ES06365.)

1142 THE EFFECTS OF LOW LEVEL POSTWEANING LEAD EXPOSURE ON TYROSINE HYDROXYLASE.

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Several recent studies from our lab and others suggest that one possible mechanisms for the detrimental effects of lead (Pb) on the CNS could be an alteration in dopaminergic (DA) systems. This study examined the effects of low level postweaning Pb exposure on tyrosine hydroxylase (TH), the rate limiting enzyme in the production of DA, in both the nigrostriatal and mesolimbic DA systems. Male Long Evans rats were exposed to 0, 100 or 500 ppm Pb acetate (n=8, 8, 8) in drinking water from 21 days of age until 171 days of age. These exposures produced mean blood lead levels of .44, 16.7 and 62.34 ug/ dl, respectively. Non-perfused brains were extracted and punches were taken to remove the SNc and VTA, while the NAcc and Str were extracted surgically. Due to the small amount of tissue, VTA samples were pooled (2 brains per sample) creating a VTA n=4 for each exposure. RNase protection assays were performed on the VTA and SNc samples to determine TH mRNA levels, while enzyme assays and western blotting were performed on the NAcc and Str samples to determine TH activity and TH protein levels. Pb exposure did not produce significant changes in either the SNc or VTA. However, there was an increase in TH activity in the NAcc with increasing Pb exposure (significance obtained between the 0 and 500 groups), while the Str showed no lead effect. There was a trend towards increasing amounts of TH protein with increasing Pb exposure, yet only in the Str were these changes significant (between the 0 and 500 ppm groups). These data suggests that while lead does not increase the amount of TH-RNA made by the dopaminergic cell bodies, it alters TH enzyme activity and protein levels in the dopamine nerve terminals, and may do so differently in the two dopamine systems. This could possibly be due to an increase in TH synthesis, a stabilizing effect on the TH protein, increased affinity for the biopterin cofactor or a combination of the three. ES07026, ES05017, ES02047.

1143 TIME COURSE AND REGIONAL SPECIFICTIY OF LEAD-INDUCED ALTERATIONS IN DOPAMINE CONTENT.

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Lead (Pb) is reported to produce differential, sometimes opposing effects on the mesocorticolimbic (nucleus accumbens (NA), frontal cortex (FC), and hippocampus (HIP)), and the nigrostriatal (striatum, brain stem and hypothalamus) dopamine (DA) metabolism (Kala and Jadhav 1994). This study sought to determine the time-dependent effects of Pb on the dopamine content of these systems during the postweaning period. Twenty-one day old male Long Evans rats exposed to 0 ppm (as sodium acetate in drinking water) and 50 ppm Pb (as lead acetate in drinking water) were sacrificed at 0, 30, 60 and 90 days. Blood lead analysis by atomic absorption spectrophotometry showed a significant difference between the control and lead-treated rats at each time point except at day 0, without any significant difference between day 30, 60, and 90, indicating that blood lead stabilizes to the Center for Disease Control and Prevention's threshold level for elevated lead burden for children by day 30 (10 μg/dl). The evaluation of tissue dopamine contents by high performance liquid chromatography indicated complex time- and region-specific changes in dopamine content: NA DA (% change from control) contents increased significantly in lead treated rats at day 30 and then decreased gradually at day 60 and 90; HIP DA contents increased at day 60 and at day 90; and FC DA contents showed insignificant decreases at day 30, 60 and 90. These data further support Pb's opposing effects within the mesocorticolimbic system and suggest time-dependent compensatory changes between NA and HIP. These results also demonstrate the critical need to explore the time course of effects of low lead exposure in greater detail. (Supported by ATSDR Cooperative Agreement # U50/ATU 398948.)

1144 INHIBITION OF PKC ACTIVITY IN RATS EXPOSED TO SUB CHRONIC LOW LEVEL LEAD (Pb).

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Our previous studies have demonstrated that low level exposure to Pb leads to specific changes in indices of operant behavior, i.e., fixed interval (FI) and fixed ratio (FR) in rats. Protein kinase C (PKC) is an important enzyme in mediating cellular transduction mechanisms and neurotransmitter release. Additionally, phosphorylation of brain proteins by PKC plays an important role in regulation of neuronal plasticity, a physiological basis of learning and memory. Under conditions which produced changes in operant behavior, we conducted studies to assess the effects of low level Pb exposure (50 ppm) for 90 days on PKC activity in various brain regions of male Long Evans rats. Results of these experiments indicate that significant region-specific Pb accumulation was associated with changes in PKC activity. These results indicate that Pb-PKC interactions may be important in learning deficits associated with low level Pb exposure. (Supported by ATSDR Cooperative Agreement # U50/ATU 398948.)

1145 ROLE OF PROTEIN KINASE C ACTIVATION IN LEAD ACETATE-INDUCED DNA SYNTHESIS IN 132 1N1 HUMAN ASTROCYTOMA CELLS.

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Lead is known to be a potent activator of protein kinase C (PKC) and the PKC signal transduction system may modulate the proliferation rate of gliomas. This study was undertaken to investigate the ability of lead to activate PKC and its downstream events in 132 1N1 human astrocytoma cells. Treatment with 50 μM lead acetate for 1 hour was able to induce PKCα translocation as evidenced by Western Blot analysis and enzyme activity assay, while PKCε was not affected. Lead treatment (1 μM to 100 μM for 24 hour), resulted in a concentration- and time-dependent increase in DNA synthesis up to 300% of basal level, as measured by ^{3}H-thymidine incorporation assay. No cytotoxicity was observed, as measured by LDH release assay. The effect of lead was blocked by two PKC inhibitors, bisindolylmaleimide and staurosporine, and also by down-regulation of PKC by a pretreatment with the phorbol ester PMA. Flow cytometric analysis using double staining with bromodeoxyuridine(Brdu)-quenchable Hoechst 33258 and ethidium bromide showed that treatment with lead or PMA stimulated the G_0/G_1 to S/G_2 phase transition resulting in increased percentage of cells in the S/G_2 phase (30% in treated group vs. 4% in control group). These results suggest that lead stimulates DNA synthesis and cell-cycle progression in human astrocytoma cells through a PKC-dependent mechanism. (Supported in part by grant ES07033.)

1146 LEAD AFFECTS EXPRESSION OF ZIFF268 IN RATS.

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Exposure to lead is associated with a significant impairment of cognitive function and a delay in behavioral development. However, the molecular and biochemical mechanism of lead toxicity is not clear. Transcriptional regulation of genes is one of the pivotal mechanisms of neuronal responses to many stimuli underlying neural plasticity. The immediate early response gene (IEG) zif268 encodes a zinc finger family transcription factor that has been shown to be a third messenger in neural plasticity. In our previous study, lead increased expression of IEGs including zif268, c-fos and c-jun in PC12 cells. Here, we investigated the effect of lead on expression of zif268 by in situ hybridization in brains of Long-Evans rats exposed at birth to 0.2% lead acetate in the drinking water. Exposure to lead resulted in higher levels of zif268 expression in the somatosensory cortex but not in the striatum, hippocampus, occipital cortex or cerebellum in 28-day old animals. Electroconvulsive seizure (ECS) was examined to study the effect of lead on the induction of zif268 mRNA expression. The expression of zif268 mRNA peaked within 30 min after ECS in nonexposed rats, dramatically declined at 1h, and returned to baseline levels within 4h. In lead-exposed rats, however, the levels of zif268 mRNA remained elevated in the dentate gyrus 1 h after ECS but declined to baseline levels at 4 h. This effect of lead was specific to the dentate gyrus since the time course of zif268mRNA decline did not change in other areas of brain, including the CA1, CA2, CA3 regions of the

hippocampus. Similar observations were made in 42-day old animals, and, in addition, ECS induced expression of zif268 in the cerebellum was also not affected by lead. These results suggest that exposure to lead interferes with the regulation of zif268 mRNA levels in specific areas of the brain.

1147 THE DNA-BINDING CHARACTERISTICS OF A SYNTHETIC APO-ZINC FINGER PEPTIDE VERSUS A rhSP1 PROTEIN IN THE PRESENCE OF VARIOUS DIVALENT METALS.

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The zinc finger motif belonging to the Cys_2-His_2 family provides structural framework for a number of critical proteins, which are essential for cellular function. This motif requires Zn(II) for its proper structural folding and activity. Perturbations in this domain either by metal competition or displacement could result in disruption of neurobiological functions, such as altered DNA-binding, changes in receptor function and channel properties. To determine whether these domains are potential targets for heavy metal perturbations, the DNA-binding activity of a rhSP1 protein and a synthetic apo-zinc finger peptide, of the type present in the transcription factor SP1, in the presence of several divalent metal ions, were analyzed using the electrophoretic mobility shift assay. Results indicate that metals with similar physiochemical configurations to Zn (Cd, Hg, and Pb) were able to complex with the apo-peptide and bind to their cognate DNA consensus sequence with high affinity and specificity. These same metals inhibited the DNA-binding activity of rhSP1 protein, which already contains the bound zinc, in a concentration-dependent manner. Both Pb(II) and Cd(II) were more detrimental to the DNA-binding of the intact rhSP1 than all the other metals tested. Class IIA metals such as calcium neither exhibited the proper peptide complexation nor did they bind DNA or interfere with rhSP1 DNA-binding ability. Therefore, we postulate that environmental heavy metals selectively modulate zinc finger proteins through structural alterations of their zinc finger motifs thus ultimately altering their function.

1148 Pb-INDUCED CHANGES IN SP1 FUNCTION INVOLVE PKC BUT NOT MAPK SIGNALING PATHWAYS.

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Cognitive deficits have been shown to result from developmental lead (Pb) exposure and may involve modulation of genes crucial for growth and differentiation. A link in these events may be the zinc-finger transcription factor Sp1 and the second messenger protein kinase C (PKC). Previous experiments from this lab have shown that Pb alters Sp1 DNA-binding and protein levels as well as PKC activity. Growth factor receptors can result in activation of PKC, thereby, initiating the mitogen-activated protein kinase (MAPK) cascade and resulting in phosphorylation of nuclear transcription factors associated with growth and differentiation. The objective of the following study was to relate previously observed changes in Sp1 with these signaling cascades and to determine their cellular location. Rat pups were exposed lactationally to Pb or control water from postnatal day 1-20 (0.2% Pb acetate in the drinking water of the dam). Animals were sacrificed at various postnatal time points, and their cerebella and whole corticies were dissected. Western blots were performed on cytosolic, nuclear and membrane protein extracts to determine changes in PKC and MAPK expression. Both nuclear PKC and MAPK showed an increasing developmental profile in the cerebellum and cortex, however only PKC patterns were modulated by Pb. No developmental patterns or effects of Pb were seen with either protein in cytosolic fractions. However, cerebellar membrane PKC protein developmental profiles were modulated by Pb. These data suggest that the effects of Pb on nuclear Sp1 involve PKC but not MAPK.

1149 Pb TOLERANCE IN C6 RAT GLIOMA CELLS.

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Astroglia serve as a depot or filter for Pb in the brain. A number of observation in cell culture indicate that astroglia are capable of adaptive responses or tolerance to the presence of intracellular Pb, including minimal effects on proliferation and viability, recovery of transiently depleted intracellular glutathione levels and buffering of intracellular Ca and Pb. C6 rat glioma cells resemble rat astroglia in culture in that both cell types accumulate Pb intracellularly from the medium. As such, C6 cells are a model for Pb accumulation by the brain. In the present study we examined a possible molecular mechanism for tolerance. Exposure of C6 cells for one week to 1 μM Pb acetate resulted in the increased abundance of a protein with the following characteristics on two-dimensional acrylamide gels: 71 kD with pI of 5.6. This protein matches HSP78 and GRP78 by protein microsequencing. The levels of five other proteins ranging in size from 37-41 kD with pIs of 6.0-6.8 declined. In addition, we isolated a 71 kD protein from Pb-exposed C6 cells by the use of a Pb-affinity column. This protein was strongly bound to Pb and could not be eluted by 500 mM imidazole unless 50 mM EDTA was applied. The protein sequence is unknown and is under investigation. These observation extend our previous results that most of the Pb retained by C6 cells is associated with high molecular weight soluble components of the cytosol, rather than with smaller components, such as glutathione or metallothionein.

1150 SYNAPTOTAGMIN AND OTHER C2-DOMAIN PROTEINS BIND LEAD WITH HIGH AFFINITY.

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Lead (Pb^{++}) has been shown to increase presynaptic neurotransmitter release from nerve terminals, and this action may cause its neurotoxicological effects during development. It has also been demonstrated that Pb^{++} potently activates protein kinase C (PKC), possibly by binding to its C2 regulatory domain that mediates calcium-dependent phospholipid binding. We hypothesized that Pb^{++} increases neurotransmitter release by activating synaptotagmin, a synaptic vesicle protein with two C2 domains that is required for synaptic vesicle exocytosis. We demonstrated that Pb^{++} promotes synaptotagmin binding to acidic phospholipids (EC_{50} ~7 nM free Pb^{++}), about 300-fold more potently than calcium (EC_{50} ~2 uM). Mutagenesis of a critical aspartate in synaptotagmin's C2A domain reduced both Pb^{++} and Ca^{++} binding to the protein. Pb^{++} (100 nM) partially protected endogenous synaptotagmin in rat brain extracts from tryptic cleavage in a manner similar to that of 10 uM Ca^{++}, further establishing that synaptotagmin is a high-affinity Pb^{++}-binding protein. We next investigated whether Pb^{++} interacts with C2 domain proteins in general. Pb^{++} promoted the binding of PKCα, synaptotagmin I, and synaptotagmin III C2A to phospholipids, but not the binding of rabphilin 3A, synaptotagmin III C2B, or Munc-13. Thus, Pb^{++} may bind and activate a subset of the dozens of proteins known to contain C2 domains, and its effects on synaptotagmin in particular may disrupt synaptic transmission during development.

1151 REGION-SPECIFIC EFFECTS OF Pb^{2+} EXPOSURE ON CENTRAL CHOLINERGIC AND CATECHOLAMINERGIC PATHWAYS.

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We previously reported that lead exposure of perinatal rats from E16 to PN21 caused a marked 20-40 % reduction in expression of ChAT activity in the septum and hippocampus and 50-90 % increase of TH activity in the hippocampus without alternations in the septal activity (Brain Res.1996). In this study the effects of Pb exposure on ChAT and TH activities were compared in different regions of the brain. The rats were maternally lead-exposed by giving 0.2 % lead acetate in drinking water to dams from one week before parturition through weaning at postnatal day 21 (PN21). Lead-exposure effected depression of ChAT activities in the septum (29%), hippocampus (24%), nucleus basalis of Meynert (27%), parietal cortex (37%), frontal cortex (21%), preoptic magnocellular nucleus (32%), striatum (24%), and spinal cord (14%), in contrast Pb had no influence on ChAT activities in the horizontal diagonal band, olfactory bulb, thalamus and cerebellum. Pb exposure resulted in increase of TH activity in the hippocampus (49%), substantia nigra (62%), striatum (18%), and locus coeruleus (137%) whereas TH activities in the septum, frontal and parietal cortices were not significantly altered. These results indicate that early developmental lead exposure decreases the ChAT activity in cholinergic areas Ch1, Ch2 and Ch4 but has slight or no effect on Ch3, Ch5, and Ch6. In contrast to its effect on ChAT activity, Pb increases activity of tyrosine hydroxylase in select catecholaminergic pathways i.e. locus coeruleus –hippocampus and substantia nigra-striatum. This suggest that Pb exposure can induce imbalance between cholinergic and catecholaminergic systems in the brain. (Supported by NIEHS grant # ES 06365.)

1152 LEAD NEUROTOXICITY IS ASSOCIATED WITH DNA FRAGMENTATION AND INCREASED BY GLUTAMATE.

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The role of glutamatergic neuronal stimulation, the production of reactive oxygen species (ROS) and DNA fragmentation in lead neurotoxicity was investigated in mouse hypothalamic GT1-7 cells. The cells were stimulated with 1 mM glutamate, 1 mM lead acetate (PbAc) and their combination for 3, 6, 12, 24, 48 or 72 hours in a cell incubator. The production of ROS and cell viability were measured at each time point in 48-well plates by using fluorescent probes, dichlorofluorescin diacetate and propidium iodide, respectively. DNA fragmentation was studied by isolating the fragmented DNA from cells cultured in 6-well plates. Isolated DNA was subjected to agarose gel electrophoresis in which typical DNA ladder formation is seen if DNA fragmentation had occurred. Glutamate alone increased ROS production compared to control during 72 hours. However, glutamate did not have a significant effect on cell viability, and did not induce DNA fragmentation at any timepoint. PbAc alone or together with glutamate did not increase ROS production during exposure time of 72 hours. Cytotoxicity of PbAc was seen at 24 hours after the beginning of exposure, thereafter neurotoxicity of PbAc was also amplified by glutamate. At this same, and at later time points, PbAc alone and together with glutamate induced DNA fragmentation. These data suggest that neurotoxic effects of lead are mediated through mechanisms other than ROS production, and that glutamatergic neuronal stimulation increases lead neurotoxicity. Because lead can mimic calcium in cellular processes, it seems possible that lead enters neuronal cells upon glutamate-induced excitation. Thus, glutamatergic receptor stimulation can be an important factor in augmented lead neurotoxicity. Moreover, lead-induced cell death may partially be mediated through apoptosis. (Supported by The Academy of Finland and University of Kuopio.)

1153 NMDAR-2A SUBUNIT PROTEIN EXPRESSION IS REDUCED IN THE DEVELOPING RAT HIPPOCAMPUS BY EXPOSURE TO Pb^{2+}.

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The *N*-methyl-D-aspartate receptor (NMDAR) has been identified as a principal target for lead (Pb^{2+})-induced neurotoxicity. Age-dependent changes in NMDAR subunit gene expression have been observed in the hippocampus of rats chronically exposed to Pb^{2+} during development (Guilarte and McGlothan, Brain Res 790:98, 1998). These changes were present at blood Pb^{2+} levels ranging from 20-60 µg/dl. In the present study, littermates were used to determine whether the changes found in gene expression were reflected in protein levels. NR1, NR2A, and NR2B subunit protein levels were measured in rat hippocampus and cortex at postnatal days (PND) 7, 14, 21, and 28 by Western blot and densitometric analyses. A treatment effect was apparent for NR2A subunit protein expression in the hippocampus ($F_{1,28}$=10.224, p<0.01). NR2A subunit protein was reduced by 40%, 19%, and 27% from control levels in PND14, 21, and 28 Pb^{2+}-exposed rats, respectively. Mean comparisons within age groups indicated that rats at PND14 exhibited the most significant reduction of NR2A ($F_{1,8}$=32.53, p<0.001). These data concur with our previous data finding reduced NR2A mRNA in hippocampal pyramidal and granule cells of Pb^{2+}-exposed rats. Pb^{2+}-exposure during development had no effect on NR1 or NR2B subunit protein expression in the hippocampus at any age. No effect was observed on any subunit in the cortex at any age. The developmental profile of the NMDAR-2A subunit protein in the hippocampus is specifically changed by chronic exposure to Pb^{2+}. These data suggest that the composition of subunits comprising hippocampal NMDAR may be altered in Pb^{2+}-exposed rats. (Supported by ES06189 to TRG and T32 ES07141 to MKN.)

1154 DEVELOPMENTAL Pb^{2+}-EXPOSURE CHANGES NMDAR1 SPLICE VARIANT mRNA IN THE RAT HIPPOCAMPUS.

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N-methyl-D-aspartate receptors (NMDARs) serve important roles in synaptic plasticity and long-term potentiation, an experimental model of learning and memory. We investigated the effects of chronic Pb^{2+} exposure on the expression of NMDAR1 splice variants in the developing rat hippocampus. The oligonucleotide probes NR1-a, NR1-b, NR1-1, NR1-2, and NR1-4 have been described previously (Laurie and Seeburg, J. Neurosci. 14:3180-3194). Pb^{2+}-exposure resulted in a robust increase in NR1-a mRNA throughout the rat hippocampus at postnatal day (PND) 14 (p<0.05). The NR1-b mRNA was increased in the CA1, CA2 and CA3 regions at PND21 (p<0.05). NR1-2 mRNA was increased at PND21 in all regions of the hippocampus except the CA3 region. Notably, expression of NR1-4 mRNA was reduced in the CA3 region ($F_{2,12}$=4.62, p<0.05) and throughout the pyramidal cell layer ($F_{2,12}$=3.896, p<0.05), also at PND21. No differences were detected in NR1-1 mRNA variant expression. These data indicate that individual splice variants of the NMDAR are selectively affected by developmental Pb^{2+} exposure in the rat hippocampus. Alternative splicing of NR1 mRNA provides molecular diversity in NMDAR assemblies and result in combinations that exhibit different pharmacological and electrophysiological properties. Pb^{2+}-induced changes in NR1 splice variant mRNA may ultimately alter NMDAR function during development. These studies support the hypothesis that NMDAR and specific NR1 splice variants are important targets for Pb^{2+}-induced neurotoxicity. (Supported by ES06189 to TRG.)

1155 ENHANCEMENT OF THE INHIBITORY POTENCY OF ZINC (Zn^{+2}) FOR THE RAT NMDA RECEPTOR CHANNEL IN THE PRESENCE OF LEAD (Pb^{+2}).

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The NMDA subtype of glutamate receptor is widely recognized to be important in synaptic plasticity and cognitive function. Consequently, the effect of Pb^{+2} on this receptor has been one focus of attempts to define the bases of exposure-induced cognitive impairments. This study examined the effects of acute exposure *in vitro* to concentrations of free Pb^{+2} and/or Zn^{+2} on NMDA receptor channel function. Tritonized cortical membranes were prepared from adult male non-exposed rats, and binding measured in 50 mM Tris-acetate (pH = 7.0) with ^{3}H-MK-801 in the presence of saturating concentrations of glutamate and glycine. The potency of Pb^{+2} to inhibit the receptor channel (IC_{50} 0.55 uM) was 2.3-fold greater than that of Zn^{+2} (IC_{50} 1.28 uM). Dissociation of MK-801 from its binding site exhibited two-component kinetics, and both components were significantly slowed in the presence of Pb^{+2} or Zn^{+2}. To determine if Pb^{+2} inhibited the receptor channel by binding to the Zn^{+2} modulatory site Zn^{+2} inhibition curves (10^{-8} – 10^{-4} M Zn^{+2}) of MK-801 binding were determined in the presence of constant concentrations of Pb^{+2}. In contrast to the rightward shift of the inhibition curve expected for a competitive interaction, increasing constant levels of Pb^{+2} produced a concentration-dependent downward shift of the Zn^{+2} inhibition curve, indicating a non-competitive inhibition by Pb^{+2} with respect to that of Zn^{+2}. Furthermore, Zn^{+2} IC_{50} values significantly decreased (1.28 uM to 0.54 uM) as a function of increasing Pb^{+2} concentration. Analogous results were obtained when Pb^{+2} inhibition curves (10^{-8} – $10^{-5.5}$ M Pb^{+2}) were determined in the presence of constant concentrations of multiple levels of Zn^{+2}. In contrast to acute Pb^{+2} exposure *in vitro* however, Scatchard analyses in hippocampal tissue from animals chronically exposed to Pb in drinking water reflected an increase of MK-801 binding sites that was significant in the 0.2% Pb group. These results indicate that: 1) Pb^{+2} and Zn^{+2} exert their inhibition on the NMDA receptor via independent binding sites; and 2) inhibition of the receptor channel may be one factor in the up-regulation of MK-801 sites found in chronically exposed animals. (Supported by ES06253, NRC, and US EPA.)

1156 EXPRESSION OF THE NMDAR1 mRNA AND ITS RELATIONSHIP TO SP1 DNA-BINDING IN THE RAT HIPPOCAMPUS FOLLOWING DEVELOPMENTAL LEAD EXPOSURE.

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Learning incapabilities are known to be produced by lead exposure. The N-methyl-D-aspartate R1 subunit (NMDAR1), which plays a significant role in learning and memory, contains several Sp1 response elements in its promotor region. Sp1, a zinc finger protein, is a transcription factor essential for transcribing genes that are needed for cellular growth and differentiation. Evidence in our laboratory suggests that lead alters Sp1 DNA-binding. The goal of these experiments was to correlate the developmental profile of Sp1 DNA-binding and NMDAR1 mRNA expression in control and lead exposed

animals. Animals were exposed lactationally to 0.2% lead acetate placed in the drinking water of the dam, from birth to weaning. Control animals were given regular water. Pups were sacrificed on postnatal days (PND) 3, 5, 10, 15, 20, and 30 and their hippocampi were dissected and used for RT-PCR analysis. In control animals, the expression of the NMDAR1 mRNA was found to be low on PNDs 3 and 5 and gradually increased with the highest levels observed on PND 20 and 30. The NMDAR1 mRNA in lead-exposed animals peaked on PND 10 and 15 and was decreased by PND 30. These results are consistent with the pattern of Sp1 DNA-binding previously reported by us. These findings suggest that lead may alter NMDA receptor expression via modulation of the function of Sp1 thereby contributing to cognitive deficits.

1157 EFFECT OF LEAD (Pb) ON TRANSTHYRETIN (TTR) AND THYROXIN IN HUMAN CEREBROSPINAL FLUID (CSF).

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TTR, a major thyroid hormone transport protein in the CNS, is mainly manufactured by the choroid plexus (CP) in brain. Our previous animal studies suggest that the sequestration of Pb in the CP is associated with a decrease in CSF TTR. The major objective of this study was to determine whether the status of thyroid hormone (T4) in the CSF was altered both as a function of CSF-Pb and CSF-TTR in humans. Seventy-nine CSF samples were collected from randomly-selected patients who were requested for clinical diagnosis of CSF chemistry. The samples were analyzed by AAS for Pb, and by RIA for TTR and T4. Results showed that CSF concentrations of TTR ranged 0.77-8.15 μg/mg of CSF proteins (3.33±0.36 μg/mg, Mean±SE, n=79); T4 ranged 0.10-6.7 ng/mg (1.50±0.19SE ng/mg, n=79); and Pb ranged 0.05-3.84 μg/dl (0.53±0.09SE μg/dl, n=59 of those above the detection limit). The linear regression analysis revealed that CSF TTR was positively associated with CSF T4 ($r=0.34$, $p<0.005$), which strengthens the role of TTR in T4 transport in the CNS. The decline in CSF TTR, however, was inversely associated with the rise in CSF Pb ($r=-0.31$, $p<0.01$). We further observed an inverse correlation between CSF T4 and CSF Pb ($r=-0.28$, $p<0.05$). While there was an age-related reduction in CSF TTR, the changes in CSF T4 or Pb did not seem to be age-associated. The current human data support our earlier observation from the animal work that Pb exposure appears to alter TTR levels in CSF, and further suggest that the alteration in CSF TTR may result in a disrupted thyroid hormone status in CSF. (Supported by NIEHS RO1 ES08146.)

1158 INTEGRATING ENVIRONMENTAL ISSUES ACROSS SUBJECTS.

B D Steinberg, B Schlegel, D Shields, L Hemminger, A R Gotsch and L Weidner. *Environmental and Occupational Health Sciences Institute, Piscataway, NJ, USA.* Sponsor: M A Gallo.

From increased health risks to determining where children can safely play, environmental health issues are becoming a large part of children's everyday lives. It is appropriate and relevant to use these issues to teach basic science, math, language arts, social studies and health concepts in schools. ToxRAP™ teaches K-9 students how to evaluate environmental health problems utilizing content and skills from many subject matters. Students apply the ToxRAP™ framework, which draws upon the practice of industrial hygiene and the risk assessment model used by the US Environmental Protection Agency, to recognize, evaluate and, when necessary, control contaminants. Through age-appropriate, hands-on, investigative activities, students develop critical thinking, decision making and problem solving skills needed to address the complex environmental health issues of today and, more importantly, tomorrow. This award-winning multidisciplinary curriculum is divided into three modules and can be easily integrated across subjects. Pilot test results showed that students could readily transfer the ToxRAP™ framework to other environmental health concerns in their lives. The curriculum is indexed to the national science education standards and is being adopted in schools across the country through the ToxRAP™ Network. Curriculum development and dissemination were funded by the National Institute of Environmental Health Sciences.

1159 KIDNEYS AND METALS, AN INTERATIVE WEB RESOURCE FOR LEARNING TOXICOLOGY AND ENVIRONMENTAL HEALTH SCIENCE.

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Chemicals & Human Health (CHH) is a web site designed to teach high school students basic principles of toxicology and research using actual experimental data. The newest CHH module, *Kidneys and Metals*, focuses on renal and metal toxicology. Research shows that students learn better when they are actively engaged in solving problems. The newest CHH module is an interactive problem set. Students are presented with problems and multiple-choice answers. Choosing the correct answer brings up information to reinforce student understanding of the concept. Choosing the incorrect answer brings up tutorials. These tutorials use different modes of presentation (text, illustrations, photographs, and animations) to help students understand the concepts and make the connections necessary for long term retention. Content includes kidney anatomy and function, membrane structure and transport, properties of metals, and mechanisms of metal toxicity. Several strategies are used to promote the new web site including the distribution of informational brochures and flyers and locating the CHH web site within another well known web resource, The Biology Project. During its initial six months, the CHH web site had over 10,000 user sessions indicating that the site is well utilized. It is anticipated that the addition of the *Kidneys and Metals* module will maintain or increase the usage of the web site. Future plans include the development of a set of data collection and interpretation activities to compliment the *Kidneys and Metals* module. Science education reform initiatives and the new national science standards call for hands-on science with a focus on problem-solving and inquiry. High school teachers can use these materials to help their students meet the new standards. (This project is supported by NIEHS Center grant ES 06694.)

1160 GENES, DISEASE AND THE ENVIRONMENT.

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With genetic polymorphisms expected to be associated with diseases such as cancer, heart disease and Alzheimer's, it should be stressed to the general public that a positive genetic test for a polymorphism, only implies that the person is at higher risk of developing the disease than is the general population, not that s/he is destined to develop that disease. A lesson plan is presented that introduces the concepts of polymorphisms to high school students and the interested lay person. Covered during the lesson are the molecular events leading up to a "change in function" protein and its associated cellular effects. One example utilized is Xeroderma pigmentosum (XP) as an autosomal recessive mutation in DNA repair genes causing unrepaired sections of DNA to persist which eventually lead to skin cancer. Also discussed are the ethical considerations of "Who should obtain information on an individual's genetic makeup?" A worker may prefer not to be overly exposed to occupationally hazardous agents if s/he had a predisposition to cancer or toxicity that may be caused by these agents. On the other hand an employer might like to know the genotype of its workers, in order to hire only those at low risk for toxicity or malignancy from exposure to particular agent(s) in the workplace. These and other topis are discussed through "hands on" activities and illustrated through patient case reports.

1161 THE SKYROCKETING IMPORTANCE OF RISK ASSESSMENT TRAINING.

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There has been an increase in the use of risk assessment in decision-making concerning the clean-up of environmentally contaminated sites, the protection of human health, and the future use of land at these sites. Subsequently, there has been an increase in the number of individuals entering the field of risk assessment who have had no formal training in this area. The concern for government regulators at the federal and state levels is that many of the personnel, currently, involved in doing health/risk assessments need further training to improve the quality of these evaluations and the data obtained. To help fulfill this need, a three-week course was implemented in 1997 at the School of Medicine and Public Health of the Ohio State University to pro-

vide risk assessors training in the areas of Environmental Health, Health/Risk Assessment, and Risk Management /Risk Communication. Attendees, came from multidisciplinary backgrounds ranging from engineering to ethics. Conclusions from the Overall Course Evaluation are that students ranked the Health/Risk Assessment segment as the most useful in their work, followed by Risk Management/Risk Communication and Environmental Health. Students, further ranked Site Investigation highest in a list of future courses, followed by Monte Carlo Analysis, Ecological Risk Assessment, PB-PK Modeling, and Hot Topics in Public Health. Also, students indicated that they liked case study presentations and tests that covered practical concepts. Evaluation of each segment of the course was also conducted. (This project was funded by Grant No. 97G003 from the Ohio Environmental Protection Agency.)

1162 HOW COPPER IONS SIGNAL TO THE TRANSCRIPTIONAL MACHINERY.

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The metal ion copper plays a dual role in biological systems. On one hand, copper plays an essential function in the active sites of a large number of critical cellular enzymes such as Cu, Zn superoxide dismutase, cytochrome oxidase, dopamine β-hydroxylase and lysyl oxidase. On the other hand, copper ions are potent toxins, through interactions with superoxide anions, resulting in the generation of hydroxyl radical resulting in damage to membranes, proteins and nucleic acids. Therefore, all cells must orchestrate the accumulation of copper for essential biochemical reactions, yet prevent the accumulation of free copper ions to cytoxic levels. My colleagues and I are interested in understanding the precise molecular mechanisms whereby cells maintain homeostatic control of copper ion levels through regulated transport, detoxification and through rapid transcriptional responses. Indeed, cells have exquisitely sensitive mechanisms for both sensing and responding to alterations in copper levels by directly signaling to the transcriptional machinery. Using yeast cells as a model eukaryotic organism, we have isolated genes encoding distinct copper sensing transcription factors that rapidly and potently activate the expression of copper detoxification genes in response to elevated copper levels. These rapid responses are achieve through the interdigitation of copper ion sensing and DNA binding domains in the copper metalloregulatory transcription factors, as well as through the use of specialized chromatin structures that poise metal induced genes in an accessible state. Furthermore, we have isolated genes encoding copper ion transporters and have identified transcription factors that activate expression of the copper transporter genes in response to copper starvation, but extinguish expression in the presence of high copper levels. The delicate balance that is required for maintaining sufficient levels of copper, while preventing copper toxicity, is dictated by the dynamic interplay between distinct copper ion sensing transcription factors and through the use of multiple layers of cellular regulation. These investigations lay the groundwork for understanding the molecular mechanisms by which mammals establish and maintain copper ion homeostasis.

1163 THE ROLE OF QUINONES IN TOXICOLOGY.

J L Bolton[1] and T J Monks[2]. [1]*Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL, USA and* [2]*University of Texas at Austin, College of Pharmacy, Austin, TX, USA.*

Quinones represent a class of toxicological intermediate which can create a variety of hazardous effects in vivo including, acute cytotoxicity, immunotoxicity, and carcinogenesis. The mechanisms by which quinones cause these effects can be quite complex. Quinones are Michael acceptors and cellular damage can occur through alkylation of crucial cellular proteins and/or DNA. Alternatively, quinones are highly redox active molecules which can redox cycle with their semiquinone radicals leading to formation of reactive oxygen species (ROS) including superoxide, hydrogen peroxide, and ultimately the hydroxyl radical. Production of ROS can cause severe oxidative stress within cells through the formation of oxidized cellular macromolecules including lipids, proteins, and DNA. Formation of oxidatively damaged bases such as 8-hydroxydeoxyguanosine has been associated with aging and carcinogenesis. Further ROS can activate a number of signaling pathways including protein kinase C and RAS. This symposium will explore the varied cytotoxic effects of quinones using specific examples including quinones produced from benzene, polycyclic aromatic hydrocarbons, estrogens, and catecholamines. The numerous mechanisms of toxicity (i.e., alkylation versus oxidative stress) will be correlated with the known pathology of the parent compound(s).

1164 BENZENE QUINONES AND BONE MARROW TOXICITY AND CHEMOPREVENTION.

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Benzene is a widespread environmental pollutant. Exposure to benzene induces toxicity to various bone marrow cell populations which can be manifested as pancytopenia, aplastic anemia, leukemia and immunotoxicity. It is well established that metabolites originating in the liver may be further bioactivated to reactive intermediates within bone marrow target cell populations. Of particular interest is the conversion of hydroquinone (HQ) to p-benzoquinone (BQ) which is highly reactive toward SH-containing molecules. Treatment of cells with HQ results in: altered cytokine synthesis, cell division, cell differentiation and viability; the inhibition of DNA topoisomerase II; the induction of mitotic recombination, chromosomal translocations and aneuploidy, The bioreactivity of p-BQ within target cells is controlled by quinone reductase and glutathione, whose activity/content vary according to: the state of cell differentiation; bone marrow cell type; and strain/species. Accordingly, the induction of quinone reductase and glutathione serves as a basis for molecular intervention against benzene induced hematotoxicity. The focus of this symposium presentation will be to discuss the experimental evidence underpining the concepts described above.

1165 ortho-QUINONES AS ACTIVATED METABOLITES OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH).

T M Penning. *Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA, USA.* Sponsor: J L Bolton.

A novel pathway of PAH activation involves the formation of reactive and redox active o-quinones catalyzed by rat and human dihydrodiol dehydrogenase (DD) members of the aldo-keto reductase superfamily. In this pathway non-K-region trans-dihydrodiols (proximate carcinogens) are diverted from CYP1A1 and undergo enzymatic NADP+ dependent oxidation to yield the corresponding o-quinone. Using rat DD, human recombinant DD isoforms, rat hepatocytes, and stable-transfectants we have shown that (+)-trans-7,8-dihydroxy-7,8-dihydro-benzo[a]pyrene is converted to a catechol, 7,8-dihydroxy-benzo[a]pyrene, which is then autoxidized via an intermediate o-semiquinone to yield benzo[a]pyrene-7,8-dione. EPR and spectroscopic measurements showed that the autoxidation event is accompanied by the formation of reactive oxygen species (ROS) e.g., superoxide anion, hydrogen peroxide and hydroxyl radical. The PAH o-quinones generated are reactive Michael acceptors and can form stable and depurinating adducts with bases in DNA, and form thio-ether conjugates with L-cysteine, mercapturic acid and GSH. Covalent adducts are likely responsible for the frame-shift mutations that o-quinones cause in the Ames test. PAH o-quinones also undergo two electron (non-enzymatic) or one electron (enzymatic) reduction steps to establish futile redox-cycles in which ROS and o-semiquinone radicals are generated multiple times. Amplification of ROS leads to cytotoxicity (reduced cell viability and survival) and genotoxicity (DNA strand scission). Strand scission is dependent upon a Cu(II)/Cu(I) redox couple and hydroxyl radical. The release of malondialdehyde upon acid hydrolysis of quinone-treated DNA points to a Criegee rearrangement with the formation of base-propenals during the strand-scission event. Since ROS are the causative agents in radiation induced carcinogenesis and are generated by PAH o-quinones and catechol estrogens they may be the common mediators of the carcinogenic event. (Supported by NIH grant CA39504.)

1166 QUINONES FROM PREMARIN® ESTROGENS: ROLE IN ESTROGEN CARCINOGENESIS.

J L Bolton. *Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL, USA.*

Endogenous estrogens as well as estrogens present in estrogen replacement formulations have been implicated in breast and endometrial cancers. The mechanism(s) of how estrogens cause these cancers remains both controversial and elusive. One mechanism could involve metabolic activation of these estrogens to o-quinones which then cause oxidation/alkylation of DNA. It has been shown that the catechol estrogens metabolites of estrone and 17β-estradiol can form DNA adducts *in vitro* and *in vivo*. The non-genotoxic 2-

hydroxycatechols seem to form mainly stable bulky adducts whereas the carcinogenic 4-hydroxylated metabolites primarily alkylate the N-7 position of guanine generating apurinic sites. The equine estrogens present in the popular estrogen replacement formulation Premarin® (Wyeth-Ayerst) can be metabolized to primarily 4-hydroxylated products. In contrast to the endogenous catechol estrogens, the 4-hydroxyequine estrogens autoxidize to semiquinone radicals and o-quinones without the need for oxidative enzyme catalysis. Both 4-hydroxyequilin and 4-hydroxyequilenin form the same highly unusual cyclic adducts with DNA. Several different types of lesions result including, bulky stable adducts with guanine and cytosine and apurinic sites resulting from adenine alkylation. Finally, 4-hydroxyequilenin in particular causes significant oxidative damage to guanine and adenine residues as well as damage to the phosphate/sugar backbone of DNA. These data suggest that DNA damage mediated by endogenous and/or exogenous catechol estrogens could contribute to the carcinogenic effects of estrogens in vivo. (Supported by CA73638-01.)

1167 QUINONE-THIOETHER MEDIATED TOXICITIES.

T J Monks and S S Lau. *Div. of Pharmacology & Toxicology, College of Pharmacy, Univ. of Texas at Austin, TX, USA.*

Conjugation of reactive electrophiles, such as quinones, with glutathione (GSH) is frequently associated with detoxication. However, quinol-GSH conjugates retain the ability to redox cycle, and the reactivity of the corresponding quinone-thioether often exceeds that of the parent quinone. Indeed, quinone-thioether-mediated DNA damage (including mutations [base substitutions/deletions] and single-strand breaks), growth arrest, cell transformation, and cell death (necrosis and apoptosis) are all linked to the generation of reactive oxygen species. Conjugation with GSH is usually efficiently coupled to the export of the conjugate from the cell, and limits the potential toxicity of quinol-GSH conjugates. In contrast, GSH conjugates are transported into cells via eiither the activity of an intact GSH transporter, or as the corresponding cysteinylglycine and cysteine conjugates following metabolism by γ -glutamyl transpeptidase (γ -GT) and dipeptidases. Cells that express either γ -GT or an intact GSH transporter will therefore be exposed to the potential toxic effects of these conjugates. Tissues susceptible to quinol-GSH conjugate induced toxicities share interesting physiological similarities. For example, γ -GT is frequently located in cells that separate the circulation from a second fluid filled compartment, such as occurs in the blood-urine (renal proximal tubule cells) and blood-brain (brain endothelial cells) barriers. Quinol-GSH conjugates are nephrotoxic, nephrocarcinogenic, and neurotoxic. In the latter case however, access to the brain appears to be mediated by an intact GSH transporter, and not by γ -GT. Similar physiological barriers exist within the body and may provide additional sites for the toxicity of these conjugates. (ES07359, ES 07247, CA58036, DA10832, GM 39338)

1168 QUINONE-THIOETHERS AND PARKINSON'S DISEASE.

G Dryhurst, X-M Shen, H Li, J H Han, L Gu, Z Yang, and F-C Cheng. *Department of Chemistry and Biochemistry, University of Oklahoma, Norman, OK, USA.* Sponsor: J L Bolton.

The characteristic motor symptoms of PD (tremor, rigidity, akinesia) are caused by the degeneration of nigrostriatal dopamine (DA) neurons. The pathological processes occur in the neuromelanin-pigmented cell bodies of these neurons in the substantia nigra pars compacta (SNc) and include a defect in mitochondrial respiration at the level of complex I and decreased α -ketoglutarate dehydrogenase. A massive loss of glutathione (GSH) is the earliest known change in the parkinsonian SNc that slightly precedes the complex I defect. Another change in the SNc of PD brains is increased activity of γ -glutamyl transpeptidase. Based on these observations and information drawn from studies of the dopaminergic neurotoxicity evoked by MPTP, methamphetamine and ischemia-reperfusion, a new mechanism will be discussed based on reactions between DA-o-quinone and cysteine. The intraneuronal reaction between DA-o-quinone and CySH is proposed to form cysteinyl conjugates of DA that are further easily oxidized to a number of dihydrobenzothiazines (DHBTs) and benzothiazines (BTs). These DHBTs/BTs include putative intraneuronal metabolites that can be accumulated by mitochondria and evoke irreversible inhibition of complex I and α -KGDH. These effects involve oxidation of DHBTs and BTs by a constituent of the inner mt membrane to give highly electrophilic intermediates that covalently bind to -SH residues of NADH-coenzyme Q1 reductase and α -KGDH thus evoking irreversible inhibition.

1169 BIOLOGIC MARKERS IN MOLECULAR EPIDEMIOLOGY: MEASURES OF EXPOSURE AND RISK FOR CANCER DEVELOPMENT.

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Although biomarkers have been used for sometime in epidemiology, recent advances in molecular biologic techniques have made it possible to detect biologic changes at low levels of exposure. Biomarkers can be used to identify exposures, effects of those exposures, and populations that may be at increased risk. Measurement of DNA adducts from polycyclic aromatic hydrocarbons, aflatoxin, and alkylating agents have been used to monitor exposure to these agents in smokers, workers, and those with environmental exposure. Lipid peroxidation produces high levels of malondialdehyde-DNA adducts which are highly mutagenic. Oxidative DNA damage is also present at high levels. Thus, DNA damage from endogenous sources may be an important factor in tumor development. Data on the relationship between DNA damage (or protein damage as a surrogate) and risk for cancer have demonstrated that these assays can predict development of disease in populations exposed to aflatoxin. Genetic susceptibility factors related to ability to metabolize carcinogens influence DNA damage levels and cancer risk. Biomarkers of effect measure mutations (HPRT or glycophorin A) or cytogenetic damage and are useful to help bridge the continuum of exposure to disease. Many of the biomarkers of exposure and early effects are reversible and can be used as tools to monitor reductions in workplace exposure or the efficacy of chemoprevention studies. The rapid expansion of large banks of stored blood samples will make possible nested case-control studies evaluating the relationship between biomarkers and disease risk and gene-environment interactions.

1170 THE RELATIVE CONTRIBUTION OF EXOGENOUS AND ENDOGENOUS EXPOSURES OF HUMANS TO CARCINOGENS AS REFLECTED BY DNA AND PROTEIN DAMAGE.

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DNA and protein adducts are now widely accepted as valid monitors of exposure to genotoxic compounds. In the case of hepatocellular carcinoma, biomarkers of exposure to aflatoxin B1 including DNA adducts proved to be highly predictive of tumour outcome in humans. Correlations between low dose exposures and DNA adduct formation in target organs have been observed for a number of carcinogens in experimental systems. However, in a recent study of N©nitrosodiethylamine in 3 inbred mice strains we showed that within strains there was a strong correlation between DNA adduct levels and tumour yield, whereas interstrain variation was less well predicted and may be determined by other susceptibility factors. Using the techniques of ^{32}P-postlabelling, immunoassay or mass spectrometry (MS) it is possible to detect DNA or protein damage (adducts) caused by genotoxic carcinogens in normal human tissues. In many cases the level of endogenously produced damage is comparable to, or greater than, that seen following exogenous exposures to carcinogens from for example occupational sources or tobacco smoking. Superior analytical methods are still required to improve the sensitivity of adduct detection for monitoring low level human exposures, and to identify the chemical nature of many of the DNA adducts, which is only indirectly achievable by the technique of ^{32}P-postlabelling. We have achieved greater selectivity and sensitivity of adduct detection in human tissues using accelerator MS (AMS), following for example administration of ^{14}C-tamoxifen (collaboration with K Turteltaub and R C Garner). We have also developed a ^{32}P-postlabelling method for the detection of phosphotriesters, which, because of their abundance and stability, may offer a further increase in sensitivity over standard postlabelling techniques for measuring adducts.

1171 APPROACHES TO THE ANALYSIS OF ENDOGENOUS DNA DAMAGE IN PEOPLE.

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Characterization of the sources of DNA damage in human beings is critical for identifying the causes of genetic diseases. A compendium of DNA damage products indicates that endogenous metabolic processes play a very significant role in genomic alteration. For example, malondialdehyde is a naturally occurring product of lipid peroxidation and prostaglandin biosynthesis that is mutagenic and carcinogenic. It reacts with DNA to form adducts to deoxyguanosine and deoxyadenosine. The major adduct to DNA is a pyrimidopurinone called M_1G. This adduct is highly mutagenic in E. coli and has been detected in liver, white blood cells, pancreas, and breast from healthy human beings at levels ranging from 1 - 120 per 10^8 nucleotides. Efforts are underway to develop high throughput methods for quantitation of MDA in population-based studies. Several different assays for M_1G have been described that are based on mass spectrometry, ^{32}P-postlabeling, or immunochemical techniques. Each technique offers advantages and disadvantages based on a combination of sensitivity and specificity. Recent advances in the application these techniques to the analysis of M_1G will be discussed as a prototype for the analysis of other endogenous DNA adducts.

1172 IMMUNOLOGIC METHODS FOR MEASURING DNA ADDUCTS: APPLICATIONS TO MOLECULAR EPIDEMIOLOGIC STUDIES.

R M Santella. *Division of Environmental Health Sciences, Columbia School of Public Health, New York, NY, USA.*

Measurement of carcinogen-DNA damage in humans can provide important information on individual exposure to various environmental, lifestyle, or occupational carcinogens. Polyclonal antisera and monoclonal antibodies recognizing specific types of DNA damage have high sensitivity and can easily analyze large numbers of samples. We are currently using an antiserum recognizing polycyclic aromatic hydrocarbon (PAH)-DNA in a competitive enzyme-linked immunosorbent assay (ELISA) to analyze lymphocyte DNA adducts in a breast cancer case-control study. The same antiserum is being used for immunoperoxidase staining of breast tissue sections to determine the correlation between target and surrogate tissue adduct levels. Investigation of the relationship between adducts and genotype for glutathione S-transferases M1 and P1 will help in understanding interindividual variation in DNA damage. Immuno-histochemical analysis of exfoliated oral and bladder cells for 4-aminobiphenyl (4-ABP)- and PAH-DNA and oxidative DNA damage (8-hydroxyguanosine) demonstrated elevated levels of damage in smokers compared to nonsmokers, suggesting these are good markers of exposure. Studies on the correlation between DNA damage and mutations in p53, a tumor suppressor gene, demonstrated an increased risk for mutant p53 in subjects with higher levels of aflatoxin B_1 or 4-ABP-DNA adducts, suggesting the importance of adducts in the etiology of cancer development. Finally, adduct measurements are also useful as intermediate biomarkers in intervention studies. For example, we recently completed an antioxidant vitamin intervention in smokers in which the endpoint is modulation of DNA damage in lymphocytes and exfoliated oral and bladder cells. Demonstration that an intervention can modulate a biomarker on the pathway to cancer development should simplify the testing of new chemopreventive agents.

1173 MOLECULAR MARKERS OF BIOLOGICAL EFFECT - USE AS MEASURES OF RETROSPECTIVE CUMULATIVE EXPOSURE AND AS PREDICTORS OF HUMAN HEALTH EFFECTS.

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The tools provided by modern molecular biology and genetics, together with ever more sensitive and sophisticated analytical instrumentation, have allowed the development of powerful new markers of early biological effects relevant to the molecular etiology of human disease. These effect biomarkers, representing both cytogenetic and molecular genetic methods, have found useful application in recent molecular epidemiological studies both for assessing the biological impact of distant and/or cumulative exposures to chemical and physical agents as well as predicting, and molecularly characterizing, subsequent clinical disease. Examples from the contemporary literature will be used to illustrate these concepts and the current "cutting edge" of laboratory methods and molecular epidemiological study design. While these tools are presently best developed in cancer epidemiology, examples using other chronic disease endpoints and adverse reproductive effects will also be presented. Finally, the presentation will include a discussion of rapidly emerging techniques and scientific concepts that represent the next generation of biological markers that promise to offer increased sensitivity and specificity, early detection of preclinical disease, population screening for identification of susceptible groups, and assessment of individual risk of disease.

1174 LINKING BIOMARKERS OF BIOLOGICALLY EFFECTIVE DOSE AND EARLY BIOLOGIC EFFECT TO DISEASE RISK IN EPIDEMIOLOGIC STUDIES.

P A Schulte[1] and N Rothman[2]. [1]*NIOSH-Taft Labs, Cincinnati, OH;* [2]*Division of Cancer Epidemiology and Genetics, NCI, Bethesda, MD, USA.* Sponsor: R M Santella.

In order to understand the relevance of biomarkers of biologically effective dose and early biologic effects in a broad public health context, it is critical to understand the biomarker-disease relationship. Although preliminary work can be carried out for some biomarkers in case-control studies, the potential for disease bias will always raise a concern about results from these studies. As such, prospective cohort studies carried out on large, generally healthy populations are the optimal approach to study the relationship between biomarker levels and disease risk. In these studies, biologic samples are collected from a defined population and banked for later analysis. The cohort is followed forward in time; subjects who develop disease are identified, and premorbid biomarker levels in these cases are then compared with those in unaffected subjects. Often, a nested case-control approach is used. Samples from cases and only a sample of the controls are analyzed, which considerably reduces the laboratory requirements and costs. These studies allow calculation of the relative risk, which provides an estimate of the increased risk in disease for a given level of a biomarker, and the etiologic fraction, which estimates the proportion of disease reflected by the biomarker. The consistent results across several, small cohort studies showing a positive relationship between chromosomal aberration levels in peripheral lymphocytes and subsequent cancer incidence or mortality suggest that at least some biomarkers of early biologic effect will prove to be relevant for predicting cancer risk. There will soon be over a million subjects with blood samples banked in cohort studies in the U.S., Europe, and Asia. These studies will provide striking opportunities for the evaluation of the relationship between a wide range of biomarkers and disease risk over the coming years.

1175 TOXICOLOGY FOR KIDS: A HOW TO GUIDE FOR TOXICOLOGISTS.

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

SOT has recognized the importance of education in the science of toxicology for students in grades K-12. While many members of SOT are interested in becoming involved in K-12 education, there is much uncertainty in what to do and how to do it. The Education Committee and its K-12 Subcommittee are committed to activities that will enhance the efforts of SOT members in their efforts. Thus, the K-12 Subcommittee has enlisted the help of several prominent scientists and educators to provide a program focused on the practical aspects of outreach programs with kids. The purpose of the workshop is to give SOT members the philosophical approaches and specific educational tools that are effective in classroom presentations. Specific hands-on demonstrations of proven techniques will be provided at the workshop.

1176 TOXICOLOGY CAN TURN KIDS ON TO SCIENCE.

N Biggart. Bonita *Vista High School, El Cajon, CA, USA.* Sponsor: G S Yost.

Toxicology is an excellent discipline with which to engage the interest of high school students because of two characteristics: it is relevant to students'

physical selves, a subject of intense interest; and secondly, it provides excellent opportunities for experimentation in the classroom. Toxicology can be included in cellular biology topics such as membrane transport, gene expression, and cancer. In addition, it is relevant to animal and human physiology as one can discuss toxicant damage in terms of tissues, organ systems, and the entire body. Finally, there are many simple toxicity experiments that can easily be performed in a classroom and that incorporate the concepts of variables, controls, assays, reproducibility, and experimental design. The inclusion of toxicology is appropriate for all aspects of biology from ecology to molecular genetics to physiology and even evolution. It is also appropriate in chemistry because toxicants are generally chemical compounds. Toxicology is an excellent way to discuss experimental design with students. Once teachers get some basic information and ideas, they can develop lots of plans.

1177 TOXICOLOGISTS SELLING SCIENCE IN THE CLASSROOM? LESSONS FROM MARKETING.

W T Klimecki. *Motorola Inc., Corporate Research Labs, Tempe, AZ, USA.*

Stripped to its bones, science education is a product in the marketplace. While this is a distasteful metaphor to some, excellent educational programs always share commonalties with excellent product marketing programs. Superior, long-term marketing programs have a good product (toxicology) which is sold by salespersons (teachers) who believe in the product, understand the product thoroughly, and successfully transfer their knowledge and enthusiasm in presentations which are tailored to their specific customers (students). These characteristics will be highlighted in this description of an outreach program at the University of Arizona, in which 5th and 6th grade students designed a project in which they collected drinking water samples weekly, analyzed the water for a variety of constituents, then statistically analyzed and presented the data. A primary goal of the program was to teach the students to use sound, scientific principles to evaluate the validity of scientific claims which are made or quoted by journalists. Drinking water analysis was chosen because it is a topic which receives frequent (often daily) coverage in the local media. For the students, drinking water quality is discussed at their dinner table; they feel it is relevant to their lives, and they found affirmation in reporting their data to their families. Students were required to collect one journalism media article weekly regarding water quality. By the end of the semester, the students became quite proficient at both identifying the critical issues and answers which were present in well substantiated scientific claims, as well as identifying their absence in poorly founded claims. The project was inexpensive, and was primarily dependent upon enthusiastic salesmanship and a collaborative, "let's try it!" spirit between academic scientists, public school teachers and administrators. (Project support: NIEHS Center Grant ES 06694.)

1178 EXPERIENCES OF AAAS IN K-12 SCIENCE, MATHEMATICS, AND TECHNOLOGY EDUCATION.

Y S George. *American Association for the Advancement of Science, New York, NY, USA.* Sponsor: G S Yost.

Since 1955, the American Association for the Advancement of Science (AAAS) has been involved in the development and implementation of science, mathematics, and technology (SMT) education programs in schools. In more recent years, AAAS has also implemented SMT programs in partnership with science museums and zoos, youth serving organizations, faith based organizations, public libraries, and businesses. Some initiatives have focused on attracting and retaining minorities, girls, and children with disabilities in the SMT education pipeline. Products developed include inquiry based SMT manuals and kits, science and mathematics benchmarks (AAAS Project 2061), children's radio show and cyber clubs, and other Internet products for teachers and parents. Thus, this presentation will highlight AAAS SMT initiatives, while focusing on lessons learned about presenting K-12 science to a diverse student population.

1179 OVERVIEW OF K-12 ENVIRONMENTAL HEALTH SCIENCE EDUCATION EFFORTS AT NIEHS.

A Dearry. *NIEHS, Research Triangle Park, NC, USA.* Sponsor: G S Yost.

A mandate in the 1990s is to reach out to students in grade school and high school to improve their science literacy. The NIEHS has thus established a priority to develop an environmental health sciences education program at the K-12 grade levels. The objective is to improve understanding of environmental health issues by all students and to expand career awareness for those interested in pursuing further education. The Institute has supported both material development and teacher training efforts. The former are designed to provide instructional materials which can be infused into existing curricula. Examples include science adventure stories, mini-magazines, teachers' guides, interactive CD-ROM software programs, student workbooks, board games, and videotapes. These materials address a variety of environmental health science topics, including toxicology, risk assessment, air pollution, global environmental problems, and career opportunities. In addition, the NIEHS seeks to enhance dissemination and effective implementation of materials pertaining to environmental health science. A necessary step in achieving this objective is the provision of teachers with requisite disciplinary and pedagogical skills. Hence, the Institute supports a series of teacher enhancement activities, which will ultimately enhance students' comprehension of and interest in environmental health sciences. A summary of these efforts and contact information will be provided.

1180 HANDS-ON DEMONSTRATIONS OF TOXICOLOGY MATERIALS.

G S Yost and C A McQueen. *Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT, USA and Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ, USA.*

Examples of education materials for classroom presentations about toxicology will be presented at workstations around the room. The examples will include demonstrations, video tapes, and other media forms that have proven useful in science education and toxicology. Toxicologists from several of the NIEHS Centers will display materials that have been developed for a target population of K-12 students. There will be information on how to obtain these materials. Bibliographic resources will also be available. The materials will provide specific, hands-on examples of the most useful kinds of educational materials that can be employed by toxicologists in classroom settings.

1181 THE IMMUNOTOXICOLOGY OF NOVEL THERAPEUTICS.

R V House[1] and K L Hastings[2]. [1]*IIT Research Institute, Chicago, IL, USA;* [2] *US FDA, Rockville, MD.*

The discipline of immunotoxicology is predicated on the assumption that alterations in immune function resulting from xenobiotic exposure–whether stimulatory or inhibitory–are potentially deleterious. Although this assumption was suitable chemicals and "traditional" drugs, novel therapeutic agents being developed which will necessitate revision of how immunotoxicology is defined. At least three properties of these agents must be considered when assessing potential immunotoxicity. First, many novel therapeutics are proteins or polypeptides, and the use of xenogenetic proteins (or even native proteins with minor chemical alterations) may result in the development of neutralizing antibodies. Second, although the putative pharmacologic mechanism of action of many of these drugs is highly specific, unanticipated toxicities have become evident. Such toxicities may result from exaggerated pharmacological activity, non-targeted immunotoxicity, or unrelated effects on systems other than the immune system. This is confounded by the fact that the physicochemical properties and mechanism of action of these novel agents are so disparate; thus, it becomes difficult if not impossible to elucidate an overall pattern of toxic activity. Third, and perhaps most important, many of these agents are intended to therapeutically modulate the immune response. This last point is critical, since it is important to distinguish between the desired pharmacological activity and undesired side-affects.

1182 IMMUNOMODULATORY BIOLOGICS: DISTINGUISHING ACTIVITY FROM TOXICITY.

J L Bussiere. *Genentech, Inc., South San Francisco, CA, USA.*

Special issues with regards to immunotoxicity testing of new monoclonal antibody therapeutics must be considered when these molecules have known immunomodulatory activities. An important consideration for biotechnology compounds is the species specificity of the molecule. As is often the case for humanized antibodies, these compounds may by pharmacologically inactive in rodents. In addition, these proteins may be immunogenic in rodents,

and cause confounding findings in the rodent studies. Because of the pleiotrophic nature of many of the immunomodulators, non-target effects on the immune system must be investigated. However, immune responses in a 'normal' animal may be very different than during a 'disease' state. The difference in the immune system of the animal must also be considered when extrapolating risk to humans, i.e., not only in basic differences in immune function, but also differences in back-up systems, compensatory mechanisms, etc. Characterizing the risk/benefit of novel monoclonal antibodies must include separating the immunomodulatory activity from the immunotoxicity. It is important to distinguish between the pharmacological activity of the drug and toxicity that may be due to exaggerated pharmacology, due to non-targeted immunotoxicity, or due to cross-reactivity with non-target tissues.

1183 TOXICITY OF THERAPEUTIC CYTOKINES: FROM ANIMAL DATA TO CLINICAL ADVERSE EFFECTS.

J Descotes. *Lyon Poison Center and ISERM U98-X, Claude Bernard University, Lyon, France.*

Because cytokines exert pleiotropic effects, they have been proposed to treat a variety of pathological conditions. These pleiotropic effects affect primarily the immune system, but also physiological networks between the immune system, such as the CNS and endocrine glands, as well as other systems. Because (therapeutic) cytokines include a marked activation of the immune system, frequent and sometimes severe adverse effects have been described. Typical examples are the acute cytokine syndrome, autoimmune diseases such as thyroiditis and lupus erythematosus, and to some extent, allergic reactions to unrelated allergens and inhibition of hepatic drug metabolism. Due to their origin, cytokines were often, but erroneously considered safe, despite differences in dose levels and duration of exposure. Preclinical toxicity evaluation was then limited to a minimum, but most adverse effects observed in humans were later found to occur in laboratory animals as well. Thanks to the experience thus gained with available therapeutic cytokines, it should be possible to better define which safety endpoints should be included in the preclinical testing and clinical trials of novel therapeutic cytokines.

1184 MECHANISMS AND APPLICATIONS OF CpG DNA.

A M Krieg. *Veterans Affairs Medical Center and University of Iowa, Iowa City, IA, USA.* Sponsor: R V House.

The immune system has evolved the ability to distinguish prokaryotic and vertebrate DNAs because vertebrates have a relative lack of umethylated CpG dinucleotides in certain base contexts (CpG motifs). When B cells or monocytes detect DNA bearing CpG motifs, they rapidly proliferate and secrete immunoglobin (B cells) or secrete Th1-like cytokines especially IL-12 and IFN-(/ß (monocytic cells) resulting in NK deviation. CpG DNA requires cell uptake into an acidifier intracellular compartment. A cytoplasmic protein that specifically binds CpG DNA has been identified. CpG DNA triggers the activation of lymphocyte Jun kinase and NF(B within 7-15 minutes. Immune stimulation by CpG DNA promotes the generation of anitgen-specific immune responses in several ways. First, CpG DNA activation of B Cells synergizes with activation through the antigen receptor. Second, CpG DNA makes B cells resistant to activation-induced apoptosis. Third, B cells and other antigen presenting cells upregulate their expression of class II MHC and other constimulatory molecules. CpG DNA induces strong antibody and cell mediated immune responses to a variety of protein antigens (including tumor antigens) whether given intramuscularly, subcutaneously, intradermally, or through mucosal administration. In fact, CpG DNA is a stronger Th1-like adjuvant than Freud's complete, but causes no local or systemic toxicity at effective doses. CpG DNA is also an effective adjuvant for neonatal immunization and for immunization to polysaccharide antigens, and is required for effective DNA vaccination. As would be expected from the cytokines induced by CpG DNA, these immune responses are strongly Th1-like with a predominance of IgG2a antibodies and strong CTl responses. The Th1-like effect of CpG DNA reverses Th2 immune responses to allergens, preventing diseases in a mouse model of asthma. On the other hand, high loses of CpG DNA can also serve as a trigger for the sepis syndrome. These studies point to human therapeutic applications for CpG ODN.

1185 IMMUNOMODULATION BY THE PROTEASE SAQUINAVIR.

K L White, Jr.[1] and D R Germolec[2]. [1]*MVC/VCU Richmond, VA, USA and* [2]*NIEHS, RTP, NC, USA.*

Saquinavir mesylate is an HIV- protease inhibitor which is used in combination with nucleoside analogs in the treatment of HIV infection. As the target population receiving this drug may already be immunocompromised, it was important to establish that drug treatment would not further impair immune function. No alterations by standard toxicological parameters, including mean body weight or body weight change, organ weights and hematology profiles were observed in B6C3F1 mice treated with up to 1200 mg/kg Saquinavir for 28 days. Antigen-specific serum IgM antibody titers were significantly increased at the 600 mg/kg dose level, while splenis IgM antibody forming cell response against SRBCs was significantly increased at 1200 mg/kg. While low doses of saquinavir produced decreases in natural killer cell activity, preliminary studies suggest that the 1200 mg/kg dose potetiated NK cell responses. Although a significant increase in the number of CD3+ lymphocytes was observed at 1200 mg/kg dose, cell mediated immune functions, as measured by the cytotoxic T lymphocyte assay and the treatment. A significant increase in the number of immature these immunomodulatory effects could alter resistance to disease and neoplasia, we examined the effects of saquinavir in a bacterial challenge and lung tumor model. Treatment with saquinavir did not alter lung tumor burdens of B16F10 melanoma cells or modify numbers of moribund animals after challenge with Listeria monocytogenes. These data suggest that in addition to its entiretrovial activity, treatment with saquinavir may potentiate specific immunologic activity.

1186 TOXICITY OF THERAPEUTIC IMMUNOSUPPRESSANTS.

K L Hastings. *Division of Special Pathogens and Immunologic Drug Products, Center for Drug Evaluation and Research, US FDA, Rockville, MD, USA.*

Numerous immunosuppressant compounds have been developed in recent years for such purposes as prophylaxis of solid organ transplant rejection and treatment of chronic inflammatory diseases suspected to be autoimmune in nature. Cyclosporin and tacrolimus are probably the best known of these compounds: they appear to act via binding and inhibition of calcinerium, a molecule critical in T-cell activation. Clinical development of these potent compounds essentially involved determining systemic exposure levels that were effacious but did not result in complete devastation of the immune system. Other toxicities have been observed related to the use of these compounds that do not appear to de obviously related to their pharmacodynamic properties. Among these adverse effects are renal, neurological, gastrointestinal, cardiac, and pancreatic toxicity, the later being associated with a syndrome known as post-transplant diabetes mellitus (PTDM). The mechanisms of some of these toxicities have been elucidated and appear, at least in the case of PTDM, to be related to the pharmacological activity of the drugs. Other immunosuppressants, which act by mechanisms unrelated to cyclosporin, are also under development and have also demonstrated unique toxicites. These adverse effects are indicative of the interrelatedness of immune and non-immune functions.

1187 EVALUATION OF ADVERSE HEALTH EFFECTS FROM DOMESTIC METHYLPARATHION EXPOSURE.

R D Cox, R L Galli, J C Kolb, F R Carlton, and A M Houpt. *Section of Medical Toxicology, Department of Emergency Medicine, University of Mississippi Medical Center, Jackson, MS, USA.*

Methylparathion was used illegally as a domestic pesticide in over 2500 homes in Mississippi. The health impact of low-level chronic exposure to organophosphates is unknown. A prospective single-blinded study was undertaken to evaluate the presence of adverse health effects in residents of these homes. Using a structured history and physical examination, 353 residents were evaluated by four physicians. Each subject was rated as to the likelihood of having acute toxicity, chronic toxicity or past/subacute toxicity using a predefined set of criteria. No subjects were judged to have evidence of acute organophosphate toxicity at the time of examination. Three subjects were felt to have a high likelihood of having suffered a past episode

of organophosphate toxicity. All three episodes occurred within one month of the initial spraying and were associated with significant dermal and/or respiratory exposure. Symptoms involved gastrointestinal ailments that resolved within one week, and respiratory and neurologic problems that persisted up to 6 months. Forty-eight subjects were judged to possibly have had an episode of past acute or subacute toxicity. No relation was found between the likelihood of past/subacute toxicity and the concentration of methylparathion on home surface sampling or to urinary para-nitrophenol levels. A positive correlation was present between the presence of toxic symptomatology and time spent in the home. This study suggests that adverse health effects do occur from domestic methylparathion exposure. Surface sampling for methylparathion or urinary para-nitrophenol levels may not be sensitive predictors of potential toxicity.

1188 IMPACT OF DOMESTIC METHYLPARATHION EXPOSURE ON CHILDREN'S HEALTH.

J C Kolb, R D Cox, R L Galli, F R Carlton, and A M Houpt. *Section of Medical Toxicology, Department of Emergency Medicine, University of Mississippi Medical Center, Jackson, MS, USA.*

Methylparathion was used illegally as a domestic pesticide in over 2500 homes in Mississippi. The impact on children's health was of particular interest since children spend more time in the home and in contact with surfaces. The effect of methylparathion on the developing nervous system is unknown. Children living in these homes were evaluated by four physicians trained in Medical Toxicology. The Denver II Developmental Test Battery was administered to children under 7 years of age. Growth percentiles were measured for children less than 10 years of age. Using a structured history and physical examination, 153 children under 18 years, including 26 under 5 years were evaluated. Growth was below the 50th percentile in 1 child and below the 25th percentile in 3 (of 61). One child (of 50), who was born prematurely, tested as developmentally delayed. Eleven children were identified whose homes were sprayed during pregnancy, 39 prior to pregnancy and 12 within the first year of life. These children did not have a higher prevalence of health problems than other children in the study. Children under two years of age had a higher prevalence of GI problems than other children. No relationship was found between health problems reported by the parents or assessed by the physicians and home surface methylparathion levels or urinary paranitrophenol levels. Although children had early exposures to methylparathion, this study does not indicate delays in development or growth, or an increase in expected childhood illnesses.

1189 THE UTILITY OF PLASMA AND RBC CHOLINESTERASE MEASUREMENTS IN THE EVALUATION OF CHRONIC ORGANOPHOSPHATE TOXICITY.

R L Galli, R D Cox, J C Kolb, F R Carlton, and A M Houpt. *Section of Medical Toxicology, Department of Emergency Medicine, University of Mississippi Medical Center, Jackson, MS, USA.*

Plasma and RBC cholinesterase levels are usually depressed in the presence of acute organophosphate toxicity and are used as clinical markers. Their utility in the evaluation of chronic organophosphate exposure is unknown. A study was undertaken to evaluate the utility of cholinesterase levels in chronic low-level organophosphate exposure. Cholinesterase levels were obtained on 310 subjects who were being evaluated for adverse health effects resulting from domestic methylparathion exposure. Cholinesterase levels were determined colorimetrically using the reaction with propionylthiocholine. Analyses were performed on plasma and whole blood (total), and the RBC cholinesterase was calculated. Analyses performed on 51 control subjects showed significant variability, with coefficients of variation (CVs) of 13.3%, 24.3% and 13.3% for total, plasma and RBC respectively. A large variability was also found in the sample population, with CVs of 14.7%, 22.6% and 20.9%. Variability over time for the same subject was less: 6.8%, 6.2% and 10.1% (total, plasma, RBC). No correlation was found between the cholinesterase measurements and home surface methylparathion levels. No correlation was found between cholinesterase levels and the presence of toxic manifestations. No trends were observed of cholinesterase levels increasing over time after individuals were moved from their homes. This study suggests that there is too much inter-subject variability in cholinesterase levels to detect subtle health problems, or that the subtle health problems produced by chronic low-level organophosphate exposure are not associated with significant depression of cholinesterase activity.

1190 BLOOD CHOLINESTERASE FROM CALIFORNIA MIGRANT HOUSING CENTER RESIDENTS USING FINGER STICKS.

B W Wilson, J D Henderson, S A McCarthy, J E Billitti and S A McCurdy. *University of California, Davis, CA, USA.*

Blood cholinesterases are commonly used to monitor exposure to anticholinesterase chemicals. This objectives of this study were to compare venous draw and finger stick samples of acetylcholinesterase (AChE) of volunteers and to measure AChE from finger stick samples of adult residents of migrant housing centers in the Central Valley of California collected early and late in the spray season. Blood was collected after wiping the fingers thoroughly with alcohol, immediately hemolyzed with a phosphate/Triton X-100 buffer, frozen on dry ice and stored in an ultra-low temp freezer. Assays were conducted with a 96 well microplate reader using the Ellman assay at 410 nm run under optimal conditions. Mean AChE values from finger stick and venous blood draws from 13 laboratory volunteers averaged 14.6 ± 1.2 and 14.7 ± 1.2 nmol/min/mg Hb, respectively. Early season AChE levels from 891 subjects were similar, averaging 14.7 ± 2.6. Late season AChE levels from 510 subjects were 12.1 ± 3.5. Data analysis with regard to work history is in progress. The results to date show reproducible blood AChE levels can be obtained from finger stick samples if due care is taken in sampling, storage, and the conditions of the assay itself. (Supported in part by NIOSH R01 0403444-02, CA Department of Pesticide Regulation Contract 95-0190, and NIOSH (CDC U07/CCU906162-02) and NIEHS (ES05707) Center Grants.)

1191 EFFECTS OF A NOVEL ORGANOPHOSPHORUS INSECTICIDE ON DETOXIFYING ENZYMES OF RAT.

M Mahaboob[1], M K J Siddiqui[2], M F Rahman[1], and K Jamil[1]. [1]*Toxicology Unit, Indian Institute of Chemical Technology, Hyderabad, AP, India.* [2]*Analytical Toxicology Division, Industrial Toxicological Research Center, Lucknow, UP, India.* Sponsor: S Hussain.

The acute and subacute oral effects of 2-butenoic acid-3-(diethoxyphosphinothionyl)- methyl ester (RPR-V) on Phase-1 and Phase-II enzymes were studies in male albino wistar rats. Acute study (3 mg/kg) caused a reduction in cytochrome P-450) content in liver and lung and also brought depletion in reduced glutathione (GSH) content and inhibition in glutathione S-transferase (GST) activity. The sub-acute study at medium (0.066 mg/kg/d for 90 days) and (0.099 mg/kg/d for 90 days) doses caused time and dose specific decrease in Cyt. P-450, cytochrome b_5 (cyt. b_5), cytochrome P-450 reductase, GST and GSH content. Where as, the UDP-glucuronyl transferase (UDPGT) activity induced significantly in liver and kidneys. The withdrawal study (28 days post-treatment) revealed that changes produced were reversible. *In vivo* GSH and CDNB activation kinetics of GST demonstrated a mixed type of inhibition. *In vitro* GSH activation kinetics of GST and CDNB revealed a non-competitive and mixed type of inhibition respectively. The present studies indicated that this new phosphorothione insecticides was affecting the normal bio-transformation of xenobiotics.

1192 CHEMICAL MIXTURES: ANTAGONISTIC INTERACTIONS OF CHLORPYRIFOS AND METHYL MERCURY.

J A Steevens and W H Benson. *Environmental and Community Health Research/RIPS, Department of Pharmacology, The University of Mississippi, University, MS.*

The toxicodynamic interactions of chlorpyrifos and methyl mercury were evaluated utilizing an aquatic invertebrate animal model, *Hyalella azteca*. Binary combinations of chlorpyrifos and methyl mercury were assessed to elucidate the mechanism of interaction. Initial studies have demonstrated that chlorpyrifos and methyl mercury interacted additively with survival as an endpoint. Additionally, accumulation experiments demonstrate that exposure to chlorpyrifos (0.11 nM) significantly ($p < 0.05$) decreases the rate of accumulation and elimination of methyl mercury (42.4 nM). Specific activity of acetylcholinesterase was determined, *in vivo*, following exposure to chlorpyrifos (0.04, 0.14, and 0.27 nM) and methyl mercury (30, 190, and 440 nM) individually and in combination. Chlorpyrifos inhibited the activity of acetylcholinesterase at 0.14 and 0.27 nM, however, concurrent exposure to methyl mercury (190 nM) antagonized the enzyme inhibition. To further investigate the antagonistic interaction, the inhibition of acetylcholinesterase was assessed *in vitro* utilizing the bioactivated form of chlorpyrifos, chlorpyrifos-oxon, and methyl mercury. Chlorpyrifos-oxon inhibit-

ed acetylcholinesterase (IC_{50} = 118 nM), however, in combination methyl mercury failed to antagonize the inhibition. Results of the *in vitro* assessment of acetylcholinesterase activity demonstrates that methyl mercury does not antagonize chlorpyrifos at the acetylcholinesterase enzyme. Mechanisms responsible for the antagonistic interaction of methyl mercury and chlorpyrifos *in vivo*, potentially exist through alterations in accumulation due to chemical-chemical interactions prior to bioactivation of chlorpyrifos.

1193 DEVELOPMENTAL NEUROTOXICITY OF CHLORPYRIFOS: EFFECTS ON NUCLEAR TRANSCRIPTION FACTORS INVOLVED IN CELL DIFFERENTIATION.

T L Crumpton, F J Seidler and T A Slotkin. *Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.*

Chlorpyrifos is a widely used organophosphate insecticide which is a suspected developmental neurotoxin. Although chlorpyrifos exerts some of its effects through cholinesterase inhibition via chlorpyrifos oxon, recent studies suggest additional, direct actions on developing neural cells. The current study explores effects of chlorpyrifos on nuclear transcription factors involved in cell differentiation using in vitro models in which formation of the oxon does not occur. HeLa nuclear extracts were incubated with the labeled consensus oligonucleotide for AP1 and Sp1 transcription factors in the presence of various chlorpyrifos concentrations. Chlorpyrifos altered the binding activity of AP1 but did not exert major effects on Sp1 DNA-binding activity. These results suggest that chlorpyrifos itself can modulate the DNA-binding activity of some nuclear transcription protein complexes. Next, chlorpyrifos was incubated with PC12 cells in either of two states: undifferentiated cells, and cells undergoing differentiation with nerve growth factor. Nuclear extracts again specific interference of transcription factor DNA-binding by chlorpyrifos. These results suggest that chlorpyrifos alters the activation of genes that are expressed during development, in part by interfering with the DNA-binding activity of transcription factors. Direct actions of chlorpyrifos on cell development reinforce the need for examination of safety independently of effects mediated by cholinesterase inhibition.

1194 EFFECTS OF NEONATAL CHLORPYRIFOS EXPOSURE ON THE DEVELOPMENT OF NEURONAL ACTIVITY.

K Dam, S J Garcia, F J Seidler and T A Slotkin. *Dept. of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.*

After routine home application chlorpyrifos (cpf), infant and child exposures can exceed acceptable thresholds. We treated neonatal rats daily on postnatal days 1-4 (1 mg/kg, sc) or days 11-14 (5 mg/kg), treatments that evoked no overt signs of toxicity. Effects on the development of cholinergic neuronal function were assessed using choline acetyltransferase (ChAT) activity and hemicholinium-3 (HC-3) binding as indices of synaptic proliferation and synaptic activity, respectively. In the forebrain, early cpf treatment caused a decrease in ChAT without affecting HC-3 binding; late treatment decreased HC-3 binding without affecting ChAT. In the brainstem, early treatment had no effect on either parameter but late treatment decreased both ChAT and HC-3 binding. Effects of cpf were not limited to development of cholinergic synapses but also involved catecholamine pathways. For dopamine, either early or late cpf treatment evoked increased synaptic activity (transmitter turnover) in the forebrain and brainstem. For norepinephrine, early treatment increased activity only in the cerebellum, whereas late treatment increased activity in all brain regions. Our results suggest that cpf exposure during the postnatal period of synaptogenesis, elicits widespread disruption of the activity patterns of cholinergic and non-cholinergic pathways. As this is the period in which patterns of synaptic responsiveness and hence, behavior, are "programmed" by neural input, the period of developmental vulnerability to cpf is likely to extend into childhood.

1195 INCREASED SPONTANEOUS LIVER TUMOR SUSCEPTIBILITY IN CYTOCHROME P450 2B1 (CYP2B1) TRANSGENIC MICE.

L D Lehman-McKeeman, D Caudill, J D Vassallo and A S Fix. *Procter and Gamble Co., Cincinnati, OH.*

There is an empirical relationship between induction of CYP2B enzymes and liver tumor promotion in rodents, particularly mice. To better define the role of CYP2B induction in liver tumor development, transgenic mice (C57Bl/6N) were engineered to over-express CYP2B enzymes by microinjection of a fusion gene consisting of rat CYP2B1 cDNA under the regulatory control of the mouse metallothionein (MT) promoter. In the present study, spontaneous liver tumor rates were determined in three unique lines of transgenic mice and compared to non-transgenic (NT) controls. Mice were housed for 2 years under standard conditions, during which time, they received no chemical treatment. Liver tumors were detected at terminal necropsy by gross evaluation and histopathological exam. C57Bl/6 mice exhibit low spontaneous tumor rates, and no liver tumors were observed in NT mice (0/9 males; 0/12 females). In 2 transgenic lines that showed no hepatic expression of the transgene, a very low incidence of liver tumors was also observed (0/26 males; 1/22 females). MT-CYP2B1[3] transgenic mice express CYP2B1 mRNA and protein in liver, with constitutive CYP2B enzyme activity about 3-times higher than NT mice. These mice also show increased mortality rates, and to date, liver tumors (adenomas and carcinomas) have been observed in 3/11 males and 2/5 females. In total, liver tumors in NT and non-expressing transgenic mice were rare, with 1 liver tumor observed in 69 mice (1/34 females (3%); 0/35 males) at 2 years of age. In contrast, liver tumors have been seen in approximately 30% of the MT-CYP2B1[3] transgenic mice, thereby suggesting that sustained CYP2B enzyme activity increases liver tumor susceptibility in the resistant C57Bl/6 strain.

1196 RECENT RETROSPECTIVE AND PROSPECTIVE STUDIES IN THE TG.AC AND $P53^{+/-}$TRANSGENIC MOUSE LINES ON CHEMICALS COMPLETED IN THE TWO-YEAR NTP BIOASSAY.

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The Tg.AC (zetaglobin/v-Ha-ras) and $p53^{+/-}$ heterozygous knockout transgenic mouse lines have been evaluated for their capability to detect and discriminate between chemical carcinogens and noncarcinogens. The Tg.AC line detects both genotoxic (GTC) and nongenotoxic(NGTC) carcinogens via topical application to the dorsal skin. Carcinogens induce skin papillomas, the reporter phenotype, that defines chemical activity. The $p53^{+/-}$ model detects GTC administered by various routes and a wide range of target organs respond to carcinogen induction. In a prospective study, eight nongenotoxic NTP chemicals, diethanolamine (DEA), oleic acid diethanolamine (OAD), lauric acid diethanolamine (LADA), coconut oil diethanolamine (COD), 1-chloro-2-propanol (CP), furfuryl alcohol (FA), pentachlorophenol (PCP) and pyridine (PY), were tested in both transgenic models before the 2-year bioassays were completed. PCP and LADA, two of the six chemicals that were identified as bioassay carcinogens, were active in Tg.AC mice, but as expected, none of the NGTC were detected in $p53^{+/-}$ mice. The bioassay NGTC that are primarily single sex/species carcinogens, especially those that specifically target mouse liver, are much less likely to be identified in the Tg.AC line than those that are genotoxic or nongenotoxic trans-species carcinogens. The fact that some liver specific nongenotoxic carcinogens (mirex, LADA, PCP) are active in Tg.AC mice and that others (N-methyloacrylamide, methylphenidate, triethanolamine, DEA) are not, suggests that these nongenotoxic mouse liver carcinogens induce tumors by different mechanisms. The correspondence of activity for human carcinogens in transgenic mice is very high. Benzene, cyclosporin A, diethylstilbestrol, melphalan, and 2,3,7,8-tetrachlorodibenzo-p-dioxin were all detected in Tg.AC mice and the two genotoxic carcinogens, benzene and melphalan, were active, as predicted, in the $p53^{+/-}$ model. These results support the premise that chemical evaluation in both transgenic lines will identify most trans-species carcinogens, which are likely to be of most concern in human risk assessment.

1197 ASSESSMENT OF A RAPID CANCER BIOASSAY USING THE *p53* DEFICIENT Tg.AC(v-Ha-*ras*) MOUSE MODEL.

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Genetically altered mouse models for toxicology and carcinogenesis experiments may provide a mechanistic basis for understanding carcinogen induced neoplasia. We conducted rapid cancer bioassays (26 weeks exposure) in this mouse model using benzene (200 µL, 2× /wk; topical), p-cresidine (0.5%, oral, diet), and TPA (1.5 µg, 3× /wk, topical) for comparison to previously conducted cancer bioassays in either FVB/N-Tg.AC(v-Ha-*ras*) or heterozygous C57BL/6-*Trp53* mice. Mortality was greatest in p-cresidine treated mice due to rapid onset of urinary bladder masses. Mortality was also observed at lower levels in both untreated and treated dose groups due to the

rapid onset of sporadic tumors or treatment related tumors. Clinical observations indicated that both benzene and TPA induced papillomas with an expected incidence and multiplicity, but benzene mice developed more test site skin and other malignancies than TPA treated mice relative to the controls. p-Cresidine treated transgenic mice (but not the nontransgenic control FVB/N mice) rapidly developed urinary bladder masses (100% males and 70% females). p-Cresidine induced body weight depression was similar in both transgenic and nontransgenic mice. Overall, these results indicate that carcinogen induction of the v-Ha-ras transgene under p53 deficient conditions may allow a rapid detection of carcinogen induced tissue specific malignancy. Both *ras* and *p53* mutations are observed in many human cancers and a mechanistic understanding of the other critical genetic events occurring in these neoplasia may help in defining carcinogen specific mechanisms of cancer induction.

1198 ANALYSIS OF THE MUTANT FREQUENCY OF NONRESPONDER TG.AC MICE.

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The v-Ha-ras transgenic Tg.AC mouse model is under consideration by the National Toxicology Program (NTP) as an adjunct or alternative to the conventional two-year bioassay for the identification of chemical carcinogens. Tg.AC mice produce skin papillomas when topically treated with chemical carcinogens but fail to do so when treated with noncarcinogens. Molecular analyses indicate a 2000 base pair Bam HI fragment composed of two inverted transgene zeta globin promoters are essential for the induced phenotypic tumorigenic response. Genotypic analysis in conjunction with test mating to FVB/N mice indicate that the 2000 base pair inverted promoter region is subject to spontaneous deletion. Using the laws of Mendelian genetics a likelihood for any given genotype to occur at any given generation was derived. Applying these methods to the known genotypes of the 12 breeding pairs from the 25th generation a retrospective mutation probability was calculated. The probability that a Tg.AC responder allele "R" will spontaneously delete to a nonresponder allele " r " was estimated to be 1.59%. The observed mutation frequency determined from outcrossing responder Tg.AC mice to FVB/N was 1.476%. The methods from this study were used to determine the highest generation number, below which the proportion of nonresponder Tg.AC mice is acceptably small. This work provides valuable information for scientist involved in breeding and maintaining transgenic mouse lines in addition to the those involved in carcinogen identification studies employing Tg.AC mice.

1199 DEVELOPMENT OF NON-ISOTOPIC DNA RFLP ANALYSIS FOR IDENTIFICATION OF NON-RESPONDER Tg.AC MICE.

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Tg.AC transgenic mouse models have been proposed as a short term in vivo alternative to the chronic bioassay to identify and characterize the mechanisms of action of carcinogens. When exposed to the tumor promoter, 12-0-tetradecanoyl-phorbol-1,3- acetate (TPA), Tg.AC mice responded with skin papillomas as a reporter phenotype. Recently, an increase in TPA-exposed non- responder (NR) Tg.AC mice has been identified. To characterize the NR phenotype, we calculated the incidence of Tg.AC mice with papillomas. Retrospectively, from 1991 to 1996 in 9 experiments, the incidence of papillomas in TPA-treated Tg.AC mice was consistently over 90%. In 1997, the incidence of papillomas precipitously dropped ranging from 44% to 65%. The NR phenotype as a function of Tg. AC genotype, TPA-dosing regiment, and generation number are now known. NR mice lack a 2.0 kb region found in responder mice. A 32P-based Southern blot assay allows for the identification of NR based on DNA band shift. We developed an analogous non-isotopic DNA Blot procedure. This methodology is accurate, sensitive, reproducible, requires less time and expense, and obviates the need for radioactivity. These data indicates that the NR phenotype first appeared in 1997 and can be genotypically identified by non- isotopic Southern analysis.

1200 TRANSFORMATION-ASSOCIATED RECOMBINATION CLONING TO EVALUATE LCR INVOLVEMENT IN TG.AC TRANSGENE EXPRESSION.

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Genetically initiated Tg.AC transgenic mice carry a transgene consisting of an oncogenic v-Ha-ras coding region flanked 5' by a mouse zeta-globin promoter and 3' by an SV-40 polyadenylation sequence. Located proximal to the centromere on chromosome 11, the transgene is transcriptionally silent until activated by full thickness wounding, UV light, or chemical carcinogens. Expression of the transgene is an early event that drives cellular proliferation resulting in clonal expansion and tumor formation. Created on the FVB/N mouse, only one of five founder lines displayed the unique characteristics now associated with the Tg.AC mouse. To explore the possible presence of a Locus Control Region (LCR) responsible for transgene expression, a library of Yeast Artificial Chromosomes (YACs) were prepared by transformation-associated recombination (TAR) cloning. Isolated YACs contained the Tg.AC transgene and flanking mouse chromosome 11 up to 200kb 5' or 3' of the transgene. Following conversion of the YACs to Bacterial Artificial Chromosomes (BACs), each YAC/BAC was assayed for its ability to promote transcription of the transgene following transfection into an FVB/N carcinoma cell line. This data provides valuable insight into the requirement of a cis regulatory region in transgene expression.

1201 EVALUATION OF THE Tg.AC AND p53 (het) MOUSE AS POTENTIAL MODELS FOR CANCER TESTING OF PROTEIN MOLECULES AND PARTICULARLY GROWTH FACTORS.

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Three studies in both p53 (het) and Tg.ac mice were designed to address false positives caused by injection or an immune response to the protein, and the response to chronically administered growth factors. In study 1, animals received either no injection, saline, pH 5.5 vehicle or human albumin (1 mg/kg). In study 2 and 3, murine epidermal growth factor (mEGF) at 0.1, 1.0 or 10 μ g/kg or recombinant human insulin-like growth factor-1 (rhIGF-1) at 0.1, 1.0 or 10 mg/kg were administered respectively. The p53 mice used in study 1 were bred at Genentech Inc, remaining animals were from Taconic. In each study, treatments were administered 3 times per week, subcutaneously for 6 months, clinical pathology and hematology were performed prior to and at 3 and 6 months and animals were necropsied at 6 months for subsequent microscopic pathology. Blood samples were also drawn after 3 months to confirm exposure to the growth factors. All Tg.ac mice were tested retrospectively for the responder phenotype by Taconic. No apparent growth factor treatment-related gross lesions were observed. Unfortunately, injection and immune-related tumor formation in the Tg.ac model (study 1) could not be evaluated due to a 16% overall responder phenotype in this study. In study 2 and 3 the overall Tg.ac responder rate was 50% males/0% females and 83% male/ 91% female respectively. In studies 2 and 3,after 10 weeks, a small number of male Tg.ac mice developed small botryoid perineal masses, which did not appear to be treatment related. Also in studies 2 and 3, a few p53 mice developed subcutaneous masses consistent with areas of probable prior irritation at the injection site. In conclusion, neither model may be sensitive to an increased tumor risk as a result of chronic administration of either mEGF or rhIGF-1. Alternatively, this may indicate that chronic administration of growth factors is not associated with an increase in tumor risk, particularly since the Tg.ac model, which would be expected to respond to potential non-genotoxic carcinogens, was not responsive to either growth factor.

1202 SIX MONTH ORAL GAVAGE AND DIET CARCINOGENICITY STUDY WITH PHENOLPHTHALEIN IN THE HETEROZYGOUS P53+/- MOUSE.

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Researchers have been investigating the use of the 6-month $p53^{+/-}$ transgenic mouse model as an alternative to a 2-year rodent carcinogenicity bioassay. In the current study, we evaluated the carcinogenic potential of phenolph-

thalein administered 26-weeks by gavage (800 and 2400 mg/kg/day) versus in the diet (2400 mg/kg/day) to heterozygous p53$^{+/-}$ mice. p-Cresidine was used as a positive control article and administered by oral gavage in corn oil at 400 mg/kg/day. Micronucleus analysis of peripheral blood smears and histopathological examination of the thymus was conducted on all animals. There was an unequivocal drug-induced incidence of thymic lymphoma in both male and female animals administered phenolphthalein by gavage or in the diet. There was a dose related increase in the incidence of thymic neoplasms and the highest incidence was observed when phenolphthalein was administered in the diet. Animals in the 800 mg/kg/day gavage group had 3/15 males and 5/15 females affected, and the 2400 mg/kg/day gavage group had 4/15 males and 8/15 females affected. Animals receiving phenolphthalein at 2400 mg/kg/day in the diet had the highest incidence of thymic lymphoma, with 12/15 males and 14/15 females affected. Micronucleus analysis indicated that phenolphthalein administered orally by gavage or diet produces elevation in the incidence of micronucleated polychromatic erythrocytes and in the ratio of PCE to NCE. This study clearly indicates that phenolphthalein rapidly induces thymic lymphomas in both male and female p53$^{+/-}$ mice irrespective of the mode of delivery (gavage versus diet).

1203 GENETIC ANALYSES OF PHENOLPHTHALEIN-INDUCED THYMIC LYMPHOMAS FROM P53+/- MICE.

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In p53 +/- mice on a C57Bl/6 background treated with 0, 200, 375, 750, 3000 or 12,000 ppm phenolphthalein (P9189), thymic lymphomas developed at a combined incidence of 0%, 5%, 5%, 25%, 100% and 95%, respectively. We compare genomic DNA isolated from 10 of these thymic lymphomas to DNA from 2 lymphomas that arose spontaneously and to surrounding normal tissue DNA. We use PCR to confirmed published *in situ* data and show that the wildtype p53 allele, present in both of the spontaneous tumors and each normal tissue, is absent from each of the 10 phenolphthalein-induced thymic tumors. We further establish that the p53 null allele is present in each of the tumors and normal tissues. We genotype over 70 simple sequence length polymorphism (SSLP) DNA loci using the tumor and normal DNAs as template for radiolabeled PCR. The amplicons are separated on acrylamide gels to compare sizes between normal- and tumor- derived amplicons. Only the results from chromosome 11 are remarkable. The sets of amplicons generated from 29 loci located on chromosomes other than chromosome 11 are consistent with the allelic homozygosity expected for the C57Bl/6 background and no tumor-specific size instability or deletions are detectable at these loci. In contrast, allelic heterozygosity is evident at 24 of 38 chromosome 11 loci surveyed. The size of each amplicon is consistent with alleles originating from either C57Bl/6 or 129 mouse strains. Most remarkable are findings that the phenolphthalein-induced thymic lymphomas show treatment-related patterns of loss of heterozygosity on chromosome 11. The data suggest treatment-related crossover and genetic recombination between chromosomes 11.

1204 CORRELATION BETWEEN SUSCEPTIBILITY TO CHEMICALLY INDUCED CARCINOGENESIS AND CELL PROLIFERATION IN THE EARLY STAGE IN HETEROZYGOUS P53 DEFICIENT MICE.

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We attempted to elucidate susceptibility to chemically induced carcinogenesis in heterozygous p53 deficient mice (p53(+/-) mice). Both p53(+/-) mice and the wild-type mice were given 0, 20, 40, 75 and 250ppm BBN in drinking water for 20 weeks. Frequency of transitional cell carcinoma and preneoplastic lesions in the urinary bladder of p53(+/-) mice was more elevated than in the wild-type mice, showing potent susceptibility to BBN-induced urinary bladder carcinogenesis. Subsequently, both mice were respectively given the following chemicals for 4 weeks; 75ppm BBN, 10ppm DMN or 0.1% DHPN in drinking water, or 25mg/ml DMBA dermal application once a week. P53 expression, or decrease of p53-dependent p21$^{waf1/cip1}$ mRNA and protein expression from the expression in the wild-type mice were observed in the all organs examined, that is, urinary bladder, liver, lung and skin in p53(+/-) mice. However, cell proliferation is accelerated only in the urinary bladder and skin. These data indicate that p53(+/-) mice show organ-specific acceleration of cell proliferation in the early stage, and the specificity has correlation to the susceptibility to chemically induced carcinogenesis.

1205 EVIDENCE FOR A CRITICAL PALINDROMIC ORIENTATION OF TRANSGENE PROMOTER SEQUENCE FOR TUMORIGENIC RESPONSIVENESS AMONG Tg.AC TRANSGENIC MICE.

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The Tg.AC transgenic mouse skin paint assay is being evaluated as a short-term carcinogenesis model as an alternate for one species in the conventional two-year bioassay for safety testing of pharmaceuticals. Tg.AC mice carry multiple copies of transgene consisting of a ζ-globin promoter fused to the v-Ha-*ras* structural gene with a terminal SV-40 polyA signal. We have noted previously that a subset (Weaver et al, 1998; Blanchard, et al 1998) of hemizygous Tg.AC mice were refractory to treatment with 12-O-tetradecanoylphorbol 13-acetate (TPA). We have also shown (Thompson et al, 1998) that all hemizygous Tg.AC mice screened contain about 40 copies of the transgene but that TPA-unresponsive mice had lost a 2 kb *Bam*HI fragment containing ζ-globin promoter sequence. Results from restriction enzyme digestion and S1 nuclease digestion experiments provide evidence that the 2 kb diagnostic *Bam*HI fragment is a palindromically oriented transgene sequence that has a propensity to form a cruciform structure. The cruciform structure appears to be both important for functional expression as well as a cause for the observed transgene instability. We also report examples of non-responders with only a small deletion in the 2 kb *Bam*HI fragment of ζ-globin promoter sequence. The locus of this short ζ-globin sequence, which is deleted asymmetrically and appears critical for regulated expression, has been identified. Systematic screening of Tg.AC breeder mice for the intact 2-kb diagnostic marker of phenotypic responsiveness is allowing Tg.AC mouse colonies to be culled of nonresponders so that the utility of this model for carcinogenicity testing can be fairly evaluated and evaluations of the carcinogenic potential of pharmaceuticals tested with this model can be made accurately. These findings provide incentive for improved transgenic mouse quality assurance monitoring. Furthermore, variants with small or large deletions of ζ-globin sequence are providing insight into the mechanisms by which Tg.AC mouse transgene expression may be regulated by carcinogens.

1206 ROLE OF TUMOR NECROSIS FACTOR α (TNFα) AND TNFα RECEPTOR 1 (TNFR1) IN SUPPRESSION OF HEPATOCYTE APOPTOSIS BY PEROXISOME PROLIFERATORS IN WILD TYPE AND PPAR$^{-/-}$ MICE.

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Peroxisome proliferators (PPs) are a class of nongenotoxic rodent hepatocarcinogens that activate the peroxisome proliferator activated receptor α (PPARα) to cause increased hepatocyte DNA synthesis, peroxisome proliferation and liver enlargement. We have demonstrated previously that PPs suppress spontaneous rat and mouse hepatocyte apoptosis and that induced by the physiological negative regulator of liver growth, transforming growth factor β (TGFβ1). In addition, we have shown that tumor necrosis factor α (TNFα) is able to suppress apoptosis and induce S-phase in cultured mouse hepatocytes, mimicking the effects of PPs. Furthermore, the neutralisation of TNFα using anti-TNFα antibodies abrogated the suppression of apoptosis by the PP, nafenopin. Here, we show that neutralisation of one of the receptors for TNFα , TNFR1 (p55) abrogated the suppression of apoptosis and induction of S-phase by nafenopin. However, neutralisation of TNFR2 (p75) was without effect. In cultured hepatocytes isolated from the PPARα knock out mouse (PPARα -/-), PPs were no longer able to induce suppress apoptosis and induce DNA synthesis. Similarly, PPs were no longer able to induce peroxisome proliferation as measured by cyanide insensitive palmitoyl CoA oxidation (PCO). However, exogenous TNFα still was able to induce S-phase and suppress apoptosis in PPARα -/- hepatocytes, but was unable to induce PCO. Thus, TNFα , acting via TNFR1, appears to mediate PP-induced growth perturbation but not peroxisome proliferation. In addition, production or release of TNFα is downstream of PPARα activation. These data suggest a role for hepatic cytokines such as TNFα in nongenotoxic hepatocarcinogenesis.

1207 DIETHYLDITHIOCARBAMATE (DDC) INCREASES SENSITIVITY OF KUPFFER CELLS TO LIPOPOLYSACCHARIDE (LPS).

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DDC, a chelator of copper and a potent inhibitor of superoxide dismutase, is widely used in agricultural, industry and clinical therapy. Previous studies have shown that DDC causes rat liver injury in rats and the pathogenesis of this injury involves, in part, activation of Kupffer cells (KCs). Moreover, we have found that DDC increased the sensitivity of KCs to LPS *in vitro*. To further investigate the role of KCs in the pathogenesis of DDC induced liver injury, KCs were isolated from male Sprague-Dawley rats by collagenase-protease perfusion and purified by centrifugal elutriation. Nitric oxide (NO) was estimated using a dichloro-fluorescin free base. Tumor necrosis factor (TNF)-α was determined by L929 cell bioassay. TNF-α and iNOS protein expressions as well as activated NF-κ B were detected by immunocytochemical analysis. Oxidative stress was detected by intracellular loading of dichlorofluorescin diacetate.

DDC (0-30 μ M) increased the secretion of NO and TNF-α by LPS-stimulated KCs in a concentration-dependent manner. DDC+LPS-treated KCs showed increases in iNOS and TNF-α immuno-reactivity as compared to that of LPS alone. The stimulatory effect of DDC was inhibited by H-7 (an inhibitor of protein kinase C) and N-acetyl-L-cysteine (an inhibitor of NF-κ B activation). DDC enhanced the nuclear translocation of activated NF-? B by LPS-stimulated KCs. There was no evidence of oxidative stress following either LPS or DDC+LPS exposure.

These findings demonstrate that DDC increases the sensitivity of KCs to LPS and suggest that DDC may affect signaling pathway upstream of protein kinase C. (Supported in part by NIEHS Center Grant ES 06694.)

1208 COORDINATE REGULATION OF INDUCIBLE NITRIC OXIDE SYNTHASE AND CYCLOXYGENASE-2 EXPRESSION IN HEPATIC MACROPHAGES DURING ACUTE ENDOTOXEMIA.

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Nitric oxide (NO) is a cytotoxic mediator produced by activated macrophages via an inducible form of nitric oxide synthase (NOSII). NO has been implicated in the regulation of a number of enzymes involved in the inflammatory response. Of particular interest is cycloxygenase-2 (COX-2), an enzyme that oxidizes arachidonic acid leading to the formation of prostaglandins, prostacyclin and thromboxane A_2. Endotoxin is a bacterially derived product known to induce hepatic injury. This is associated with an infiltration of macrophages into the liver which release mediators such an NO and eicosanoids which are thought to contribute to endotoxin-induced tissue damage. In the present studies we analyzed mechanisms regulating production of these mediators in the liver during acute endotoxemia. To induce acute endotoxemia, rats were injected with Escherichia coli lipopolysaccharide (5 mg/kg, i.v.). Hepatic macrophages were isolated 3-48 hr later. We found that macrophages from control and endotoxin treated rats readily produced NO in response to inflammatory stimuli. Treatment of rats with endotoxin resulted in a time-dependent increase in NO production. This was correlated with increased NOSII expression by the cells as determined by Western blot analysis. Maximum NO production and NOSII protein were observed 3 hours after endotoxin treatment of rats. Following endotoxin administration, we also found that COX-2 expression in macrophages was increased. As observed with NOSII, maximal expression was observed 3 hours after endotoxin treatment of rats. To determine if NO is involved in the regulation of COX-2, we used aminoguanidine (AG), a relatively specific NOSII inhibitor. Treatment of macrophages with AG reduced NO production by the cells. In contrast, AG had no effect on NOSII or COX-2 expression by the macrophages. These data suggest that activation of both NOSII and COX-2 may be important in the pathophysiological response of hepatic macrophages to endotoxin. Moreover the activity of these oxidases appears to be regulated coordinately. (NIHGM34310, ES06897 and the Burroughs Wellcome Fund).

1209 POTENTIAL ROLES OF AP-1 AND NF-κB IN ITO CELLS DURING LIVER FIBROGENESIS.

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Exaggerated connective tissue accumulation occurs during hepatic fibrosis that is associated with a loss of functional hepatocytes. AP-1 is a key transcription factor which is involved in the positive and negative regulation of the interstitial collagenase (MMP-1) and α1-(1) procollagen gene expression, respectively. During liver injury NF-κB activates IL6 gene expression in Ito cells, which in turn stimulates NF-κB in hepatocytes and prevents them from apoptosis. We have investigated the AP-1 and NF-κB binding activities, by gel mobility shift assays, in Ito cell lines derived from normal (NFSC) or cirrhotic (CFSC) rat liver. We found constitutive AP-1 binding activity in NFSC, but not in CFSC cells. A different pattern of NF-κB binding was found in CFSC cells compared to that in NFSC cells, which is due to a non-phosphorylated protein complex. Our data indicate that changes in the AP-1 and NF-κB in Ito cells, during liver injury, may represent a mechanism by which cell injury stimulates collagen production, inhibits collagen degradation and causes apoptosis in hepatocytes. This chain of events is likely to potentiate the process of liver fibrosis. (Supported by NIH R01 AA09300.)

1210 EFFECT OF ENDOTOXIN PRETREATMENT ON OXIDATIVE STRESS, COLLAGEN AND CYTOKINE SECRETION OF HEPATIC STELLATE CELLS EXPOSED TO ETHANOL AND ACETALDEHYDE.

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Endotoxins (LPS), are believed to play a major role in alcohol-induced liver injury. Plasma levels of LPS are increased after acute ingestion of ethanol and in patients with alcoholic liver disease. Alcohol also enhances oxidative stress in the liver which can either independently stimulate the release of cytokines or amplify the effect of LPS. The objective of the present study is to determine the effect of endotoxin pretreatment on the oxidative stress, collagen and cytokine secretion of rat stellate cells treated with ethanol (EtOH) and acetaldehyde (Ac). Media containing 1μ g/ml LPS were added to rat hepatic stellate cells (CFSC-2G) 24hs after seed, and replaced 48 hs later by media containing LPS+ EtOH (50 mM) or LPS + Ac (175 μ M). Measurements were done at least trice 24 hs later. Lipid peroxidation was measured as malondialdehyde production in the presence of thiobarbituric acid. Reduced glutathione was quantified by means of the Ellman reaction, and collagen secretion by the hidroxiproline micromethod. TNF-α , IL-1β , IL-6 and TGF-β_1 were determined in the media by ELISA using commercial kits (R&D). Lipid peroxidation damage did not increase as a result of endotoxin pretreatment. However, GSH content diminished 43% in EtOH and 83% in acetaldehyde endotoxin pretreatment.. LPS pretreated cells increased collagen secretion. Among cytokines, IL-6 presented sensible differences with endotoxin pretreatment . There were not found TNF-α or IL-1β secretion in any experimental condition. LPS pretreatment of rat hepatic stellate cells treated with EtOH and Ac increases oxidative stress and collagen secretion. These findings support a convergence of LPS damage, oxidative stress and cytokine pathways in the fibrogenic process. (Supported by grant from CONACYT # 400200-5-25175M.)

1211 THE ROLE OF NUCLEAR FACTOR-KAPPA B IN CARBON TETRACHLORIDE INDUCED LIVER INJURY.

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Carbon tetrachloride (CCl_4) is a well-characterized centrilobular hepatotoxicant. Recent studies have shown that the toxicity of CCl_4 is mediated, in part, by tumor necrosis factor-alpha (TNF-α), a macrophage derived inflammatory mediator. Thus following CCl_4 administration to mice there is a time-dependent induction of serum and hepatic TNF-α. Moreover, mice lacking the gene for TNF-α are protected from CCl_4 induced hepatotoxicity. The promoter region of the TNF-α gene contains binding sites for several different transcription factors including NF-κB. This transcription factor in composed of a family of proteins, including two homologous subunits: p50 and

p52. These bind to the p65 subunit to form an active p50/p65 or p52/p65 heterodimer. In the present studies we analyzed the role of NF-κB in liver injury induced by CCl_4 using knockout mice lacking the genes for the p50 or p52 subunits. Treatment of wild type control C57/BL6 mice with CCl_4 (0.3 ml/kg, i.p.) resulted in a time-dependent induction of centrilobular hepatic necrosis reaching a maximum at 24h. This was correlated with increased serum transaminase levels. CCl_4 was found to be significantly less effective in inducing hepatotoxicity in mice lacking the gene for the p50 NF-κB subunit. In contrast, knockout mice lacking the gene for the p50 subunit were not protected from CCl_4-induced liver injury. These data suggest the TNF-α induced signal transduction mediated by the p50 NF-κB subunit is an important regulator of CCl_4-induced liver injury (Supported by NIH GM34310 and Burroughs Wellcome Fund.)

1212 DIPHENYLENEIODONIUM, AN NADPH OXIDASE INHIBITOR, PREVENTS EARLY ALCOHOLIC LIVER INJURY BY CHRONIC INTRAGASTRIC ETHANOL EXPOSURE IN RATS.

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Chronic ethanol administration enhances free radical formation in the liver. Reactive oxygen species could be involved in triggering liver injury due to alcohol by activation of transcription factors, such as nuclear factor κ B (NF-κ B), which regulate production of inflammatory cytokines by Kupffer cells. The purpose of this study was to determine whether NADPH oxidase is involved in early alcoholic liver injury. Male Wistar rats were fed a high-fat diet with or without ethanol (10-18 g/kg/day) continuously for up to 4 weeks using an intragastric enteral feeding protocol developed by Tsukamoto and French. Diphenyleneiodonium (DPI; 1 mg/kg/day) or vehicle (5% glucose, 0.2 ml/day) was administrated by subcutaneous injection for 4 weeks. DPI did not effect body weight or cyclic patterns of ethanol in urine. Mean urine ethanol concentrations were 251 ± 33 mg/dl and 262 ± 22 mg/dl in ethanol- and ethanol+DPI-treated groups, respectively. Ethanol treatment for 4 weeks caused about 4-fold increase in serum AST levels (253 ± 42 IU/L). DPI treatment blunted this increase significantly by about 55% (115 ± 15 IU/L). Ethanol treatment also caused severe fatty infiltration, inflammation, and mild focal necrosis in the liver after 4 weeks (pathology score: 7.2 ± 0.1). However, these pathological changes were blunted significantly by DPI (pathology score: 2.2 ± 0.3). Activation of NF-κ B was assessed with an electrophoretic mobility shift assay. After 4 weeks of ethanol treatment, NF-κ B activity in hepatic nuclear extracts was increased significantly nearly 2.5-fold over control. This increase was blunted significantly by DPI with values only 25% as high as ethanol alone. These results indicate that DPI prevents early alcoholic liver injury, most likely by inhibition of free radical formation by NADPH oxidase which prevents activation of NF-κ B.

1213 COMPARATIVE HEPATIC EFFECTS OF DIFFERENT LIPOPOLYSACCHARIDES FOLLOWING REPEATED DOSING IN RATS.

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Lipopolysaccharide (LPS) is rapidly cleared by hepatic Kupffer cells and high doses induce the release of a variety of cytokines which result in hepatotoxicity and other systemic effects. The objective of this study was to compare the chronic effects of LPS from two bacterial strains, each with different LPS agonistic properties, on Kupffer cells to the Kupffer cell changes caused by beta-glucan. LPS from *E. coli* (ecLPS) and *Rhodobacter sphaeroides* (rsLPS) were given i.v. (0.6 and 6 mg/kg) to male SD rats (3/group) for 7 days. rsLPS and its lipid A are reported to elicit no cytokine synthesis from macrophages *in vitro* and to prevent LPS-dependent production of tumor necrosis factor. Beta-glucan (bGLU) was given i.v. (30 mg/kg) as a positive control. Control rats received 5% dextrose. Body weights, clinical pathology, and hepatic histology data were collected from each rat. Rats were killed on day 15. ecLPS (6 mg/kg) was lethal and caused hepatocellular necrosis and Kupffer cell vacuolation (KCV), with increased ALT, AST, ALP and GGT. ecLPS (0.6 mg/kg) caused only minimal KCV and inflammation; there were acute increases (day 2) in hepatic enzymes. rsLPS caused a dose-responsive increase in KCV associated with Kupffer cell hyperplasia/hypertrophy and inflammation, but no increases in liver enzymes were observed. bGLU hepatic lesions were manifest by florid granulomas which most likely originated from Kupffer cells; there were no increases in liver enzymes. Nonlethal doses of lipopolysaccharides, with differing agonistic properties, resulted in dramatically different Kupffer cell responses in this 15-day study. Because rsLPS is a very weak LPS agonist, higher doses resulted in greater Kupffer cell exposure resulting in a response that appears unique when compared to ecLPS in this study. It is possible that longer-term exposure to low doses of ecLPS could elicit similar responses as rsLPS. Neither ecLPS nor rsLPS caused granulomas similar to bGLU.

1214 ACUTE EXPOSURE TO DIMETHYLNITROSAMINE RESULTS IN ALTERED HEPATIC EXPRESSION OF CYTOKINE- AND APOPTOTIC-ASSOCIATED GENES.

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We have shown that following acute dimethylnitrosamine (DMN; 5.0 mg/kg) exposure, serum cytokine levels of tumor necrosis factor-α (TNFα), interleukin (IL)-1β and IL-6 increase. In addition, acute high dose DMN has been previously shown to result in apoptosis as measured by DNA laddering. However, the mechanism of apoptosis and pathway(s) involved have not been characterized. TNFα is a potent regulator of apoptosis, thus, mice deficient in both p55 and p75 TNF receptors (TNFR double knockout; DKO) were obtained. We examined the role of TNFα following acute high dose DMN (100 mg/kg) exposure on hepatic mRNA expression of proinflammatory cytokine, cytokine receptor and apoptotic genes. RNase protection assays showed that WT (TNFR intact) mice exposed to DMN had increased hepatic mRNA expression of TNFα and transforming growth factor (TGF)β 2. DMN also increased expression of the following cytokine receptors: IL-1RI, IL-1RII, TNFRp55, and TGFβ RII, whereas TNFRp75, IL-6Rα , and TGFβ RI remain unchanged. WT and DKO mice showed up-regulation of the apoptosis promoting genes, bax and Fas, and the anti-apoptosis gene bcl-X_L, while decreased expression of the apoptosis inhibiting gene, bcl-W. Another anti-apoptosis gene, bfl1, showed increased mRNA expression with DMN exposure in WT mice, but not in DKO mice suggesting TNFα signaling is capable of protective mechanisms during DMN-induced hepatotoxicity. In addition, histological evidence indicates that signaling through TNFRp55 is required during hepatic inflammatory responses as well as contributes to liver damage following acute DMN. Thus, our results show that acute high dose DMN alters the hepatic expression of different cytokines and their receptors as well as genes associated with apoptosis. (This work was supported by NIH Grant ES08395.)

1215 INCREASED SENSITIVITY OF TUMOR NECROSIS FACTOR-α (TNF-α) KNOCKOUT MICE TO ACETAMINOPHEN (AA) IS MEDIATED BY NITRIC OXIDE (NO).

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TNF-α a macrophage-derived cytokine reported to contribute to chemical-induced tissue injury associated with inflammation. In the present studies, we used mice lacking the gene for TNF-α or the TNF-α p55 receptor to analyze the role of this cytokine in AA-induced hepatotoxicity. Mice were treated with 300 mg/kg AA, ip and sacrificed 1-12 hr later. In control animals treated with anti-TNF-α antibody, AA-induced hepatotoxicity was reduced. In contrast, TNF-α knockout mice exhibited significant increases in serum transaminase levels within 2 hr of AA treatment which corresponded to more severe liver injury and higher mortality rate when compared to wild type control (WT) mice. These data suggest that low levels of TNF-α may be hepatoprotective. NO is a cytotoxic agent known to be involved in AA-induced hepatotoxicity. Thus, following AA administration, inducible nitric oxide synthase (NOS II) expression and hepatocyte NO production are increased. Moreover pretreatment of animals with aminoguanidine, which blocks NOS II, abrogates AA-induced hepatotoxicity. To analyze the role of NO in the hypersensitivity of TNF-α knockout mice to AA, we measured levels of NOS II protein and mRNA in the liver. Although there were no differences in NOS II mRNA levels between TNF-α knockout and WT control mice, NOS II protein increased more rapidly in the TNF-α knockout mice as determined by immunohistochemistry and Western blot. In addition, aminoguanidine pretreatment reduced AA-induced tissue injury in TNF-α knockout mice. These data suggest that the hepatoprotective effects of TNF-α may involve negative regulation of NO production in the liver. (Supported by NIH grants GM34310, ES06897 and the Burroughs Wellcome Fund.)

1216 CARDIOPULMONARY TOXICITY OF INSTILLED NICKEL, VANADIUM, AND IRON IN MONOCROTALINE-TREATED RATS.

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Previous studies from this laboratory have demonstrated a biphasic *hypothermic response* to intratracheal instillation (IT) of residual oil fly ash (ROFA) particles in healthy and cardiopulmonary-compromised rats. This response consisted of both an acute (0–6 h post-IT) and delayed (12–72 h post-IT) hypothermia that was accompanied by bradycardia and an increased incidence of cardiac arrhythmias. Other studies using constituents of ROFA have implicated three transition metals, iron (Fe), nickel (Ni), and vanadium (V), as the primary mediators of ROFA-induced pulmonary inflammation. The present study examined the roles of these metals, both individually and in combination, in producing the hypothermic response and pulmonary inflammation in monocrotaline (MCT)-treated Sprague-Dawley rats (male, 60 d, n=4/group). Doses were chosen to match those of the individual metals contained within the highest dose of ROFA from the earlier studies, i.e., 105 μg $Fe_2(SO_4)_3$, 263 μg $NiSO_2$, and 245 μg VSO_4; control rats (n=4) received acidified saline vehicle (pH=2). Three weeks prior to metal IT, all rats were implanted with radiotelemeters capable of continuously monitoring ECG and core body temperature (T_{CO}). Two weeks pre-IT, MCT was injected (60 mg/kg, *ip*) to induce pulmonary vasculitis and hypertension. Bronchoalveolar lavage and lung tissue samples were collected 96 hours post-IT and assayed for inflammatory markers. Fe-treated rats showed no significant change in HR and T_{CO} from control animals. Rats dosed with V displayed an acute hypothermic response for the initial 4 hours post-IT (Δ_{max}=2.1°C↓ ; 60 bpm↓). Ni produced a delayed response from 18–72 h post-IT (Δ_{max}=2.4°C↓ ; 110 bpm↓). Lethalities (n=2) only occurred in the Ni-treated group. Cardiac arrhythmias were seen concurrently with hypothermic episodes in the V- and Ni-treated animals. These results suggest that these metals are important mediators of the toxic effects of ROFA, and that the biphasic aspect of the hypothermic response may result from combined effects of the individual metals.

1217 RESIDUAL OIL FLY ASH (ROFA) ENHANCES ALLERGIC AIRWAY RESPONSES TO HOUSE DUST MITE (HDM) VIA TH2 CYTOKINE UPREGULATION.

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Epidemiological studies have shown an association between elevated levels of particulate matter (PM) air pollution and increased morbidity, hospital visits, and use of medication in asthmatics. Residual oil fly ash (ROFA) is a product of combustion and a soluble particulate containing several transition metals including vanadium, nickel, and iron. We have previously shown that in a Brown Norway rat model of HDM–induced pulmonary allergy, animals exposed to ROFA prior to HDM sensitization had enhanced allergic pulmonary responses to HDM challenge compared to animals sensitized to HDM alone. In this study we examined TH2 cytokine levels in the bronchoalveolar lavage fluid (BALF), lung tissue, and lymph nodes of rats instilled via the trachea with 200 or 1000 μg ROFA 3 days prior to local sensitization with 10 μg HDM. Cytokine levels were assessed by ELISA assay of the BALF and lung homogenates at day 2 after sensitization, and at day 2 after challenge. Cytokines were also measured by semi–quantitative RT–PCR in the lung and bronchial lymph nodes at days 2 and 7 after sensitization and at day 2 after challenge. TNF–α, IL–6, and IL–10 were upregulated in the BALF and lung homogenates of ROFA+HDM–treated rats compared to HDM controls 2 days after challenge. IL–4 mRNA was upregulated in the lymph node 2 days after challenge, and TNF–αmRNA was upregulated 2 days after sensitization, and IL–10 and IL–5 mRNA were upregulated 7 days after sensitization in the lung tissue of the ROFA+HDM–treated group. These data suggest a mechanism for particulate–enhanced sensitization to allergens via increased expression of TH2 cytokines, and may explain the exacerbation of asthma following PM excursions. (This abstract does not reflect US EPA policy.)

1218 EXPOSURE TO DIESEL EXHAUST PARTICLES (DEP) COMPROMISED THE PULMONARY CLEARANCE *OF LISTERIA MONOCYTOGENES* IN RATS.

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In this study, we tested the hypothesis that exposure to DEP may impair lung defense against *Listeria monocytogenes* infection. Male Sprague-Dawley rats were dosed with either 5 mg/kg of DEP or saline intratracheally for 3 days, then intratracheally instilled with ~ 5,000 *Listeria*/rat. At 3, 5 and 7 days post-*Listeria* instillation, the rats were killed; the left lobe of lungs and the spleen were excised; and the numbers of Listeria in the tissues were determined. The remaining lung underwent bronchoalveolar lavage. Nitric oxide (NO) was measured in AM-conditioned supernatant. All rats survived and no significant lung inflammation and injury were noted in these rats. The pattern of *Listeria* clearance from the lungs was different between DEP- and saline-pretreated rats. At 3 days post-*Listeria* exposure, the number of bacteria in the lungs of DEP-treated rats (~ 55,000 *Listeria*/left lobe) was lower than that of saline-treated rats (~ 110,000 *Listeria*/left lobe). In contrast, the number of *Listeria* in the lungs was significantly higher in the DEP-treated rats compared to the saline-treated rats at 5 and 7 days post-*Listeria* exposure (~ 17,000 *Listeria*/left lobe vs ~ 8,500 at 5 days; ~ 7,000 vs ~ 1,500 at 7 days). Exposure to DEP alone resulted in increased NO production by AM. However, the NO production among *Listeria*-infected rats was not different. These results show that DEP exposure compromises the pulmonary clearance of *Listeria* and increases the susceptibility of hosts to this infectious pathogen. This decreased *Listeria* clearance in rats after DEP exposure does not seem to be dependent on reactive nitrogen intermediates.

1219 ROLE OF ALVEOLAR MACROPHAGES (AM) IN PARTICLE-INDUCED INFLAMMATION: STUDIES IN AM-DEPLETED RATS.

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Macrophages present in the lungs orchestrate many of the responses to inflammatory stimuli, such as particles, through release or induction of several proinflammatory molecules. We report here on a method of alveolar macrophage (AM) depletion in rats by exposure to liposome-encapsulated clodronate via intratracheal inhalation. Rats were anaesthetized with halothane, cannulated with a 14-gauge catheter, and attached to a rodent respirator system. Inspiratory pressure was set to 15 cm H_2O and a solenoid valve set the inspiratory phase to 1.5 sec and the expiratory phase to 0.5 sec. The liposomes containing clodronate were then delivered in aerosol form using a low-flow generator. In order to asses the role of AMs in response to inflammatory stimuli, one group of rats received 75 μ g crystalline SiO_2 by intratracheal instillation 3 days after liposome exposure. Twenty-four hrs after silica instillation, several indices of inflammation were assessed in bronchoalveolar lavage fluid. Rats exposed to liposomes had 80.5% fewer AMs as compared to rats exposed to saline-filled liposomes with no significant neutrophil (PMN) influx (0.7% of total cells in sham-exposed controls). Rats exposed to silica alone demonstrated significant lung inflammation (4.0 x 10^6 PMNs), while those exposed to silica after being depleted of AMs had less than half that number (1.7 x 10^6). We conclude that although AMs play a crucial role in orchestrating the response to particles, there may be other cell types such as epithelial cells which contribute to the particle-induced inflammatory cascade.

1220 PARTICULATE MATTER (PM) AS AN IMMUNOSUPPRESSIVE FACTOR THAT CAN EXACERBATE ONGOING PULMONARY INFECTIONS.

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Infection, specifically pneumonia, contributes substantially to the increased mortality among elderly individuals following exposure to PM. Studies were performed to test the hypothesis that alterations in pulmonary immunologic mechanisms important for resistance against infectious pneumonia contribute to the observed increase in PM-induced mortality in the elderly. For these studies, the effects of inhaled concentrated ambient PM2.5 from New York City air upon rats infected with *Streptococcus pneumoniae* 48 hr prior to exposure were examined. The results indicated that a single 5 hr exposure to PM induced time-dependent increases in pulmonary bacterial burdens and numbers of circulating WBC, and decreases in levels of lavageable neu-

trophils/cytokines and bronchus-associated lymphoid tissue compared to that in infected air-exposed control rats. Moreover, exposure of infected rats to PM increased the extent of pneumonia-associated lung lesions, as well as the incidence of septicemia in rats for up to 18 hr following PM exposure. Taken together, these findings suggest that exposure of rats with fulminating *S. pneumoniae* infections to concentrations at or somewhat greater than the promulgated 24 hr NAAQS of 65 μg PM2.5/m3 can exacerbate the disease process and compromise host ability to adequately "handle" an ongoing pneumococcal infection. These changes, in turn, could possibly contribute to the increased incidence of pneumonia-related deaths observed in PM-exposed elderly populations. (Supported by HEI #94-03A.)

1221 RESIDUAL OIL FLY ASH (ROFA)-INDUCED PULMONARY INFLAMMATION AND MUCOUS CELL METAPLASIA IN RATS CORRELATES WITH LEACHABLE VANADIUM CONTENT.

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Increases in particulate air pollution are linked with increased hospital admissions and emergency room visits for cardiopulmonary diseases. Intratracheal instillation of residual oil fly ash (ROFA), an emission source particulate, induces pulmonary inflammation, cytokine/chemokine expression, and airway epithelial injury. F344 rats were intratracheally instilled with 0, 50 or 200 μ g of ROFA containing high (ROFA6) or low (ROFA2) amounts of leachable Vanadium to examine the effect of this transition metal in ROFA-induced inflammation and mucous cell metaplasia. Rats were sacrificed 3 d and 7 d after instillation. Right lung lobes were lavaged and recovered fluids analyzed for inflammatory cells, LDH activity, and total protein content. RNA from right lung lobes was analyzed for MIP-2 and MCP-1 mRNA levels. The left lung was processed for light microscopy and morphometric quantitation of intraepithelial mucosubstances in pulmonary airways. Both ROFA2 and ROFA6 induced dose-dependent increases in recovered neutrophils and lymphocytes that were greatest 7 d after instillation. All ROFA6-instilled rats had significantly more neutrophils than ROFA2-instilled rats. Only ROFA6 induced an increase in LDH activity and total protein levels in lavaged fluids. Both ROFA6 and ROFA2 induced dose- and time-dependent increases in MCP-1 and MIP-2 mRNA levels. ROFA6, but not ROFA2, induced mucous cell metaplasia with significant increases in stored mucosubstances in the surface epithelium lining main axial pulmonary airways. These data suggest that ROFA-associated inflammation and mucous cell metaplasia is dependent on the amount of Vanadium associated with the particles and emphasizes the need to consider the chemical/physical characteristics of ambient air particulates when assessing potential pulmonary toxicity.

1222 RESIDUAL OIL ASHES WITH DIFFERING COMPOSITION ELICIT PRO-INFLAMMATORY CHEMOKINES IN THE LUNG AND HEART.

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Residual oil ash (ROA) is a significant environmental hazard and contributes to airborne particulate matter. In the present study, toxicity profiles for two ROAs differing in composition were examined. Residual oil ash 1 (ROA1) had a lower carbonaceous component and high vanadium content; ROA2 was rich in carbon and included high amounts of nickel and manganese. HYPOTHESIS: The greater presence of vanadium in ROA1 will result in more severe pulmonary injury. METHODS: Rats were lightly anesthetized and intratracheally instilled with 2.5 mg/kg ROA1 or ROA2 or PBS. Animals were sacrificed at 4, 24, and 48 hours (hr) post-instillation (PI). Bronchoalveolar lavage (BAL) was performed and BAL cells as well as lung and heart tissues were retained for RNA isolation and Northern analysis. RESULTS: Significant ROA-induced pulmonary inflammation was observed in BAL samples at all time points with neutrophil and eosinophil influx and increased biochemical markers of inflammation ($p < 0.05$). These changes were significantly more severe in the ROA1 group for several parameters, particularly at 4 hr. Macrophage inflammatory protein (MIP)-1α and MIP-2 mRNA levels were markedly increased in ROA-exposed BAL cells at 4 hr; expression was present but declining at later timepoints. Lung and cardiac tissue exhibited very low MIP-1α and -2 mRNA levels at 4 hr PI only. There was no difference in the level of mRNA expression of these chemokines between the two ROA groups. CONCLUSION: ROA1 appears to be a more toxic pro-inflammatory particle indicating that composition is an important determinant of pulmonary response. The presence of vanadium or lack of carbon content may play a role in this differing response. Secondly, these data indicate that ROA induces pulmonary inflammation by induction of pro-inflammatory chemokines. (Supported By: ES00002, ES08129, HL05947, and HL54958.)

1223 DISTRIBUTION OF RETAINED PARTICULATE MATERIAL IN RAT AND HUMAN LUNGS.

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Rats and monkeys differ in patterns of particle retention in the lungs after identical exposures to diesel exhaust (DE) or coal dust at a single concentration (2 mg respirable material/m^3) resulting in moderate lung burdens. Here we extend the morphometric studies of particle retention to humans and examine the influence of exposure concentration on retention patterns in rats and humans. Histologic lung sections from a previous study in rats and from the National Coal Workers' Autopsy Study were examined morphometrically to estimate the volume percentage of particulate material in defined anatomic compartments. The rats had been exposed 7 h/day, 5 days/wk for 24 mo to DE at 0.35 (low), 3.5 (medium), or 7.0 (high) mg soot/m^3; the 24-mo soot lung burdens were 0.6, 12, and 21 mg, respectively. The human lungs sections were from 1) nonsmokers who did not work as miners, 2) nonsmoking coal miners who worked under the current standard of ≤ 2 mg dust/m^3 for 3 – 20 y (mean = 13 y), and 3) nonsmoking coal miners who worked under the former standard of < 10 mg dust/m^3 for 33 – 50 y (mean = 40 y). The volume percentage of the lung occupied by particulate material was 0.2, 1.6, and 3.7% in the low, medium, and high exposure concentration rats, respectively. The particulate material was predominately retained in macrophages in the alveolar and alveolar duct lumens, with 82, 81, and 85% of the material being in lumens and 18, 19, and 15% of the material being interstitial in the low, medium, and high exposure concentration rats, respectively. The volume percentage of the lung occupied by particulate material was 0.3, 1.8, and 10.3% in the non-miners, coal miners under the current standard, and coal miners under the former standard, respectively. Fifty-seven, 68, and 91% of the particulate material was interstitial, and the remainder was in lumens, in the non-miners, coal miners under the current standard, and coal miners under the former standard, respectively. These results show that chronically inhaled diesel soot is similarly retained predominately in lumens of rats over a wide range of exposure concentrations and resultant lung burdens. In non-miners and coal miners, chronically inhaled particulate material is retained primarily in the interstitium, and the percent of the material in the interstitium is related to dose (exposure concentration, years of exposure, and/or lung burden). (Research sponsored by the Assoc. of German Car Manufacturers (VDA).)

1224 RESPONSE TO ULTRAFINE AND FINE PARTICLES IN THE LPS-PRIMED LUNG.

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Bacterial endotoxin (LPS) is an environmental contaminant and product of infection which has potent inflammatory and priming effects in organs such as the lung. Low levels of urban particles are associated with increased morbidity in susceptible populations. We hypothesize that ambient ultrafine particles are more effective than fine particles in causing adverse effects. To test this, we have compared the response of young (8 wks) and old (20 mos) F344 rats to two different sizes of TiO_2 both with and without prior exposure to LPS. Rats were exposed to LPS (0.02 μ g deposited for young rats; 0.01 μ g for old rats) via inhalation and then immediately to particles via intratracheal instillation (50 μ g ultrafine TiO_2 (~20 nm) or fine TiO_2 (~250 nm)). Animals were sacrificed 24 hrs after exposure and inflammatory parameters in bronchoalveolar lavage fluid were assessed. Priming with LPS resulted in a significantly greater PMN response in the lungs of particle-exposed rats as compared to LPS or particle exposure alone; this was true for both young and old rats. Additionally, the ultrafine particles produced greater inflammation by themselves and with LPS priming as compared to the fine particles. We conclude that inhaled LPS can act to prime the lung to subsequent particle exposure and that ultrafine TiO_2 has greater inflammatory potential than fine TiO_2.

Due to the ubiquitous nature of both endotoxin and low-toxicity dusts such as TiO_2, the interaction between the two shown here could aid in understanding the impact on health of such exposures in sensitized individuals.

1225 TISSUE INJURY INDUCED BY INHALED FINE PARTICULATE MATTER AND PEROXIDE IS ASSOCATED WITH ALTERED OXIDATIVE METABOLISM IN ALVEOLAR MACROPHAGES.

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Inhalation of fine particulate matter (PM) has been positively correlated with mortality and morbidity in humans. The overall objective of our studies is to analyze the role of particle-associated peroxides in fine PM-induced toxicity. An aerosol consisting of ammonium sulfate (AS), a major component of fine PM in the eastern US, was used as a model. An aqueous solution of AS and hydrogen peroxide (H2O2) was atomized, conditioned to 85% relative humidity and introduced into a 6-port nose-only exposure chamber. The resulting aerosol contained 415 $\mu g/m^3$ AS with a particle number distribution that peaked at 0.3 μm in diameter, as measured by Lasair Optical particle sizer, and 17 ppb of gas-phase peroxide in equilibrium with the particle phase. Gas-phase and particle-phase peroxide concentrations were calculated using total peroxide concentration, Henry's law constant and aerosol water content. Sprague-Dawley rats were exposed to particle-free air or aerosols consisting of AS or AS plus H2O2 (17 ppb) (AS+H2O2) for 2 h. Rats were sacrificed 22 h post-exposure. We found that inhalation of AS aerosols had no effect the number, viability or composition of cells or lactate dehydrogenase (LDH) levels in bronchoalveolar lavage (BAL) fluid relative to air exposed rats. In contrast following inhalation of AS aerosols, an increase in serum LDH levels and BAL protein levels was observed and a decrease in superoxide anion production by alveolar macrophages. Whereas the presence of 17 ppb H2O2 in the aerosols caused no change in cell viability, cell composition or LDH levels in BAL relative to air and AS exposed rats, the effect of AS on superoxide anion production was reduced. These data suggest that components in fine PM may be important in the toxicity of these materials. (Supported by the Health Effects Institute and a National Research Service Award from the NIH).

1226 SPECIES DIFFERENCES IN THE PULMONARY RESPONSE TO PARTICLES.

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A number of low solubility particles produce lung tumors in rats after chronic inhalation. These particles have not been shown to be genotoxic, indicating that the rat tumor response may result from a secondary, nongenotoxic mechanism. It has been hypothesized that the rat lung response to these particles may be due to the recruitment, activation, and subsequent release of damaging mediators by the inflammatory cells. Recent studies have suggested that hamsters and mice may be less sensitive to the inflammatory and tumorigenic effects of low solubility particles than rats. To investigate species differences in lung particle response, this study compared the effects on rats and hamsters to α quartz and carbon black. Briefly, rats and hamsters were intratracheally instilled with saline, 0.2, 2, or 20mg of α quartz or carbon black. Seven, 28, and 56 days after exposure bronchoalveolar lavage (BAL) was performed and the BAL fluid was characterized for cell number, type, LDH and total protein. Additionally, lung tissue was evaluated for the expression of various cytokines, as well as levels of metallothionein and glutathione. Both species showed dose related increases in neutrophils, LDH, and total protein after α quartz and carbon black exposure, however, the changes were significantly greater in the rat. Additionally, rats showed greater expression of several pro-inflammatory cytokines and lower levels of the anti-oxidants metallothionein and glutathione. These differences in cytokine expression and anti-oxidant production may contribute to the apparent species differences in tumor response.

1227 4-AMINOBIPHENYL (4ABP) HEMOGLOBIN (Hb) ADDUCTS IN MATERNAL SMOKERS: ROLE OF PASSIVE SMOKE EXPOSURE ON THE FETUS.

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Previous studies from this laboratory have focused on the qualitative and quantitative assessment of maternal:fetal exchange of a variety of tobacco smoke carcinogens including 4-aminobiphenyl, benzo(a)pyrene, and the tobacco specific nitrosamines NNN and NNK. In recent studies we have extended these observations into investigating the effect of "passive" smoke exposure on the *in utero* development of the fetus. In this study we examine the effect of "passive" exposure to tobacco smoke and the subsequent formation of both maternal and fetal hemoglobin adducts, specifically 4ABP adducts, resulting from this exposure. Women nonsmokers were enrolled in the study who were found to be exposed to passive smoke >8 hours/day either in the workplace or home environment. Upon admission for labor and delivery, maternal urine was obtained for cotinine assessment as well as maternal blood for Hb adduct determinations. Fetal bood was obtained via the umbilical vein for assessment of fetal exposures to tobacco smoke carcinogens. The results indicated that women exposed to passive smoke during pregnancy had significantly elevated levels of 4ABP ((maternal) 104.3 ± 26.5) and ((fetal) 46.2 pg/ gm Hb) adducted to both maternal and fetal Hb when compared to nonsmokers ((maternal) 34.6 ± 9.6; (fetal) 12.4 ± 5.9 pg/gm Hb). However, these values were found to be significantly lower than the maternal (375.2 ± 29.3) and fetal values (162.4.9 ± 31.5 pg/.gm Hb) of 4ABP adducts obtained in 1 - 2 pack per day smokers. These results demonstrate that exposure to "passive" cigarette smoke is a concern during pregnancy and the levels of Hb adduct detected as a result of passive smoke exposure warrant further investigations as to the health effects of in utero exposure to passive smoke

1228 REGIONAL DIFFERENCES IN HEMOGLOBIN ADDUCTS FROM AROMATIC AMINES IN CHILDREN.

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The origin of hemoglobin (hb) adducts from aromatic amines in nonsmokers is largely unknown. Recently, the contribution of environmental tobacco smoke (ETS) exposure has been shown to be negligible (Falter et al., Biomarkers 3:35,1998). Regional differences relating to population and/or traffic density have been postulated to be of more importance. Therefore, hb adducts of 4-aminobiphenyl (4-ABP) as well as o-, m-, and p-toluidine were analysed in children from three different sized Bavarian cities, Munich (1.3 mill. inhabitants; N=34), Augsburg (0.25 mill. inhabitants; N=126) and Eichstätt (13,000 inhabitants; N= 65). ETS exposure was estimated from self-reported smoking of parents and other household members. Additionally, urinary cotinine was determined in Augsburg children. 4-ABP-hb adducts were significantly higher in children from Munich (31±6 pg/g hb, $p<0.001$) and Augsburg (27±17, $p<0.05$) compared to Eichstätt (21±12). In children from Augsburg and Munich, adducts from o-, m-, and p-toluidine were similar and 33-44% higher than in children from Eichstätt ($p<0.05$). 4-ABP and toluidine adducts were not influenced by ETS exposure. In children from Augsburg urinary cotinine highly significantly correlated with ETS exposure (Spearman rank correlation: r=0.69) but not with 4-ABP-hb adducts (r= 0.077) or adducts from o-, m-, and p-toluidines (r=-0.17, -0.18, -0.20). In children, hb adducts from aromatic amines are strongly influenced by regional differences whereas ETS is not an important source of these adducts.

1229 TOXICITY OF DEGRADABLE CHAFF COUNTERMEASURES.

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United States military aircraft and ships currently use aluminized glass chaff as a passive countermeasure for radar guided threats. Heightened concern for the health impact of deployed chaff on human and animal habitats has led to the development of an environmentally friendly, degradable chaff (EcoChaff(TM)). EcoChaff(TM) is an aluminum coated degradable vitreous

oxide (DVO(TM)) material. Aluminum, the principle toxic component of EcoChaff(TM), has been implicated in the etiology of at least one neurodegenerative disease, although data supporting this hypothesis are inconclusive. In an effort to assist in human health and ecological risk assessment of the geographical areas over which the new chaff will be deployed, current studies are evaluating the effects of human exposure to EcoChaff(TM). Preliminary analysis of sediment samples taken from the Naval Research Laboratory-Chesapeake Bay Detachment (NRL-CBD), an area over which aluminized chaff has been deployed for over twenty years, suggests that sediment bioavailable aluminum levels are not significantly different from background levels. Further studies will focus on the reactivity of the aluminum coating of EcoChaff(TM) under conditions which mimic the gastrointestinal (GI) tract environment using a physiologically-based extraction bioassay. Aluminum absorption by the GI tract and deposition in nervous tissue will also be monitored in *in vivo* experiments. (Funding Sponsor: Naval Air Warfare Center Aircraft Division, Patuxent River, MD.)

1230 MONITORING DEGRADATION OF POLYURETHANE FLEXIBLE FOAM IN A LANDFILL SIMULATOR.

C Bartlett, D Aul, M S Blough, M D'Andrea, J Hauck, A L Kennedy, E G Minkley and W E Brown. *Carnegie Mellon University, Pittsburgh, PA, USA.* Sponsor: M F Stock.

Polyurethane foams are found in many common products such as car seats and mattresses. Disposal of these materials has raised the question of the stability of the polyurethane in landfill environments and the potential for aromatic amine formation. For this study, a paper-based, recirculating, landfill simulator was used to monitor the fate of both fresh toluene diisocyanate-based flexible foam and cured auto shredder residue (ASR) under anaerobic, methanogenic conditions. Leachate taken at regular time intervals from the simulators was monitored specifically for the presence of toluene diamine (TDA) using GC/MS. Duplicate simulators were run for each experimental condition and monitored for 650 days. The flexible foam was added to the simulators at concentrations up to 9% (w/w) and TDA-spiked simulators were processed in parallel as positive controls. For the foam simulators, detectable levels of TDA were found in leachate with initial flow-through, reached a maximum after 26 days, and returned to below detectable levels (0.0001%) at 500 days. At maximum levels, an average of 0.001% of the flexible foam mass was measured in the leachate while no detectable TDA was found in simulators containing ASR. At a maximum point of elution, the average recovery from TDA spiked simulators was 3.5% of the TDA initially added to the simulator. These results indicate greater than 95% of the added TDA is bound in the simulator. No evidence of polyurethane foam degradation under landfill conditions was observed.

1231 TOTAL EXPOSURE AND BIOLOGICAL EFFECT ESTIMATES IN A TEXAS COMMUNITY NEAR FORMER URANIUM MINING AND MILLING WASTES.

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Background: The northern section of Karnes County, Texas has been the site of uranium mining/milling since the early 1960s, producing serious concern in the local residents as to possible long-term health consequences. Experimental Design: We conducted a human health risk assessment on the local population utilizing molecular epidemiology and total exposure assessment strategies. Multiple pathways and routes of exposure to the local residents were considered and include soil, water, indoor dusts, indoor air, ambient air, and foodstuffs grown in the area. The long-lived radionuclide uranium-238 (^{238}U) and progeny, as well as lead isotopes were measured in order to evaluate the extent of contamination from mining/milling facilities. Thorium-232 (^{232}Th) was quantified simultaneously and served as a reference. Results: Concentrations of uranium in yard soil (n=98) of residents near mining/milling areas were higher in the surface (0.34-15.7 mg/kg) compared to subsurface (0.17-4.95 mg/kg) and higher than control soil surface (0.13-0.26 mg/kg) or subsurface (0.13-0.23 mg/kg). Distribution of ^{232}Th, indicated the increase in ^{238}U is anthropogenic. Water samples (n=8) from individual water wells indicate contamination into the local aquifer near a mining/milling facility. Indoor dust samples (n=27) revealed higher uranium concentrations in homes from the target area (27.1-570.3 μg/kg) compared to the control (9.1-80.1 μg/kg). Experimental gardens revealed bioaccumulation of uranium in vegetables proportional to soil concentration, resulting in higher concentrations of ^{238}U in vegetables from the target area. Total exposure evaluation indicates that the target population receives 5-times the individual total daily dose (TDD) of ^{238}U while the TDD of ^{232}Th was not significantly different, based on statistical distribution. Furthermore, cumulative individual dose (CID) of ^{238}U was up to 11-times higher in the target population compared to control. Analysis of 8-OHdG DNA adducts and chromosomal translocations by FISH analysis in lymphocytes of residents indicated no excessive damage in the target population compared to the control. Conclusion: Despite the documented uranium contamination, biomarkers of genetic damage did not indicate serious health threats. (Supported by NIH T32E507254.)

1232 NARCOSIS AND REACTIVITY: COMPARING TWO DIFFERENT MODES OF ACTION IN AQUATIC TOXICOLOGY.

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In environmental risk assessment it is a challenge to correctly classify a chemical regarding its mode of action (MOA) with little or no mechanistic information. In the present work, we use a series of acrylates and methacrylates to illustrate a strategy to compare the importance of two MOAs and thereby identify the predominant cause of acute fish toxicity. Acrylates are known to be Michael acceptors and may therefore react with glutathione (GSH) causing GSH-depletion in vivo (reactive mechanism). On the other hand, acrylates may also act by a non-specific mechanism (narcosis). The following two, physiologically meaningful, parameters were calculated in order to estimate the contribution of these two mechanisms to the overall acute toxicity: (i) a lipid normalized body burden for narcosis and (ii) the potential degree of GSH depletion for reactive toxicity. The degree of GSH depletion is represented by the product of the reactivity towards GSH and the exposure concentration. This model was validated with four model compounds and an in vivo study. For both modes of action, a critical value was established.

1233 EFFECT OF ESTRADIOL AND CORTISOL ON Na^+K^+ATPASE AND FLAVIN-CONTAINING MONOOXYGENASE IN EURYHALINE FISH.

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Euryhaline animals exposed to compounds bioactivated by FMO (i.e. aldicarb) are more susceptible to toxicity at higher salinity than fish residing in lower salinity. Previous studies in our laboratory have shown a direct relationship between flavin-containing monooxygenase (FMO) expression and osmotic pressure in several species of euryhaline fish. However, it is unclear how salinity regulates expression of FMO(s). Na^+K^+ATPase is also directly related to osmotic pressure and is regulated by a number of hormonal signals including 17-β estradiol (E2) and cortisol (CO). In order to determine the effect of these compounds on FMO expression, sexually immature Atlantic flounder (*Platichthys flesus*) received abdominal implants of 5 mg of E2 and CO and were maintained in ambient seawater for 18 days. Serum was sampled for hormone and osmolality measurments, and gill was sampled for Na^+K^+ATPase and FMO mRNA expression. Serum E2 levels were nearly 10-fold greater than untreated and sham-operated controls (n =4). Alpha subunit expression of gill Na^+K^+ATPase mRNA was significantly reduced ($p \leq 0.05$) by 75% when compared to controls. FMO activity (trimethylamine oxygenase) has also been shown to be reduced by E2 treatment. These data indicate that Na^+K^+ATPase and FMO are controled by similar hormonal signals and supports the hypothesis that FMO may play a role in cellular osmoregulation as well as xenobiotic biotransformation. (This work was supported by the Ray Lankester Investigatorship of the Marine Biological Association of the United Kingdom.)

1234 CHARACTERIZATION OF HYDROQUINONE AND CATECHOL FORMATION USING HEPATIC MICROSOMES FROM THREE SPECIES OF FISH.

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The in vitro metabolism of phenol at 11 °C has been studied using juvenile and immature adult rainbow (*Oncorhynchus mykiss*), immature adult brook (*Salvelinus fontinalis*), and immature adult lake trout (*Salvelinus namaycush*) hepatic microsomal preparations. Incubations were optimized for time, cofactor concentration, pH, and microsomal protein concentration. Formation of phase I ring- hydroxylation metabolites, i.e., hydroquinone and

catechol was quantified by the use of high performance liquid chromatography with dual channel electrochemical detection. Sample preparation and chromatographic conditions were optimized to achieve the separation and sensitivity required for the analysis of these very labile products. Metabolism of phenol over a range of substrate concentrations (1 to 150 mM) was quantified in an attempt to calculate the Michaelis-Menten kinetics constants K_m and V_{max}. Results indicate a difference in K_m values between species, with an apparent enzyme affinity for substrate greatest in rainbow trout, followed by brook trout, and less in lake trout. These experiments provide a means to quantify metabolic activation of xenobiotics in fish, to compare activation reactions across aquatic species, and to provide a basis for the prediction of metabolic pathways and rates in fish.

1235 EFFECT OF ß-NAPHTHOFLAVONE INDUCTION UPON 3,4,3',4', TETRACHLOROBIPHENYL BIOAVAILABILITY IN *IN SITU* PREPARATIONS OF CHANNEL CATFISH.

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Fish are exposed to multiple agents in the diet, including persistent polychlorinated biphenyls and polycylic aromatic hydrocarbons-type P4501A inducers. These studies examined the relationship of intestinal induction with xenobiotic bioavailability. Systemic bioavailability of dietary [^{14}C]-3,4,3',4', tetrachlorobiphenyl (TCB) and intestinal metabolic abilities were evaluated using an *in situ* isolated perfused intestinal segment of the catfish. In addition to controls, catfish were preexposed *in vivo* for 10 days to dietary ß-naphthoflavone (BNF) at a low (10 mg/kg diet) or high dose (50 mg/kg diet). Upon BNF pretreatment, a [^{14}C]-TCB (60 µM) solution was administered via linoleic acid (10mM) micelles into isolated intestinal segment. Following a 60-min perfusion, the efferent blood, intestinal contents and mucosa were analyzed for [^{14}C] content. Assays of total P450 content and aryl hydrocarbon hydroxylase (AHH) and glutathione-S-transferase (GST) (1-chloro-2,4-dinitrobenzene, CDNB; ethacrynic acid, EA) activities were determined on mucosal subsamples. Dietary preexposure to BNF had no effect upon the systemic bioavailability of [^{14}C]-TCB, with controls showing blood values of 751 ± 263 pmol TCB/mL, while low and high dose BNF-treated animals exhibited 646 ± 80 and 612 ± 41 pmol TCB/mL. Similarly, BNF preexposure did not influence either [^{14}C]-TCB levels in the mucosal and postinfusate tissues, or P450 content and GST activity. AHH activity was significantly increased 9.2 and 12.5-fold after exposure to low and high doses of BNF. In conclusion, there was no correlation of intestinal monooxygenase induction by BNF with systemic TCB bioavailability. (Funded by NIEHS P42 E5 07375.)

1236 MULTIGENERATION REPRODUCTIVE EFFECTS OF DI-*N*-BUTYL PHTHALATE IN JAPANESE MEDAKA (*ORYZIAS LATIPES*).

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Japanese medaka, a freshwater teleost, was used to examine multigeneration reproductive effects of di-*n*-butyl phthalate (DBP). The endpoints included mortality, histopathologic changes, growth, gonadal-somatic index (GSI), sexual development, fecundity, embryonal development, vitellogenin induction and hepatic microsomal testosterone metabolism. The F_0 generation was first exposed as 14-day old larval fish. Exposure consisted of feeding DBP or 17 β-estradiol (E2) in dry flake food, at a daily ration 5 % body weight. There were seven treatment groups, with two replicates (n=20). The treatment groups included an ethanol control, 0.5, 5, 50 µg DBP/g flake food, and 0.05, 0.5, 5 µg E2/g flake food. The F_0 and F_1 generation were fed each treatment through sexual maturation. All E2 0.05 µg/g fish were phenotypic females, as confirmed histologically. No eggs were produced by E2-treated fish. DBP had no major effects in the F_0 generation. DBP at 50 µg/g decreased F_1 generation ovary weight, GSI, egg production, and in the F_1 generation males reduced testes weight and GSI. DBP at 50 µg/g caused an increase in microsomal protein levels, liver weight, and hepatic-somatic index in both F_1 generation males and females. Evaluation of F_1 and F_2 eggs from DBP-treated groups, showed normal embryonic development. The male to female ratios in all DBP groups were similar to the control. DBP caused reproductive toxicity in the second generation medaka offspring, following chronic dietary exposure, to environmentally relevant concentrations. (ES-22330, ES-29782, NJAES-01126)

1237 DIFFERENCES IN HEPATIC VITAMIN E CONCENTRATIONS IN THREE FISH SPECIES.

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Vitamin E (Vit E) or tocopherol is a lipid soluble vitamin with antioxidant properties. Various studies have shown increased susceptibility to oxidative stress when organisms have been raised on low Vit E diets. While there is a considerable amount known on the Vit E status in mammals, there is limited information on normal concentrations in aquatic organisms. Such information is necessary in making inferences as to the susceptibility of aquatic species to oxidative stress. Endogenous concentrations of Vit E were monitored in laboratory-reared male and female brook trout (BkT, 800-1400 g) (*Salvelinus fontinalis*), rainbow trout (RbT, 100-1000 g) (*Oncorhynchus mykiss*), and lake trout (LkT, 600-900 g) (*Salvelinus namaycush*) raised on similar diets. Specifically, Vit E was measured in whole liver homogenate and microsomes by HPLC with UV detection. Hepatic microsomal Vit E concentrations in male BkT and RbT tended to be higher than females when comparing fish of similar sizes ~800-1200 g, (BkT: 13 and 6 ug/g microsomal protein; RbT: 4.5 and 1.5 ug/g microsomal protein, respectively). Female RbT (500-900 g) had lower Vit E/g liver than male RbT (500-900 g). Additionally, concentrations of Vit E found in these females were similar to those found in younger, smaller female RbT (100-300 g). The male LkT had hepatic Vit E concentrations lower than males of the other species, but similar to those measured for female RbT. This study indicates hepatic Vit E may vary with sex and species despite similar diets. While previous studies indicated susceptibility to oxidative processes may be linked to lower dietary Vit E, this study raises the question of a possible inherent species specific susceptibility factor. Further testing is needed to verify the significance of Vit E differences among species.

1238 FISH HEPATIC ENZYMES IN A TROPICAL LAGOON FROM PUERTO RICO.

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San Jose Lagoon is one of the most contaminated estuary environments in Puerto Rico. Contaminants measured in sediments show levels of PCB's (450 ug/kg), Chlordane (34.5ug/kg), Phalate Esters (16,000 ug/kg), PAH's (475 ug/kg), Lead (750 ug/kg) and Mercury (4.9 ug/kg). This Lagoon has been declared a National Estuary by the USEPA. A major clean up plan for the system is being established and remedial action will soon begin. In order to follow up with the effectiveness of the clean up operation, a hepatic enzyme biomarkers profile for the stripped moharra (Diapterus plumieri) has been obtained. Cytochrome P-450, cytochrome b5, NADH cytochrome b5 reductase, NADPH cytochrome P-450 reductase, cytosolic and microsomal Glutathione-S-Transferase (GST) together with Liver Somatic Index were examined from fish collected in five stations throughout the San Jose Lagoon and at a reference site in La Parguera, Puerto Rico. The induction of a specific isozyme, P-450IAI was determined by inmunoblot assay and other enzyme activities by spectrophotometric assays. Of all parameters measured, cytosolic and microsomal Glutathione-S-Transferase were found to be good enzyme indicators of pollution. An immunoblot of cytochrome P-450IAI showed induction of CYP1A1 in most of the fish from stations in the Lagoon. These results were compared to the reference site which showed no cross-reactivity with basal levels. Cytochrome P-450, NADH and NADPH reductases were also sensitive but not at the same level as cytosolic and microsomal GST. Some differences were observed between male and female fish. The liver somatic index was found to be considerably lower (1/2) at the control site than any of the experimental stations from San Jose Lagoon, this finding was statistically significant for all stations. This research serves as a baseline for future studies for the assessment of the remediation efforts of the San Juan Bay Estuary Program. The findings of this research may be useful for comparison of enzyme activities between temperate and tropical species.

1239 EFFECTS OF DIAZINON CONTAMINATINO IN *ICTALURUS PUNCTATUS*: BIOACCUMULATION AND TERATOGENIC MONITORING IN THE ECOLOGICAL FOOD CHAIN.

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Environmental contamination of organophosphates in the aquatic environments has reached epic portions since the late 1980s. Beginning in 1989 in the southern states of America, an unusually high record of organophosphate contamination was reported in the Mississippi River and surrounding areas. One OP in particular that was noticed in extremely high concentration levels was Diazinon. This OP is used as a basic insecticide/herbicide for controlling unwanted mosquitoes, weeds, and other annoying organisms and plants. However, recent biochemical evidence has shown that Diazinon has a strong tendency to induce high contamination levels in various aquatic animals, particular the members of *Ictalurus punctatus*-(known commonly as the Channel catfish). In 1997 it was reported that extremely high levels of this pesticide was found to exist in these species for a long period of time. In our study it was shown that Diazinon has a strong tendency to bioaccumulate in the tissues and muscle cells of these fish and thus induce teratogenic changes in fish morphology and structural development. It was also shown that exposure periods greater than 96-hrs tend to produce random movement behavior in these animals as well as increased dietary in-takes, thus inducing a severe increase in weight. It was also shown through CG/MS analysis that the lower fillet regions of these aquatic animals tended to have an extremely high concentration level of Diazinon before cooking (>45 ppms) and an even higher level after cooking ($90 \geq 101 \geq 117$ ppms) either through frying or boiling or stem cooking. Moreover, our study clearly shows that in cases where the pregnant female members of these aquatic animals were exposed to this contaminant for a period of six months or greater, the offsprings produced appeared to have four sets of fins, three eyes, or no development of fins or eyes as far as teeratogenic effects are revealed. In terms of the bioecological food-chain, male humans ingesting these contaminated fish over a three to twelve month period on a course of at least twice a day-(lunch and dinner) were known to develop extremely low sperm counts nd to have developed problems with nephritic stones appearing in the kidneys. In human females (18 to 51 yrs old) who absorbed an exceptionally high dietary in-take of these animals for a monthly period, exhibited severly painful urination problems as well as kidney infections.

1240 PHASE I AND II ENZYME AND ACTIVITY LEVELS IN THE GUMBOOT CHITON *CRYPTOCHITON STELLERI* FOLLOWING EXPOSURE TO A DIETARY BROMO-PHENOL, LANOSOL.

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The effects of diet and other non-anthropogenic effects on biochemical defenses and their relationship to susceptibility have been largely ignored in wildlife populations. Lanosol (1,2-dihydroxy, 3,4-dibromo-5-(hydroxymethyl)-benzene), found in the alga *Odonthalia dentata*, was administered to gumboot chitons (*Cryptochiton* stelleri) by oral gavage at 0, 1, 2.5, 5, or 10 mg/kg. *C. stelleri* feed on *O. dentata*, but it is avoided by other herbivores. After four days digestive gland microsomes were probed for expression of homologous isoforms of CYP1A, CYP3A, and CYP2 using a variety of anti-peptide, anti-trout (provided by D. Buhler) and commercial antibodies. No expression of CYP1A or CYP2-like proteins was observed. Expression of a CYP3A-like protein was increased by approximately 30% over control at 2.5, 5, and 10 mg/kg doses. Microsomal ethoxy-coumarin-*O*-deethylase activity was highly variable and did not change with dose. Estradiol hydroxylase activity showed an increasing trend with dose, while testosterone hydroxylase activity showed a decreasing trend with increasing dose. Antibodies raised against three mammalian glutathione-S-transferase (GST) families, α, μ, and π, (provided by E Gallagher) did not recognize any cytosolic protein from chitons. GST activity (CDNB conjugation) showed a decreasing trend with increasing lanosol dose. These results show that exposure to the brominated-phenol, lanosol, may alter expression and activity of some phase I and II biotransformation enzymes in chitons which may provide a selective dietary advantage for this species.

1241 TOXICITY STUDIES WITH POMACEA PALLUDOSA (APPLE SNAIL) AND CATHARANTHUS ROSEUS AXENIC PLANT ROOTS CONTAINING TRINITROTOLUENE.

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Trinitrotoluene (TNT) reduced (remediated) by the axenic plant roots of Catharanthus roseus and its potential toxicity to Pomacea palludosa (Apple snail), an aquatic mollusk indigenous to the Florida marshes, was studied extensively. These mollusks though they consumed quite readily the uncontaminated C. roseus axenic roots, they showed a definite aversion to the roots that had been grown in media containing various concentrations of TNT, ranging from 10-80 ppm. After the Apple snails were force fed these contaminated roots with TNT, they all succumbed within 48 hours to concentrations greater than 40 ppm. The smaller snails succumbed within 24 hours after ingesting these roots, while the larger (older) snails succumbed within 48 hours. Upon necropsy they all revealed an enlarged intestine with obvious large gas cysts. The livers of most mollusks also had necrotic lesions. Upon Gas Chromatographic chemical analyses of the contaminated axenic C. roseus roots we were able to clearly demonstrate the presence of 2-amino-4, 6-dinitrotoluene and 4-amino-2, 6-dinitrotoluene. The intestinal contents of the deceased snails similarly demonstrated the presence of the metabolites of reduced TNT, including a large concentration of phenols. We therefore have clearly demonstrated that though the axenic roots of Catharanthus roseus can absorb TNT after reducing it to monoaminated-dinitrotoluene, these metabolites are toxic to the Pomacea palludosa aquatic mollusk with a LD50 calculated value of 25 ppm.

1242 TOXICITY STUDIES WITH POMACEA PALLUDOSA (APPLE SNAIL) AND MYRIOPHYLUM AQUATICUM CONTAINING TRINITROTOLUENE.

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Trinitrotoluene (TNT) reduced (remediated) by Myriophyllum aquaticum and its potential toxicity to Pomacea palludosa (Apple snail), an aquatic mollusk indigenous to the Florida marshes, was studied extensively. These mollusks consumed quite readily the uncontaminated M. aquaticum plant, and they showed no aversion to the plants that had been grown in media containing various concentrations of TNT, ranging from 10-50 ppm. After the Apple snails were allowed to forage on plants containing 40 ppmTNT , they did not show any toxic effects in the period of 90 days. When compairing the acute toxic effects observed with the same animal model and the TNT containing axenic roots of Catharanthus roseus to the absence of any acute or chronic toxicity with TNT contaminated M. aquaticum plants, the great potential of this latter aquatic plant system for the removal and remediation of TNT from the water, is self evident. Liquid Chromatography coupled to electronspray negative ion atmospheric pressure ionization revealed monoaminated dinitrotoluene complexed with disaccharide and trisaccharide molecules. The necropsied mollusks revealed no significant pathologic findings, though the TNT was easily traceable in their intestinal contents. It should be noted that no TNT or any metabolite were detected in any of the other tissues of the apple snails.

1243 BIOENERGETIC EFFECTS OF LOW-DOSE CYANIDE ON HOMING PIGEONS (*COLUMBA LIVIA*):A MODEL FOR MIGRATORY BIRD STUDIES.

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Modern gold mining techniques generate large quantities of cyanide-containing waste solutions which are released into the environment to form large ponds, some greater than 300 acres in size. These ponds are often used as watering sites by migratory birds and pose a potential danger to these birds. Our laboratory has shown that mallard ducks exposed to cyanide levels comparable to those found at many of these sites, and considered safe, had significantly reduced tissue ATP levels and significant biological damage. To assess how this non-acutely lethal biological damage might affect migratory birds at a population level, we treated homing pigeons with cyanide in order to determine the cyanide dosages resulting in comparable loss of tissue ATP in pigeons as observed in the mallard ducks. Tissue ATP levels were

determined by the luciferase-luciferin bioluminescence assay at 25° C. Using these homing pigeons and the previously determined cyanide dosages, we tested the impact of cyanide exposure on flight time. Pigeons were taken into the wild, given one of two dosages of sodium cyanide, and released. Individual bird return times were recorded by scanning their leg bands as they returned to the loft. The data show that flight times increase with increasing cyanide dosages, and with flight distance. These results suggest that this may be good model for assessing migratory bird exposure and toxicity to environmental pollutants. (These studies were funded by NIEHS grant 1P42ESO5951 and by USDA Hatch Grant NEV0720.)

1244 PHYTOTOXIC EFFECTS OF SHORT-TERM EXPOSURE TO RDX IN WILD/COVER PLANTS.

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Plants comprise most of the biomass in the biosphere and those growing on sites with RDS (hexahydro-1,3,5-trinitro-1,3,5-triazine) contaminated soil could be exposed to varying RDX concentrations. Limited information is available on the response(s) of terrestrial vascular plants to RDX exposure. Research conducted to date on the fate and effects of RDX in terrestrial vascular plants has focused on food crop plants. Short-term (5-10 days) phytotoxicity experiments were conducted using ten wild/cover plants to evaluate the effects of RDX exposure on early life stage growth and development (seed germination/seedling emergence, root and shoot length and morphological abnormalities). The experiments were conducted in two large walk-in growth chambers using a completely randomized experimental design. Chamber temperatures were either 23 or 26 +/- 2.0° C and the photo period was 14:10 (L:D). Treatments and controls (unamended or solvent) were replicated three times (ten seeds per replicate). Four grasses (rye, fescue, orchard grass, Kentucky blue grass), five legumes (red clover, white clover, lespedza, sanfroin, small burnett), and one forb (sunflower) were planted in a Grenada soil amended with graded concentrations (0 to 4000 ppm) of RDX (military grade). Plants were watered but not fertilized during the experiments. The Statistical Analysis System was used to perform statistical analyses. Phytotoxic effects were observed more frequently in dicots than in monocots. Sunflower plants were the most sensitive to RDX. Sunflower plants had significantly longer shoots (5.8 - 300ppm or shorter roots (500-4000 ppm) than control plants. Other phytotoxic effects observed included chlorosis, necrotic spots, malformed leaves or roots, and inappropriate pigmentation. Overall, phytotoxic effects were not as prevalent in most of the grass species.

1245 EFFECT OF MERCURY BODY BURDEN ON GENERAL AND REPRODUCITVE HEALTH OF LARGEMOUTH BASS (MICROPTERUS SALMOIDES) FROM THREE LAKES IN NEW JERSEY.

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The influence of mercury body burden, a ubiquitous metal in aquatic ecosytems, on the general and reproductive health of wild fish populations has not been well studied. Threfore, we determined serum androgen levels, body weight, length, condition factor, gonadosomatic and liversomatic indices, serum cortisol levels following stress, and intrarenal nuclear diameter in male largemouth bass (Micropterus salmoides) collected in April 1997 from three bodies of water in New Jersey: Assunpink Lake (containing low levels of mercury), Manasquan Reservoir (containing moderate levels of mercury), and Atlantic City Reservoir (containing high levels of mercury). The mean mercury content in fish from Assunpink Lake was 0.30 µg-Hg/g-bw, from Manasquan Reservoir 1.23 µg-Hg/g-bw, and from Atlantic City Reservoir 5.42 µg-Hg/g-bw. Inter-lake and intra-lake analyses indicated that mercury tissue concentrations were positively associated with 11-ketotestosterone serum concentrations and negatively associated with testosterone serum concentrations. While this relationship was statistically significant, a cause-effect relationship could not be established because other contaminants were not measured in the fish. Body weight, length, condition factor, and gonadosomatic and liversomatic indices were similar for all three lakes. In contrast to previous findings by others, mercury did not grossly impair adrenocortical function, indicated by intrarenal nuclear diameter and serum cortisol levels following stress. The findings of this study suggest that while elevated levels of mercury in fish potentially alter androgen profiles, they do not decrease other indicators of general and reproductive health.

1246 GENE EXPRESSION AS A POTENTIAL BIOMARKER OF INORGANIC MERCURY EXPOSURE IN TERRESTRIAL ECOSYSTEMS.

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Biomarkers have shown tremendous benefit in human health risk assessment but their potential has not yet been fully evaluated in ecological settings. Studies with aquatic ecosystems suggest biomarkers can provide benefits in ecological evaluations as well, but few studies have examined terrestrial systems. This research is part of ongoing efforts to investigate the utility of molecular biomarkers in terrestrial ecotoxicology. The present study examines potential changes in gene expression of several stress response related genes (hsp70, metallothionein [MT], p21) in *Tenebrio molitor* beetles in response to inorganic mercury ($HgCl_2$) exposure. The study design was based on conditions found at Hanford Nuclear Reservations in order to allow laboratory developed biomarkers to be validated in the field, where conditions are more variable. Beetles were exposed to four different levels of $HgCl_2$ (0, 0.5, 5.0, and 50 ppm in "soil") for 12 days. RNA was extracted from each insect and analyzed by a Reverse Northern technique. No dose response patterns in gene expression were noted. Northerns were performed to validate the Reverse Northern findings, but no specific binding was noted, suggesting non-specific binding in the Reverse Northerns. In order to test a more specific probe, oligonucleotides were generated from high species homology regions of hsp70, MT, and p21 and Northern analyses were performed. In addition, a Northern was performed using a desiccation stress gene (DSP28) cloned from *Tenebrio molitor* as the probe. Results showed a dose response pattern with $HgCl_2$ exposure and expression of both DSP28 and the p21 oligonucleotide. Further experiments are underway to investigate alternative hsp70 and MT homology regions. The development of gene expression biomarkers could give subclinical and sensitive data at the both the species and ecosystem level. (Supported by USDOE DE-FC01-95EW55084 and NIEHS T32ESO-7032.)

1247 EFFECTS OF INORGANIC MERCURY AND ARSENIC CONTAMINATION ON *POECILIA RETICULATA* : HEALTH EFFECTS IN THE BIOECOLOGICAL FOOD-CHAIN.

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Bioaccumulation of inorganic mercury and arsenic in the basin and rivers along the Mississippi River stations between Baton Rouge and New Orleans is a serious environmental issue. For many years the Mississippi River was viewed as a large dumping bed for pesticides, metals, runoff debris and chemicals that were washed down from the northern and mid-sections of the United States. In 1996 a toxicological study revealed that a large concentration of inorganic mercury and arsenic was found at six industrial sampling stations in the Mississippi River between Baton Rouge and New Orleans. The concentration levels of mercury and arsenic found was reported as 0.67 ppbs (Hg) and .92 ppms (As). In our study we showed the effects that these high concentration levels of contaminants had on a species of aquatic animals known as *Poecilia retilculata*, commonly known as feeder fish. Inductively Coupled Plasma-Mass Spectrometer-(ICP/MS) analysis revealed that these aquatic animals had extremely high concentrations of mercury in their fillet tissues, stomach tissue, and liver regions. Additionally, it was revealed through ICP/MS analysis of the whole fish body over a twelve month period that a arsenic accumulation in the lower and upper fillet regions were extremely high-(1.00 ppms per fish examined). Also, the fish exposed to mercury developed severely boggling eyes in which the pupils had the appearance of white dot indicating a high concentration of mercury. Aquatic chemical analysis revealed that these animals have the ability to convert inorganic mercury into methyl mercury as to make absorption through the body cells and tissues easier. Analysis of *Macropterus salmoides*-(Smallmouth Bass)who consumed these contaminated fish revealed that these animals experienced a high concentration of mercury in their fillet and liver tissues.

1248 EFFECTS OF HIGH-LEVELS OF INORGANIC MERCURY ON *PROCAMBARUS CLARKII:* ECOLOGICAL AND HEALTH IMPLICATIONS FOR HUMAN CONSUMPTION.

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Recently high concentrations of mercury have been found in the area of Honey-Island Swamp, an are in the heart of Bay St. Louis, Mississippi. This area is of great economic importance to the crawfish industry in this region. Red crawfish beds are located throughout the Honey-Island Swamp. The effects of low to relatively high concentration levels of inorganic mercury on these aquatic animals has been astonishing. Through a series of biochemical reactions involving a process known as respiration, these and many other aquatic animals are able to convert inorganic mercury to methyl mercury as to make absorbtioninto the body more accessible.In our two year research study it was show that both male and female members of *Procambarus clarkii* have a string tendancy to absorb high concentrations of inorganic mercury and convert these inorganic levels to methyl mercury. It was also shown in our study through the use of ICP/MS-(Inductively Couples Plasma / Mass Spectrometer) that once absorbed into the bodies of these aquatic animals, the methyl mercury is then stored in the hepatic, tail, and fatty abdominal regions as absorbtion through these regions takes place. Severe abnormalities in the eye sizes of these aquatic animals as well as changes in their dietary in-takes were very noticable during this study. our study also showed that when females-(ages 18 through 45) ingest these contaminated animals over a six month to one year period on a semi-daily basis began experiencing trouble with their monthly menstrual cycles as well as problems with mild to excessive bleeding when their cycles commenced. For women who were past the age of 45 (>45), they experience excessive periods of sweating as well heat flashes on regular intervals after their dietary in-take was completed. These women also experience a marked increase in high-blood pressure as well as irritability from even the most insignificant of events. Additionally, it was shown that in the case of males (ages 17 through 51) ingesting large dosages of these contaminated animals on a normal dietary in-take for one-year they experienced severe headaches as well as urination problems. In some extreme cases where the male subjects ingested more than three pounds of these aquatic animals on a daily basis, they experience severe problems with vision and blurriness.

1249 HYPOXIA-LIKE EFFECTS OF NICKEL IN HUMAN CELLS.

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Environmental or occupational exposure to nickel compounds represents a serious danger to human health. In searching for intracellular physiological pathways affected by nickel compounds we have cloned a new human gene Cap43, that was highly inducible by soluble and insoluble nickel compounds in human bronchoalveolar cells. The same gene was found to be highly induced by homocysteine in human endothelial cells. Further studies showed that nickel, but not homocysteine was able to universally induce expression of the mRNA for this gene in cells of different tissue origin. Here we report that the expression of Cap43 is greatly enhanced under hypoxic conditions with a time course similar to that observed following nickel exposure. In addition nickel induced the expression of another gene, glyceraldehyde 3-phosphate dehydrogenase, which was also induced by hypoxia. We conclude that nickel exposure causes a hypoxia-like stress in human cells. The molecular mechanisms involved in these effects of nickel are being addressed.

1250 A COMPARISON OF THE MOLECULAR CYTOTOXIC MECHANISMS OF COPPER AND CADMIUM.

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The molecular cytotoxic mechanisms of hepatocyte cell lysis induced by $CuCl_2$ and $CdCl_2$ have been compared. "Reactive oxygen species" formation (dichlorofluorescin oxidation) increased much more rapidly with Cu than Cd likely because of redox cycling properties of Cu. Furthermore Cu but not Cd also rapidly oxidized hepatocyte GSH. A loss of mitochondrial membrane potential was an early event in the cytotoxic mechanism of both metals which along with cell lysis was prevented by SOD, catalase, desferal, dimethyl sulfoxide and antioxidants. This indicates that "reactive oxygen species" formation and lipid peroxidation were involved in the loss of mitochondrial membrane potential and cytotoxicity. Cu cytotoxicity was readily prevented by the endocytosis inhibitors methylamine, monensin, chloroquine and 3-methyladenine which suggests that cytotoxic Cu concentrations are stored in lysosomes and/or that ROS formed by the Cu caused a lysosomal leakage of digestive enzymes. Cd cytotoxicity was not affected by endocytosis inhibitors. Both metals induced apoptotic death signals as cell lysis was prevented by the cycloheximide (a protein synthesis inhibitor), actinomycin D or a tetrapeptide caspase protease inhibitor. This signalling may arise from a mitochondrial permeability transition (MPT) as cytotoxicity was prevented by the MPT inhibitors cyclosporin A, carnitine or trifluoperazine. In short, our results suggest that Cu and Cd induced cell lysis or necrosis shares common death signals initiated by mitochondrial or lysosomal injury with apoptosis but lacks the highly organized sequence of events that results in cell packaging required for apoptosis morphology.

1251 CADMIUM TOLERANCE IN PARASITE-SUSCEPTIBLE AND-RESISTANT STRAINS OF *BIOMPHALARIA GLABRATA*, AN INTERMEDIATE HOST FOR *SCHISTOSOMA MANSONI.*

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Biomphalaria glabrata is a tropical, freshwater snail that can act as an intermediate host for the trematode parasite that causes schistosomiasis. Different genetic strains of *B. glabrata* display both parasite susceptibility and resistance traits. Here, the relative cadmium tolerances in different life-history stages of parasite-susceptible NMRI and -resistant BS90 strains were examined in this species. Eggs, juveniles and adults were exposed to control and cadmium concentrations of 0.05 to 2.0 micromolar. Survival analysis showed that increasing cadmium concentration caused significant decreases in time-to-death in the three stages. Time-to-death was significantly shorter in all three stages of the parasite-resistant BS90 in comparison with the parasite-susceptible NMRI, indicating higher cadmium tolerance in the latter. To determine the relative sensitivity of the egg, juvenile, and adult stages, time-to-death was compared at 0.25 micromolar cadmium, which was common to all three. Stage significantly effected time-to-death, with the order of cadmium resistance as adult>juvenile>eggs. BS90 has a longer time-to-hatch than NMRI under normal conditions. Time-to-hatch in cadmium exposed eggs was increased in both strains but the effect was greater in BS90 as reflected in the significance of the interaction term (strain x cadmium). Percent hatch significantly decreased with increasing cadmium; BS90 was again more sensitive than NMRI. These results showed that cadmium tolerance was significantly effected by strain and life-history stage. The parasite-resistant strain was less cadmium tolerant than the parasite-susceptible strain. Use of this species for pollutant monitoring as proposed by others will need to consider this source of variability. Furthermore, the interaction of metal resistance and parasite susceptibility may have implications for human health since cadmium exposure favors the survival of the parasite-susceptible strain.

1252 PREVENTION OF MICROBIAL PROLIFERATION ON HEPA FILTRATION MATERIAL.

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Microbial growth in air handling systems can lead to exposure to bacteria, fungi, and viruses. Moisture retention gives rise to conditions favorable for microbial proliferation. Prevention of microbial growth would be beneficial. In prior studies adsorption of an antimicrobial to filters resulted in weak attachment of the agent to the filter surface. This attachment can be strengthened by first coating the HEPA (High Efficiency Particulate Air) filtration media with a colloid that binds tightly to the borosilicate glass fiber surface, followed by the binding of an antimicrobial agent to the colloid via cation exchange reactions. The ability of smectite clay to swell and form a gel-like colloid suspension in H2O allows for easy application to surfaces by a dipping process. Direct observation by scanning electronmicroscopy has shown that the clay does not obstruct the filter pores. Smectite clays have a net negative layer charge balanced by cations such as Na+ , Ca2+ , and Mg2+. Cation exchange reactions are used to bind the quaternary ammonium

antimicrobial compound, hexadecyltrimethyl ammonium (HDTMA), to the colloid on the filter surface. The colloid bound HDTMA had the same antimicrobial activity as the native HDTMA against *P. aeruginosa* in the Standard Plate Count assay. The antimicrobial activity of the treated HEPA filtration material was assessed using AATCC Method 100-1993 with *P. aeruginosa* as the test organism, and with an ATP luminescence assay. Both assays have confirmed the antimicrobial activity of the treated HEPA filters. Leachability of the HDTMA is prevented by the colloid pretreatment of the HEPA filter. This procedure can be utilized to impart antimicrobial activity to any surface that can be coated with clay or any other colloid possessing a significant cation exchange capacity. (This work was supported in part by grants from the Clay Minerals Society, the Western New York Safety Conference, and the University at Buffalo.)

1253 TOXICITY OF CHLOROACETIC ACID (MCA) IN RATS UNDER CONDITIONS OF TOXICOKINETIC STEADY-STATE APPEARS TO OCCUR ACCORDING TO HABER'S LAW OF INHALATION TOXICOLOGY.

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The rate of release of MCA was determined from surgically implanted mini-pumps. Three rats were implanted with ^{14}C-MCA filled mini-pumps. A 16-hr delay in the beginning of delivery of MCA was created by adding a polyethylene catheter (I.D., 0.76 mm; O.D., 1.22 mm) to the pump, which was filled with saline. The two liquids were separated by a small air bubble. Blood samples were collected from the tail vein at 0.25, 0.5, 1, 2, 4, and 8 hrs following the activation of the delivery of MCA from the mini-pumps. Radioactivity was determined in plasma. Results indicated constant release of MCA from the pumps. Subsequently a study was conducted to determine the time-to-effect of MCA in adult male Sprague-Dawley rats (160-175 g). Rats (5 animals per dose group) were injected with highly toxic loading doses (75, 100 and 125 mg/kg) of MCA followed by a maintenance dose delivered via the osmotic mini-pumps which were surgically implanted subcutaneously. After 16 hours of recovery from surgery, loading doses were administered into the tail vein. The beginning of delivery of MCA from the osmotic pumps was synchronized with the administration of the loading doses. Following the delivery of the loading doses, animals were closely monitored to determine the exact time-to-effect (coma, death). Preliminary results indicated that *dose x time = constant* is also valid for this highly water-soluble small molecule under conditions of toxicokinetic steady-state.

1254 METHYL-t-BUTYL ETHER (MTBE) EFFECTS ON PLASMA LUTEINIZING HORMONE (LH) IN GONADECTOMIZED MALE RATS.

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MTBE, a widely used automotive fuel oxygenate, increased Leydig cell adenomas in male rats chronically exposed to high doses by inhalation (8000 ppm) or gavage (1000 mg/kg) but has had little or no activity in most genotoxicity tests conducted thus far. Several other recognized mechanisms of rodent Leydig cell chemical carcinogenesis ultimately involve chronic hyperstimulation of these testosterone-producing cells in the testes by LH from the anterior pituitary gland (Clegg et al., 1997). Like other ethers, MTBE causes CNS depression, and specific effects on the pituitary gland (weight changes, ACTH droplet accumulation in female mice) have also been reported with chronic high MTBE exposures. We report here results of studies on direct effects of MTBE on the hypothalamic-pituitary unit with respect to LH production and release using orchiectomized Sprague-Dawley rats with/without testosterone levels maintained by silastic implants containing testosterone propionate. No consistent or statistically significant differences between MTBE-exposed and vehicle control groups were found in mean plasma LH as measured by rat-specific RIAs 2-5 hr after and again 5 days after daily high MTBE gavage doses (800 mg/kg) sufficient to cause ataxia and sedation. We have previously observed reduced plasma testosterone levels in month-long MTBE studies using this dose administered to intact rats. These new findings do not support a mechanism involving a primary effect of MTBE on the hypothalamus and/or pituitary causing alteration of LH levels directly, although a more prolonged MTBE exposure period and more frequent LH sampling may be necessary to detect subtle cumulative effects.

1255 ACUTE TESTICULAR TOXICITY OF MTBE AND BREAKDOWN PRODUCTS IN LAB MICE.

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Concern for MTBE in drinking water prompted studies of its toxicity by University of California scientists. Our laboratory studied acute oral MTBE exposure and testicular function of male mice using a fecal testosterone ELISA biomarker from fecal ether-extracts. Testosterone baseline and human chorionic gonadotropin (hCG) stimulated levels were determined before, during and after exposure. Other parameters included animal and testis weights, end point serum testosterone, and histopathology of the testis by light microscopic examination of formalin fixed tissue sections. Groups of 5 mice were orally dosed with 0, 400, 1000 or 2000 mg/kg of MTBE in canola oil three times over a 5 day period. Three mice were dosed with cadmium (Cd), a known testicular toxicant, as a positive control. A final fecal testosterone sample was taken on day 7, blood sampled, and the testes removed. There was no change in unstimulated and hCG stimulated fecal testosterone at any treatment levels, nor were there any differences in end-point serum testosterone levels, body and testis weights between untreated and MTBE dosed animals. The only histological difference between control and 2000 mg/kg exposed animals was a small increase in gross disruption of tubules in the 2000 mg/kg group (6 ± 3%, mean ± SD, $p \leq 0.05$). Cd controls exhibited decreased testis weight, fecal and serum testosterone, and histopathological damage ($p \leq 0.05$). In addition to MTBE, we studied two UV/peroxidase breakdown products, tert-butyl alcohol and tert-butyl formate, in collaboration with Dr Thomas Young, UCD. Animals were run through a similar battery of tests as MTBE with little difference found between treated and untreated animals. These studies revealed no dramatic effects of high dose levels of MTBE or its major breakdown products on male hormone levels or testicular histology in mice. Whether chronic dosing would have elicited a response remains to be tested. (Supported by University of California Toxic Substances Research and Teaching Program; special funding by the State of California (SB521).)

1256 ALTERATIONS IN ENDOCRINE ACTIVITY IN MALE SPRAGUE-DAWLEY RATS FOLLOWING ORAL ADMINISTRATION OF METHYL *t*-BUTYL ETHER.

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Methyl *t*-butyl ether (MTBE) is an oxygenated fuel additive used to decrease carbon monoxide emissions during combustion. Both occupational and environmental exposures to MTBE are substantial due to its large production volume and widespread use. MTBE is a nongenotoxic carcinogen that induces Leydig cell tumors (LCT) in male rats. The mechanism of MTBE-induced LCTs is not known, however LCTs induced by other nongenotoxic chemicals has been associated with a disruption in the hypothalamus-pituitary-testicular (HPT) axis. The objective of this study was to determine if MTBE alters HPT feedback mechanisms in male rats. Nine week old male Sprague-Dawley rats were administered MTBE by gavage at 0, 250, 500, 1000, or 1500 mg/kg/day for 15 or 28 consecutive days and sacrificed one hour following the last dose. Treatment with MTBE for 15 or 28 days did not effect body weight, however high-dose (1500 mg/kg/day) animals exhibited clinical signs of toxicity throughout the study. Relative testis, adrenal gland, liver, and kidney weights were increased in the high-dose animals. No testicular lesions were observed at any dose level. Interstitial fluid testosterone was decreased at 1500 mg/kg/day in the group treated for 15 but not 28 days. Serum triiodothyronine was significantly decreased at 1000 and 1500 mg/kg/day and a dose-related decrease in serum luteinizing hormone was observed at 28 days. These results suggest that alterations in endocrine feedback signals due to MTBE are only apparent at high-dose levels.

1257 USE OF URINE AND PLASMA GH ASSAYS TO ASSESS THE GH SECRETION PATTERN IN MONKEYS AFTER DOSING WITH BIM 28041, A NEW PEPTIDIC hGRF RECEPTOR AGONIST.

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Plasma and urinary Growth hormone (GH) levels were monitored in Cynomolgus monkeys treated for seven days with BIM 28041 [(hexenoyl trans 3)0 hGRF (1-29) NH2] in order to evaluate the efficacy and the safety

of this new peptidic human Growth Releasing Factor (hGRF) receptor agonist. Three groups of three monkeys each were treated with the vehicle or with the BIM 28041 either by daily subcutaneous injections or by continuous intravenous perfusion. GH was quantified by a radio-immunoassay in blood samples collected on days 1 and 7 at designated times and in urine samples collected from day 7 to day 8 during the last exposition period. In treated animals, GH plasma levels reached the high control levels corresponding to peak secretion values during all the exposure period. The urinary levels of GH indicated that the pituitary was only transiently stimulated after subcutaneous administration of the test compound, and that the continuous intravenous perfusion leaded to a continuous stimulation of the pituitary gland. No desensitization of the receptors nor exhaustion of the cells were observed through the administration period, and the amount of GH instantaneously released during the bursts of secretion was not increased above the physiological levels.

1258 PERSISTENT INCREASE IN ADRENAL OUTPUT WITHOUT CONCURRENT GONADAL ALTERATIONS IN MALE RATS THREE DAYS FOLLOWING ACUTE D,L-FENFLURAMINE EXPOSURE.

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We have demonstrated that acute d,l-fenfluramine (FEN) exposure has neurotoxic effects as evidenced by increased glial fibrillary acidic protein under hyperthermic conditions three days post-treatment. Previous research has shown that serotonergic agonists such as FEN can activate the hypothalamic-pituitary-adrenal (HPA) axis. In this study we examined the effect of FEN on HPA basal activity and gonadal output. Adult male Sprague-Dawley rats were administered 15 mg/kg FEN dissolved in saline every two hours for a total of four s.c. injections at either an ambient temperature of 21° C or 32° C. Control animals received saline at an ambient temperature of 32° C. Rectal temperatures were monitored on the day of dosing. As expected, the animals given FEN at 32 ° C exhibited hyperthermia as compared to the controls. Three days following the exposure period, trunk blood was collected immediately upon removal from the home cage. Corticosterone, aldosterone, and testosterone in plasma were analyzed in duplicate by RIA. Regardless of ambient temperature exposure, both FEN conditions had elevated corticosterone relative to controls. Aldosterone titers were only greater for the animals administered FEN at the higher ambient temperature. No effect was observed on the testosterone levels at this time period. These data suggest that the HPA axis shows increased responsivity following acute FEN exposure. Whether this increased activity is from a peripheral activation of the adrenal gland or a central elevation in hypothalamic or pituitary hormones remains to be determined. (Supported by NIH grants ES07051 and DA06733.)

1259 EFFECTS OF SHORT-TERM IN VIVO EXPOSURES TO POLYBROMINATED DIPHENYL ETHERS ON THYROID HORMONES AND HEPATIC ENZYME ACTIVITIES IN WEANLING RATS.

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Polybrominated diphenyl ethers (PBDEs), used as flame retardants, are ubiquitous environmental contaminants. Sharing similar structures as thyroid hormones, PBDEs act as endocrine disruptors via alterations in thyroid hormone homeostasis. We examined thyroid hormone concentrations and hepatic enzyme activity in weanling rats exposed to two commercial PBDE-mixtures, DE-71 (mostly tetra- and penta-bromodiphenyl ethers) and DE-79 (mostly octabromodiphenyl ether). Postnatal rats (PND 28) were orally administered various doses of DE-71 (0.3, 1, 3, 10, 30, 100 and 300 mg/kg/day) or DE-79 (0.3, 1, 3, 10, 30, 60 and 100 mg/kg/day) for four days. The rats were sacrificed on the 5th day, and serum and liver samples were analyzed for circulating thyroxine (T4), triiodothyronine (T3), or liver microsome ethoxy- and pentoxy-resorufin-O-deethylase (EROD and PROD) activities. Though the DE-71 treated groups did not exhibit significant changes in body weight, enlargement in liver as well as up to 20-30 folds induction in EROD (ED50= 11 mg/kg/day) and PROD (ED50= 8 mg/kg/day) were found . In comparison to controls, serum T4 levels in the DE-71 or DE-79 treated groups were dramatically reduced in a dose-dependent manner. Serum total T4 was decreased maximally about 80% in the highest dose, with an ED50 of approximately 21 mg/kg/day for DE-71 and DE-79. However, dose-related effects in serum T3 levels were less apparent; maximal reduction was 25% at the highest dose. There were no significant alterations in serum TSH levels for the DE-71 treated animals. TSH concentrations in animals treated with DE-79 were not examined. The present study demonstrates that commercial PBDE-mixtures interfere with the thyroid hormone system via Ah- and non-Ah-receptor activation. Non-PBDE impurities in the mixtures may be responsible for the EROD induction.

1260 THE RELATIVE CONTRIBUTIONS OF SYNTHESIS AND INACTIVATION TO THE LOWERING OF SERUM TESTOSTERONE LEVELS BY KETOCONAZOLE.

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At sufficiently high doses, the fungicide ketoconazole can significantly lower serum testosterone levels. This effect is generally attributed to the ability of ketoconazole to inhibit gonadal enzymes involved in testosterone synthesis. In an effort to identify potentially contributing mechanisms to this toxicity, the effects of the fungicide on adrenal and gonadal secretion and hepatic metabolism of androgen was evaluated. These studies were also used to substantiate our recent observation that the ratio of hepatic testosterone 6α:15α hydroxylase activities provides a superior indicator of the androgen status of CD-1 mice. Mice were orally administered dosages of ketoconazole (0 to 80 mg/kg) every 24 hours for 7 days. Four hours after the initial treatment, serum testosterone levels decreased and the hepatic 6α:15α hydroxylase ratio increased in a dose- dependent manner. This effect was accompanied by an overall decrease in the gonadal secretion and the hepatic biotransformation of testosterone. Twenty-three hours after the initial dosing, serum testosterone levels, gonadal testosterone secretion, and hepatic testosterone biotransformation had recovered. These parameters were also comparable to controls 7 days after treatment; although, adrenal secretion of androstenedione was suppressed in a dose-dependent manner. These results demonstrate the following. 1) The effects of ketoconazole on serum testosterone levels are transient. 2) Reductions in serum testosterone levels are due to decrease gonadal secretion of testosterone and not increased hepatic biotransformation. 3) The reduced secretion of androstenedione by the adrenal glands following ketoconazole treatment alone is not sufficient to decrease testosterone levels. 4) The hepatic 6α:15α hydroxylase ratio can serve as a biomarker of androgen deprivation even after short-term testosterone suppression.

1261 INTERACTIVE EFFECTS OF DDE, DIELDRIN AND METHOXYCHLOR ON HORMONE SYNTHESIS IN BASS GONADAL CULTURES.

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A number of recent studies have reported that combinations of environmental contaminants exhibit greater than additive effects on endocrine-responsive tissues or biochemical systems (i.e. chemicals interact synergistically in producing effects). However, some of these studies have very low power to assess interactions because they only minimally characterized the dose-response curves of the individual agents or attempted to assess the effects of combinations of agents using only single dose ratios. We tested the hypothesis that p',p'-DDE, (DDE) dieldrin (DDL) and methoxychlor (MCL) interact to produce effects on hormone synthesis in gonadal tissue cultures harvested from female large mouth bass, a species whose reproductive fitness has relevance to ecosystem health in Florida. We characterized the concentration-response relationships of these agents on 11-ketotestosterone (T) and estradiol production over a 4-log concentration range. Combinations of the agents were tested at 0.01, 1, 100, and 10,000 ppb in the culture medium in a full-factorial, block treatment design. Gonadal tissue was harvested from female bass (2-3 years of age) during the peak reproductive season (February - April) to maximize steroidogenic activity and available gonadal mass, minced and incubated in culture medium with or without test agents for 48 hours. Duplicates of each treatment were performed in each of three experiments using gonadal tissue from a different female. Estradiol, testosterone, and 11-ketotestosterone were measured in aliquots of culture medium by validated RIA procedures. MCL alone produced a slight, dose-dependent decrease in testosterone. In the presence of increasing concentrations of DDL, MCL increased T production in a dose-dependent fashion. DDL alone had no effect on T production. Other main and interactive effects were not statistically significant.

1262 THE EFFECT OF BLEACHED KRAFT MILL EFFLUENT EXPOSURE ON THE METABOLIC ELIMINATION OF TESTOSTERONE AND THE PRODUCTION OF VITELLOGENIN IN THE FATHEAD MINNOW (*Pimephales promelas*).

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Recent studies have determined that certain bleached kraft mill effluents (BKME) reduce fecundity in fish but as yet, there is no known mechanism of action. In the present study, we evaluated whether estrogenicity, antiestrogenicity, or altered testosterone metabolic clearance associated with the BKME might contribute to this toxicity. Fathead minnows (*Pimephales promelas*) were exposed through one life-cycle to BKME. Immediately prior to spawning, a subset of these fish were evaluated for the metabolic clearance of testosterone. For the remaining fish, daily egg production was measured during 6 weeks of spawning and at the end of the study, blood plasma was assayed for the presence of vitellogenin (VTG). Egg production decreased significantly with increasing effluent concentration compared to control at 50 and 100% effluent with an IC25 calculated at 21% effluent. There was no significant decrease in VTG levels in females and no VTG was detected in males, indicating that this BKME is neither antiestrogenic or estrogenic, respectively. Testosterone was elimnated from the fathead minnow as a variety of hydroxylated and conjugated derivatives. The rate of elimination of six of these metabolites was significantly altered as compared to controls. Elimination rates of three of these metabolites significantly correlated with decreased egg production. These results demonstrate that the metabolic elimination of testosterone may contribute to the reproductive toxicity and may serve as a predictive biomarker of reproductive toxicity associated with BKME.

1263 INTERACTION OF POLYCYCLIC MUSKS WITH AMPHIBIAN AND FISH ESTROGEN RECEPTOR.

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Galaxolide (HHCB) (1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(g)-2-benzopyran), Tonalide (AHTN) (7-acetyl-1,1,3,4,4,6-hexamethyltertraline) and Celestolide (ADBI) (4-acetyl-1,1-dimethyl-6-tert butylindane) are synthetic polycyclic musk fragrances. As the substitutes for natural musks, these compounds are widely used in detergents, perfumes and cosmetics. As a result of their inherent lipophilicity (Kow: 5.7 for AHTN, 5.9 for HHCB) and environmental persistence, these compounds have been detected in surface waters (ng-µg/l) and various tissues of aquatic organisms (mg/kg lipid). In view of this situation, the questions were asked whether these compounds have embryotoxic and teratogenic activity and if they could interact with the endocrine system of amphibians and fish. To address these questions, embryo-larvae of *X. laevis (X.l.)* and zebrafish (*D. rerio, D.r.*) were exposed to AHTN (0.09-1 mg/l; 0.09-2 mg/l, respectively) and HHCB (0.09-1 mg/l; 0.07-4 mg/l, respectively) for 96 h, using established assay procedures (ASTM, E1439-91, 1991, p.1-11; Dietrich et al., 1998, Toxicol. Sci. (42-1S), p259). In addition, the capability of these compounds to inhibit ^{3}H- estradiol binding to hepatic estrogen receptors of *X.l.* and rainbow trout (*O. mykiss, O.m.*), was investigated. 100% mortality occurred at the highest testing concentration for AHTN and HHCB in both species. *D.r.* embryos demonstrated a generalized ventro-dorsal curvature and tail flexure in the distal portion following exposure to AHTN or HHCB (EC_{50} = 0.2-0.3 mg/l). However no malformation was observed for AHTN and HHCB in *X.l* Estrogen receptors were prepared from both species using previously published methodology, the K_Ds determined for *X.l.* and *O.m.* were 30 nM and 3 nM, respectively. While no competitive inhibition was observed for ADBI in either species, a 1 mM concentration of AHTN inhibited E2 binding to the E-receptor in *X.l.* and *O.m.* by 40% and 30%, respectively, and 1 mM HHCB inhibited E2 binding by 40% and 20%, respectively.

1264 P-*TERT*-OCTYLPHENOL AND/OR VINCLOZOLIN INDUCED-INTERSEX IN DEVELOPING MEDAKA (*ORYZIAS LATIPES*).

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Endocrine-disrupting compounds may alter reproductive development. To study the effects of two environmentally relevant compounds, five replicates of stage 10 medaka (*Oryzias latipes*) embryos (N= 5 per replicate) were bath-exposed to 0 µg/L, 10 µg/L, 100 µg/L, 250 µg/L, 500 µg/L, 750 µg/L and 1000 µg/L of either the estrogenic surfactant, p-*tert*-octylphenol (OP), or the antiandrogenic fungicide, vinclozolin (*V*), and reared at 25°C until one day after hatch. In addition, exposure to a 50/50 mixture of both OP (500 µg/L) and *V* (500 µg/L) was performed. Ten fish per exposure and corresponding control groups were sampled at one month after initiation of the experiment. A significant increase in time to hatch was associated with exposure to10 µg/L, 100 µg/L, and 250 µg/L of *V*. Only the 100 µg/L OP group shown delayed hatch. Gross examinations under dissecting microscope revealed no signs of acute or developmental toxicity. Microscopic examination revealed eosinophilic protein droplets in kidneys of males and some females exposed to OP and *V* alone or as a mixture. There was no significant difference in sex-ratio between exposure or control groups. The most significant finding of this study was the presence of intersex gonads in medaka exposed to OP and *V* at concentrations of 250 g/L and higher. Intersex gonads were also seen in fish exposed to the 50/50 mixture of octylphenol and vinclozolin. The presence of intersex in a stable, sexually dimorphic species suggested an estrogenic or antiandrogenic effect of OP and *V* such that the male germ cells have been sexually reversed to female ova in affected individuals. Results from the three-month-old sampling will be reported. This study constitutes the first report of laboratory-induced intersex in fish exposed to vinclozolin.

1265 CYANAZINE (CYN) AFFECTS NOREPINEPHINE (NE) RELEASE IN PHEOCHROMOCYTOMA (PC12) CELLS.

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We have previously found that the chlorotriazines i.e.Cyanazine, Simazine and Atrazine disrupt the ovulatory surge of luteinizing hormone in rats. Consistent with our hypothesis that this effect on pituitary hormone secretion is centrally mediated, we found that hypothalamic NE is decreased by these compounds. In the present study, we used PC12 cells to examine the possibility that the synthesis of NE is altered by a direct action of Cyanazine on dopamine-β hydroxylase (DBH). Cells were incubated in the presence of the CYN, the DBH inhibitor fusaric acid (FA), or the $GABA_A$ receptor agonist muscimol (MU). MU had no effect between 0.01 µM thru 10 µM, whereas FA blocked the release of NE at 10 µM to 250 µM following 6 h to 48 h exposure. NE release was significantly increased by 100 µM CYN, but NE concentration was either decreased or not different than respective controls at 400 & 800 µM. The increased NE release occurred from 12 h to 48 h of exposure with a maximum increase at 24 h. Intracellular DA was decreased by CYN in a dose-dependent fashion. These data suggest that CYN does alter the NE activity *in vitro* but the mechanism of action remains to be determined. (This abstract does not reflect U.S. EPA policy.)

1266 ENDOMETRIOSIS IN RHESUS MONKEYS IS ASSOCIATED WITH ALTERED LIVER CYTOCHROME P450 ENZYME ACTIVITY IN RELATION TO SERUM ESTRADIOL AND PROGESTERONE LEVELS, AND DIOXIN EXPOSURE.

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Endometriosis (EM) is a disease in which endometrial tissue is found outside of the uterus. Rier, et al. (FAT 21:433-441, 1993) showed a link between EM and exposure to dioxin (TCDD) (2,3,7,8-tetrachlorodibenzo-p-dioxin) in a surviving subgroup (17/24) of rhesus monkeys exposed to 0, 5, or 25 ppt TCDD/day in their feed from 1977-1982. In 1996, the 12 remaining monkeys were oophorectomized and cycled on hormones to mimic 2 menstrual cycles. They were sacrificed mid-way through the 2nd mid-secretory phase. Liver tissue, and serum estradiol (E_2), progesterone (P_4) levels were provided (Dr. Sherry Rier), and liver microsomes were used to measure CYP1A1 protein (western blotting), CYP1A1 activity (7-ethoxyresorufin-O-deethylase), and dimethylbenzanthracene (DMBA) metabolism (HPLC). Total DMBA metabolism increased with E_2 in monkeys without disease (-EM) (n=4) (R^2=0.94; p<0.03), but was unrelated to E_2 in monkeys with disease (+EM) (n=8). The trend was similar for P_4. The ratio of CYP1A1 activity: protein increased with P_4 (R^2=0.60; p<0.025) and P_4/E_2 (R^2=0.86; p=0.001) and decreased with E_2 in (+EM) monkeys, and was unrelated to P_4 or E_2 in (-EM) monkeys. EM alone resulted in decreased DMBA metabolism (p<0.0003) and E_2 (p<0.02). In (-) EM monkeys, TCDD exposure resulted

in decreased E_2 ($p<0.05$) and total DMBA metabolism ($p<0.01$), and in (+ EM) monkeys, exposure resulted in a decrease in E_2 with no effect on total DMBA metabolism. The results suggest that (-EM) and (+EM) monkeys show different profiles of cytochrome P450 activity in response to E_2, P_4 and TCDD (Sponsors: Fairleigh S. Dickinson, Jr. Fndn.; Endometriosis Assoc.; NIEHS #ES08539-01.)

1267 EFFECT OF DIETARILY ADMINISTERED ENDOCRINE ACTIVE AGENTS ON HEPATIC CYTOCHROME P450 (CYP450) AND ESTROGEN RECEPTOR ALPHA (ERα) EXPRESSION IN MALE AND FEMALE RATS.

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In addition to interacting with steroid receptors, endocrine active agents may act through other mechanisms. The objective of this study was to examine the effect of several endocrine active agents on hepatic CYP450 and ERα expression. Male and female rats were exposed to methoxychlor (MXC), genistein, or vinclozolin dietarily from gestational day 7 through postnatal day 50. Rats were sacrificed and liver microsomes and cytosolic fractions were prepared. CYP2C and CYP3A levels were determined by ELISA and Western blotting. Testosterone metabolism was also examined. Cytosolic ERα levels were determined by immunprecipitation followed by Western blotting. MXC decreased CYP2C expression in males at the highest dose (1000 ppm) with no effect on CYP3A. In male rats, genistein treatment resulted in an increase in CYP2C at 250 ppm and a decrease at 1250 ppm with no effect on CYP3A. No effects on CYP2C or CYP3A were observed in MXC or genistein treated female rats. Vinclozolin increased CYP3A expression in male rats with no effect on CYP2C. In female rats, vinclozolin increased CYP2C and CYP3A. Testosterone metabolism was altered by all compounds. Cytsolic ERα expression in the liver was also modified by all componds. MXC increased male ERα expression with no effect in females. Genistein decreased ERα expression in males and increased ERα in females. Vinclozolin increased ERα in female rats and had no effect in males. These studies suggest that endocrine active agents have differential effects on hepatic CYP450 and ERα in male and female rats. These changes could affect response to endogenous or exogenous hormones by altering metabolism and level of receptor available.

1268 ESTROGEN-MEDIATED TRANSACTIVATION VIA ESTROGEN RECEPTOR (ER)-Sp1 INTERACTIONS: COMPARISON OF WILD-TYPE AND MUTANT MOUSE ER.

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Estrogen receptor (ER)-Sp1 protein complexes mediate hormone-induced transactivation of several genes through binding only GC-rich sites or composite elements containing both an estrogen responsive element half site ($ERE_{1/2}$) and GC-rich motifs [$ERE_{1/2}(N)_xGC$-rich]. The role of ER monomer or homodimer in ER/Sp1 action is unknown. This study utilizes wild-type and mutant mouse ER (MOR) expression plasmids in ER-negative MDA-MB-231 human breast cancer cells to further define functional ER-Sp1 interactions. MDA-MB-231 cells were transfected with constructs containing a consensus GC-rich (pSp1) or $ERE_{1/2}(N)_{23}GC$-rich [$pERE_{1/2}(N)_{23}Sp1$] oligonucleotide inserts linked to a bacterial chloramphenicol acetyltransferase (CAT) reporter gene; E2 did not induce CAT activity. However, in cells cotransfected with wild-type MOR and pSp1 or $pERE_{1/2}(N)_xSp1$, there was a 2.2- and 3.2-fold induction of CAT activity, respectively. Cotransfection with several MOR mutants including L511A, C241A/C244A, R507A, and progesterone receptor (PR) DNA binding domain were also investigated. E2-responsiveness were observed for L511A using both constructs; C241A/C244A was functional only with $pERE_{1/2}(N)_{23}Sp1$, and remaining plasmids were inactive with both pSp1 and $pERE_{1/2}(N)_{23}Sp1$. These results coupled with ongoing studies with ERE-containing constructs will define promoter-specific requirements for a functional ER and the role of ER as a monomer or homodimer. (NIH CA76636)

1269 THE APPLICATION OF GENOMICS ARRAY TECHNOLOGY TO UNDERSTANDING TOXIC MECHANISMS; A CASE STUDY USING CARBON TETRACHLORIDE.

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We have examined the potential of genomics technology to examine the influence of carbon tetrachloride on hepatocyte gene expression. HepG2 (human hepatoma cell line) cells were exposed for 8 hours to either carbon tetrachloride (0.18% v/v) or to the vehicle (dimethyl formamide, 0.25% v/v) alone. RNA (polyA+) was then prepared and used as a template for first strand cDNA synthesis in the presence of radioactive nucleotide. These complex probes were individually hybridised to commercially available genomic arrays ("ATLAS", Clontech) and the resulting hybridisation patterns were visualised and quantitated using a Molecular Dynamics phosphorimager. Of the 580 genes on the array, approximately 40 genes showed differential transcriptional levels following normalisation of the signals to housekeeping genes (ubiquitin, Phospolipase A2, alpa tubulin, beta actin etc). These differentially regulated genes included putative onco/tumour suppressor genes (c-fms, mas, p55CDC, L-myc-2), stress response genes (STK2, RAB-6, P450), transcription factors (ASH-1, RAD-8) and growth factors (IL-8, Stem Cell Factor). This technique clearly represents an important tool in furthering our understanding of the mechanisms of carbon tetrachloride toxicity.

1270 GENE EXPRESSION PATTERNS AND OXIDANT DAMAGE.

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Gene expression patterns have the potential to reveal mechanisms of chemically induced disease. To test this proposal, *in vitro* responses to 1,3,5-trinitrobenzene (TNB) were used. TNB induces testicular atrophy (rat), neural degeneration (rat), and methemoglobinemia (multiple spp), *in vivo*. The mechanisms responsible for these changes are not well understood, and may include superoxide generation. Changes in gene expression were determined in human HEP-G2 cells after 4 and 12 hrs exposure to 125 μ M TNB. Exposure to this concentration results in a minor reduction in cell viability at 4 hrs as assessed by the MTS assay and a distinct nitroblue tetrazolium reduction, consistent with superoxide generation. At 4 hrs, H&E stained cultures exhibit loss of cytoplasmic lipid droplets and decreased numbers of mitotic figures. At 12 hrs, there is reduced mitosis and individual cell death and at 24 hrs cultures show total cellular necrosis. Messenger RNA from 4 and 12 hr cultures was reverse transcribed to ^{33}P-labelled cDNA and hybridized to a high-density cDNA expression array (Atlas Human Array, Clontech, Palo Alto, CA) containing 588 genes. There were marked treatment-related changes, with both up and down regulation of specific genes, and clear differences between 4 and 12 hrs. At 4 hrs, several functional classes of genes were affected including early response (c-jun, FRA-1, ETR101), stress response (HSPs 40, 60 and 70), and DNA repair/synthesis (GADD 45, GADD 153). Similar changes were seen after 12 hrs, with significant differences including upregulation of the p21 gene, an important cell cycle inhibitor. These data indicate that a powerful oxidant induces transcriptional changes consistent with stress response, mitotic arrest and DNA repair. Studies of related compounds will be needed before a mechanism-specific pattern of gene expression can be defined.

1271 GENE EXPRESSION PATTERNS INDUCED IN HEP-G2 CELLS INDICATE THAT DNA DAMAGE MAY PLAY A MAJOR ROLE IN PARQUAT-INDUCED MITOTIC ARREST AND CELL DEATH.

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The mechanism of cell death induced by paraquat (PQ) in target tissues (lung, kidney and liver) remains to be determined. Patterns of gene expression, which may shed light on mechanisms of chemically-induced cell damage, were investigated in HEP-G2 cells exposed to 0 or 600 μ M PQ (EC_{50} for cytolethality) for 4, 12 or 24 hrs. Confluent HEP-G2 cultured on collagen coated 150 mm polystyrene dishes, containing a glass cover slip (for morphology studies) and 30 ml DMEM-10%FCS (depth 1.7 mm), were maintained at 37° C, 5% CO_2 for 48 hr, fresh medium for 24 hrs, and then PQ added in 3 ml DMEM. Following exposure to PQ, cover slips were fixed in absolute ethanol at -20° C, stained with H&E, and examined by light microscopy. Cells were lysed, poly A^+ RNA purified and reverse transcribed (^{33}P-dATP). ^{33}P-cDNA probe was hybridized to a 588 gene expression array (Atlas Human cDNA Expression Array, Clontech, Palo Alto, CA) and read

on a phosphorimager. Image intensities were determined and levels of up or down regulation estimated to yield expression patterns. H&E stained cover slips revealed PQ-induced loss of intracellular droplets, abnormal metaphase, absence of telophase stages of mitosis (control ~10/high power field) at 12 and 24 hrs, and scattered degenerating cells. Gene expression patterns included evidence of early response (↑ c-jun, ↑ fra-1), DNA damage and mitotic arrest (↑ GADD45, ↑ p21, ↓ cyclins, ↓ top2). Genes on the array expected to respond to oxidative stress (e.g. Cu/Zn SOD, GPx1, and GSH reductase) were unaffected, as were apoptosis associated genes (e.g. adenosine A1r, bcl-x, apo-1, caspases, p53). These observations are consistent with a significant role for DNA damage in PQ-induced cell death (Dusinska et al., Carcinogenesis, *19*,809-812) and mitotic arrest. (This publication may not necessarily reflect the views of the US EPA, and no official endorsement should be inferred.)

1272 THE USE OF GENOMICS TECHNOLOGY TO INVESTIGATE GENE EXPRESSION CHANGES INDUCED IN CULTURED CELLS IN THE PRESENCE OF XENOESTROGENS.

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Recent research on mechanisms of estrogen action have highlighted complexities not being addressed by simplistic *in vitro* testing. We have developed a genomics array approach to investigate the transcriptional changes which occur in cultured human uterine cells on exposure to estrogens. cDNA "probes" from approximately 600 genes were prepared, PCR amplified and spotted on a nylon membrane to generate a "genomics" array. Human uterine cell lines (SK-UT 1) were then cultured in the presence or absence of estradiol before mRNA was prepared. The mRNA from control and estradiol treated (10^{-8} M, 4h and 24h) cells was then used in a first strand cDNA synthesis reaction to generate complex radiolabelled cDNA probes which in turn were individually hybridised to genomics arrays. The hybridisation patterns were then visualised and quantitated using a Molecular Dynamics phosphorimager. Comparison of the resultant patterns allowed the identification of changes in gene expression in control versus estradiol treated cells by normalising signal levels against background and housekeeping genes. We propose that this approach is a useful technique to investigate the changes in gene expression induced by exposure to xenoestrogens, and may facilitate the development of *in vitro* assays for estrogenicity.

1273 DEVELOPMENT OF A TOXICOLOGICAL GENE ARRAY AND QUANTITATIVE ASPECTS OF THIS MICROARRAY TECHNOLOGY.

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DNA microarrays are high-density arrays of cDNA bound to a solid substrate. DNA arrays have proven to be a powerful system for monitoring the expression levels of thousands of genes simultaneously. Many genes are induced or repressed by toxicants and this pattern of gene expression can lead to a better understanding of underlying reactions of cells to injury as well as the processes associated with repair. Moreover, this approach is likely to yield new insights of the mechanism by which chemical toxicants interact. In this report, we describe efforts underway to examine the quantitative aspects of this microarray technology. In addition we describe the development of a "toxicology chip" that may be useful for detecting the response of mouse lung and liver to a variety of environmental toxicants including chemical mixtures. Additionally this chip may provide a mechanism for the rapid assessment of ecosystem health in relation to toxicants. Expression levels are being compared for several genes (i.e. *Cyp1a1, Cyp1a2*) using either standard Northern analysis or cDNA arrays. As expected, maximal upregulation of *Cyp1a1* in response to B-napthoflavone occurred at eight hours post treatment A dose response study is being conducted using B-napthoflavone to determine the sensitivity of the cDNA arrays as compared to Northern Blot Analysis. (Supported by NIEHS 04699 and TSR&TP.)

1274 TOXICOGENOMICS: TOXICANT IDENTIFICATION AND CLASSIFICATION USING cDNA MICROARRAY TECHNOLOGY.

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We have developed a novel approach to the identification of potential toxicants and their putative mechanism of action. This approach utilizes cDNA microarrays to measure toxicant-induced gene expression profiles. In defined model systems, treatment with known agents such as polycyclic aromatic hydrocarbons, dioxins, peroxisome proliferators, estrogenic compounds, or oxidant stressors, can provide a gene expression "signature" on a microarray which represents the cellular response to these agents. These same systems can then be treated with unknown agents to determine if one or more of these standard signatures is elicited. The data derived from this approach is inherently mechanistic, and can be used to suggest a general mechanism of action for suspected toxicants. In addition, these studies may lend insight into the necessity for testing the agent in the more expensive, time-consuming traditional bioassays. We have designed and manufactured a "Toxicant Response Chip" (*ToxChip*) which contains approximately 2100 genes involved in cellular responses to toxic insult. Included on this microarray are cell cycle genes, oxidative stress genes, xenobiotic metabolizing enzymes, DNA replication and repair genes, hormone-responsive genes, and apoptotic cell death genes, as well as many others gene categories. In addition, we have attempted to include the entire complement of genes known to be responsive to *[Ah]*-receptor agonists and peroxisome proliferators. Our initial studies using this general approach have focused on the gene expression signature elicited by different types of oxidant stressors in human cell lines.

1275 USE OF A GENOMIC TECHNOLOGY FOR ASSESSMENT OF TOXICITY.

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Analysis of differential gene expression on microarrays of key target tissues or cells may reduce the cost of toxicity testing required for the regulatory approval of new products. These assays may therefore allow higher throughput processing of compound candidates. In the present study, Sprague-Dawley rats were treated with three known hepatotoxins at doses known to cause toxicity: benzo(a)pyrene, clofibrate and acetaminophen. Compounds were administered i.p. and liver, brain, kidney, lung, spleen and pancreas were harvested at the following timepoints: 12 hr., Day 1, 3, 7, 14 and 28. Physical signs, liver/body weight ratios and serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were monitored. Fluorescently-labeled cDNA made from RNA isolated from the tissues was used for hybridization onto a microarray containing primarily rat liver and kidney gene sequences. Different gene expression profiles were observed for each compound.

1276 QUANTITATIVE ANALYSIS OF GENE EXPRESSION ARRAY DATA FROM TCDD TREATED MOUSE HEPATOMA CELLS.

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cDNA array technology represents an efficient means to simultaneously analyze the expression of hundreds of genes. However, many current methodologies are not reproducible and appropriate quantitative analysis has not been described. Using a commercially available cDNA array, the effect of 100 nM 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on gene expression in mouse Hepa 1c1c7 cells was examined. The hybridization signal intensities (HSI) from two replicate experiments were quantitated on a PhosphoImager. Results from conventional non-parametric methods of analysis and a more powerful parametric approach using a General Linear Model (GLM) were then compared. The non-parametric analysis of HSI data used global means of the background corrected HSI values to normalize each data point for direct comparison. Treatment-related effects on gene expression were then ranked by magnitude for each replicate. Rank correlation between replicates was 0.23 ($P<.05$) indicating poor reproducibility between experiments. Using a GLM, the variation in HSI was partitioned into treatment, replicate,

group, and interactions between them as classification effects and random effects of genes within group. The model was analyzed using the GLM procedure of SAS. Least Squares Means (LSM) for the genes within group were computed using Tukey adjustments. Genes were ranked according to the magnitude of the LSM. This model accounted for 94% of the variation in HSI. All of the effects in the model were significant (P<.0001). The two methods gave different rankings for the effects on gene expression. The probability of observing a treatment effect on each gene could not be calculated using the non-parametric analysis. However, the GLM approach provides a mechanism for hypothesis testing on target genes which is more consistent with the experimental design.

1277 DETERMINATION OF ACOX GENE EXPRESSION *IN VITRO* AND *IN VIVO* FOLLOWING TREATMENT WITH PEROXISOME PROLIFERATORS USING THE BRANCHED DNA TECHNIQUE.

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Branched DNA (bDNA; Quantigene, Chiron) is a novel technique that facilitates the monitoring of gene expression in a microtiter plate format using non-radiolabeled probes specific for the target gene. To assess this technique for monitoring gene expression in cell cultures following drug exposure, isolated rat hepatocytes in primary culture were treated with subtoxic concentrations of bezafibrate (BZ, 0.5-250 μ M), clofibrate (CL, 10-250 μ M) and ibuprofen (IB, 10-100 μ M) for 6, 24 and 48 hours. All three drugs caused elevations in acyl-CoA oxidase (ACOX) gene expression with BZ being the most potent. BZ caused approximately 15-20 fold induction at 25 μ M and above. A microtiter plate assay was also developed to monitor ACOX enzyme activity in rat hepatocytes. In primary cultured hepatocytes, BZ caused 2-3 fold increase in ACOX enzyme activity at 100 μ M and above at 24 and 48 hours. To investigate the applicability of the bDNA technique for monitoring gene expression during *in vivo* studies, adult male Sprague-Dawley rats were treated with BZ (100 mg/kg/day) in 0.2% hydroxypropyl methylcellulose (HPMC) for 3 days by oral gavage. Liver homogenates from animals treated with BZ showed approximately 5 fold increase of ACOX gene expression over untreated animals. In contrast, ACOX gene expression in 0.2 % HPMC treated animals were similar to untreated controls. Moreover, for comparison GAPDH gene expression was also monitored in liver tissue from BZ treated animals. BZ had only a minimal effect on GAPDH gene expression (1.5 fold) compared to untreated rats. The *in vitro* data obtained through the bDNA technique for ACOX gene expression were similar to previously reported data obtained with the Cytostar assay. In conclusion, results presented here show that the bDNA technique would allow us to screen for changes in gene expression following exposure to drugs *in vitro* and *in vivo*.

1278 GENE EXPRESSION CHANGES AS A MARKER FOR CISPLATIN INDUCED APOPTOTIC CELL DEATH IN RAT AND HUMAN LIVER SLICES.

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Cisplatin is a DNA damaging agent that induces cell death through an apoptotic mechanism. Molecular and cellular indicators of apoptosis were measured in precision cut rat and human livers slices (8 mm in diameter and ~250 μm thick) exposed to cisplatin (0, 20 and 40 μM) for up to 18 hours. Molecular evidence for the initiation of apoptosis was apparent as early as 3 hours with a rise in p53 gene expression in the 40 μM cisplatin treated slices. Elevated expression levels of p53 continued, and was statistically significant, through the 6 hour time point. An increase in p53 protein (p53p) levels, a DNA binding transcriptional activator that is induced upon DNA damage, followed at 6 hours and reached a maximum 2 fold induction by 9 hours of treatment with 40 μM cisplatin. Activation of cellular growth arrest, as evidenced by the ~ 2.5 fold increase in levels of Gadd45 message, (9 - 12 hrs, 20 & 40 μM cisplatin) followed as part of the apoptotic cascade initiated by p53p induction. Genes regulating cell cycle progression (c-myc, c-fos) were also effected by cisplatin (20 & 40μM) treatment. Repression of c-myc expression (~50% at 40μM, & ~25% at 20μM by 12 hrs) preceded the elevation of Gadd45 expression. The early response gene c-fos exhibited a continual, dose dependent increase in expression throughout the time course in both cisplatin treated groups and was ~8 fold elevated over control by 18 hours in the 40 μM cisplatin treated slices. Genomic DNA degradation into 180-220 bp oligomers, a hallmark event in apoptosis, was detectable by 12 hours with 20 μM cisplatin. Liver slice viability, as measured by RNA stability and the leakage of LDH and α-GST proteins, was significantly decreased by 18 hr of culture, ~15% & ~30% in the 20 and 40 μM cisplatin treated groups, respectively. The results obtained in this study are consistent with the findings using immortalized cell lines, and demonstrate the apoptotic mechanism of cisplatin induced cell death in liver slices at both the cellular and molecular level.

1279 PHENOTYPIC AND GENOTYPIC VARIATION IN SERUM LIPID PARAMETERS IN CYNOMOLGUS MONKEYS.

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Non-human primates are a frequently used model in the development of novel therapeutic agents for the treatment of serum lipid disorders and atherosclerosis. A high level of homology occurs between human and cynomolgus monkey proteins involved in lipoproteins and lipoprotein metabolism, however, little is known about phenotypic and genotypic variation, with regard to serum lipid parameters, within cynomolgus populations. Serum cholesterol, triglycerides, HDL-C, HDL-C, VLDL-C, apo A1, apo B, and Lp(a) were measured in 222 feral cynomolgus monkeys (109 F, 113 M) from 2 different geographic sources, Vietnam (146) and Mauritius (76), and housed in 3 separate facilities: Ann Arbor, MI (69); Mississauga, Ontario (76); and Alice, Texas (77). PCR-RFLP methods were used to genotype polymorphic loci in Apo A-I, Apo B, Apo C-III, Lp(a), and Apo E genes. A number of statistically significant phenotypic differences were found. Most notably, serum Lp(a) and LDL levels in monkeys from Mauritius were 54% and 20% lower, respectively, and serum triglycerides were 25% higher than monkeys from Vietnam. All monkeys from Mauritius had the wild-type (CC) Apo B gene while, 17% of the Vietnamese monkeys had the heterozygous polymorph (CT). In addition, 10% of the Vietnamese monkeys had a novel polymorph. No other polymorphisms were noted, however, results indicate that the least common allele in humans appears to be the predominant allele in monkeys at one of the Apo A loci.

1280 PEROXISOME PROLIFERATOR-INDUCED SIGNALING PATHWAY CHARACTERIZATION USING NULL MOUSE MODELS AND cDNA ARRAYS.

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Peroxisome proliferators (PP) induce alterations in homeostatic growth mechanisms in the livers of mice and rats resulting in increases in hepatocellular proliferation, decreases in apoptosis, and increases in hepatocellular adenomas and carcinomas. PP modulate hepatocyte growth through the activation of PP-activated receptor α (PPAR), the central mediator of PP actions in the liver. A tumor necrosis factor α (TNF)-dependent pathway has been hypothesized to contribute to PP-induced effects on hepatocyte growth. We further characterized and determined the importance of these pathways in hepatocyte growth modulation using mice which lacked functional genes for PPARα, TNF, or TNF receptor II (TNF RII). Mice were treated with WY-14,643 and assessed for changes in hepatocellular proliferation by BrdU labeling and mRNA levels using the Atlas blot (Clontech) or a cDNA array containing many known targets of PP-induced changes in gene expression. Wild-type and TNF RII-null mice exhibited indistinguishable increases in liver to body weight ratios and cell proliferation. Increases in liver to body weight ratios were abolished in TNF-null mice but WY-induced markers of peroxisome proliferation were unchanged. Thus hepatocyte growth could be uncoupled from peroxisome proliferation in this mouse model. Examination of gene expression after WY exposure demonstrated a number of genes involved in cell proliferation, apoptosis, and metabolism were altered in wild-type mice but not in PPAR-null mice. Metabolism genes included members of the glutathione-S-transferase family, whose proteins have been shown to be altered by PP in earlier studies. Our work demonstrates that PP modulate hepatocyte growth through PPAR- and TNF-dependent pathways and shows that null mouse models and cDNA arrays are a powerful combination to define signaling pathways important in tumor promotion and carcinogenesis.

1281 IDENTIFICATION OF GENES DIFFERENTIALLY EXPRESSED IN BENZO[A]PYRENE TREATED VASCULAR SMOOTH MUSCLE CELLS: IMPLICATIONS IN ATHEROGENESIS.

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Uncontrolled proliferation of vascular smooth muscle cells (vSMCs) is a hallmark of the atherosclerotic process in both humans and experimental animals. In this context, we have previously shown that serial passage of vSMCs treated with low doses of benzo[a]pyrene (BaP) is associated with induction of highly proliferative, atherogenic phenotypes. To define the molecular basis of this response, differential display polymerase chain reaction (DD-PCR) has been used to identify early target genes in murine vascular SMCs following challenge with 3 uM BaP for 8 hr. A total of 170 differentially expressed cDNAs were excised, 111 of which were successfully re-amplified. Sixty four of these cDNAs have been successfully sequenced and examined for homology to known genes. Among 19 cDNAs abundant in control but not BaP-treated cells, the following genes have been identified: (1) Mus musculus mRNA with high homology to human Aac11 mRNA, a novel gene that inhibits apoptosis after growth factor withdrawal (four copies); (2) Mouse aldose reductase gene (four copies); (3) Mus musculus polypeptide GalNAc transferase-T1 (ppGaNTase-T1) (one copy); (4) Mus musculus Ccth mRNA for CCT eta subunit (chaperonin containing TCP-1) (one copy); and (5) Mouse mitochondrial genome (one copy). In contrast, of 45 cDNAs from BaP-treated cells, only one gene, mouse retrotransposon L1Md, was identified (five copies). The remaining 40 cDNAs did not exhibit homology to any known gene, but contained several human retrotransposon elements. Confirmation for differential expression of all matched cDNAs was successfully completed by Northern analysis. Of interest was the finding that a pattern characteristic of the mouse retrotransposon L1Md Northern signal was detected among the 40 unmatched cDNAs from BaP-treated cells. These results demonstrate that prominent changes in gene expression occur in vSMCs following acute BaP challenge, including the activation of retrotransposons and repression of an apoptosis inhibitor. These alterations may contribute to the genetic and epigenetic changes that set the stage for the early stages of chemical atherogenesis. (Supported in part by NIH grants ES 04849 and ES 09106.)

1282 CLONING AND CHARACTERIZATION OF GENES REGULATED BY QUINOL-THIOETHER MEDIATED OXIDATIVE STRESS IN HL-60 CELLS.

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Glutathione conjugates of hydroquinone induce oxidative stress and apoptosis in cultured human promyelocytic leukemia (HL-60) cells. In order to understand the signal transduction pathways and the role of gene expression in this process, differential screening of cDNA libraries was performed. Two representative cDNA libraries (containing 1.5 x 10^6 clones each) were constructed in ZAP expression vectors using mRNA isolated from untreated HL-60 cells and HL-60 cells treated with 200 μM of 2,3,5 *tris*-(glutathion-S-yl)hydroquinone (TGHQ) for 30 min, 60 min and 120 min. Positive (TGHQ treated) and negative (untreated) cDNA probes with high specific activity (1-1.5x10^8 cpm/mg) were synthesised using superscript reverse transcriptase II. The size distribution of cDNA probes was 0.5-2.0 kb. Primary differential screening of clones from both untreated and TGHQ treated cDNA libraries has identified several clones which are regulated by TGHQ. Results of the screening show that TGHQ has both suppressive and inductive effects on gene expression. They also indicate that, at least in the initial 2 hrs after treatment, TGHQ has no effect on the expression of approximately 90% of genes, has a moderate effect on 5-10% of genes, and a dramatic effect on 0.5% of genes, suggesting that TGHQ modulates specific gene expression in HL-60 cells. The identity and expression pattern of some of these differentially regulated genes will assist in understanding the molecular mechanisms of action and toxicity of quinol-thioethers, and the regulation of the cellular stress response to these compounds.

1283 ZEBRAFISH TRANSCRIPTION FACTORS ACTIVATE POLLUTION-INDUCIBLE RESPONSE ELEMENTS AND DRIVE REPORTER GENE EXPRESSION.

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Many classes of environmental pollutants are found at significant levels in the aquatic environment and, as a result, pose a threat to human health through consumption of contaminated fish and/or shellfish, as well as exposure in drinking water. Aromatic hydrocarbon, heavy metal, and potent electrophilic environmental pollutants alter cellular transcription via interaction with specific transcription factors that recognize specific DNA response elements (AHREs, MREs, EPREs, respectively) in the enhancer regions of certain genes. Zebrafish transcription factors from ZEM2S embryonic cell cultures are able to recognize these DNA response elements, as determined by electrophoretic mobility shift analysis. Transient transfection of ZEM2S cells with luciferase reporter constructs, in which expression is driven by these same response elements, imparts dose-dependent gene induction upon exposure to a variety of chemicals within each of these three classes of inducers. TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin), Cd^{++} (cadmium) and tBHQ (*tert*-butylhydroquinone) were used as model compounds for the induction of constructs containing AHRE, MRE and EPRE sequences, respectively. Maximal inducibilities were 13-fold for TCDD, 4-fold for Cd^{++}, and 9-fold for tBHQ. Inducible promoters of orthologous genes from several species are able to impart dose-dependent reporter gene activity to varying degrees and, surprisingly, the fish-derived promoters are not necessarily the most effective. Our data suggest that these reporter constructs might be useful for the generation of a transgenic environmental sentinel for biological monitoring. Such a sentinel will be capable of distinguishing specific classes of polluting chemicals within the complex mixtures found in the aquatic environment. (Supported in part by NIH Grants RO1 ES07058 and P30 ES06096.)

1284 TOXICOLOGY IN SILICO: IN SEARCH OF TARGET GENES FOR THE NUCLEAR ARYL HYDROCARBON RECEPTOR COMPLEX.

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Upregulation of metabolic and detoxification enzymes by the nuclear aryl hydrocarbon receptor (AhR)/aryl hydrocarbon receptor nuclear translocator (Arnt) complex is dependent upon transcriptional activation through binding to upstream enhancers, termed dioxin response elements (DRE). This mechanism, however, does not reasonably explain the tissue-, sex- and species-specific toxicity of AhR agonists, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin. Therefore, other potentially DRE-regulating genes were searched for in the Eukaryotic Promoter Database by searching for the core DRE sequence (5'-GCGTG-3'). An IUPAC search strategy revealed the existence of 1831 DREs in the 5' flanking region of 353 vertebrate, 104 invertebrate and 132 viral genes. Due to the functional importance of nucleotides flanking the core DRE sequence, we attempted to estimate the functionality of the extended DRE sequence string (5'-N7GCGTGN7-3') using MatInspector software. Each DRE sequence string was scored according to its relative similarity to a precompiled position weighted nucleotide distribution matrix derived from 25 selected AhR:Arnt binding sequences. Matrix similarity scoring of 10 bona fide DREs produced a cutoff score of 0.701, suggesting similarity scores above this value may be biologically functional. Matrix analysis of 1831 DREs resulted in 939 DREs in 423 promoters having scores above the cutoff value, which represent genes whose DREs have potential functionality. Subsequent ranking of DRE frequency and matrix similarity score prioritize these genes for subsequent experimental verification of DRE functionality. Due to the conserved nature of DRE binding, our results also suggest a role of the AhR:Arnt complex in viral gene regulation and the existence of ancestral AhR and Arnt orthologues in invertebrates, a hypothesis recently verified.

1285 ROLE OF THE ARYL HYDROCARBON RECEPTOR AND P450 METABOLISM IN THE REDOX REGULATION OF *c-Ha-ras* BY BENZO(A)PYRENE IN VASCULAR SMOOTH MUSCLE CELLS.

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Previous studies in this laboratory have identified *c-Ha-ras* as a critical

molecular target of benzo(a)pyrene (BaP) in the dysregulation of vascular smooth muscle cell (vSMC) proliferation and differentiation. Acute challenge of vSMCs with BaP is associated with transcriptional activation of *c-Ha-ras*, a response mediated via electrophile responsive cis-acting elements (EpRE) within the promoter region of the gene. Because activation of EpREs involves aryl hydrocarbon receptor (AhR)-regulated events, we hypothesized that up-regulation of *c-Ha-ras* mRNA levels is mediated by oxidative metabolites of BaP that compromise redox regulation of the gene. To test this hypothesis, experiments were conducted to evaluate the inducibility of *c-Ha-ras* in vascular SMCs by oxidative metabolites of BaP and related products. BaP-3,6-quinone (0.3μM), a cytochrome P450 derived metabolite of BaP, and H_2O_2 (25 and 50μM), a by-product of oxidation and redox cycling, enhanced *c-Ha-ras* gene expression. Analysis of CyP1A1 and CyP1B1 mRNA levels in "wild type" vSMCs$^{+/+}$ and AhR "knock-out" vSMCs$^{-/-}$ by RT-PCR and Northern revealed constitutive expression of CyP1B1, but not CyP1A1, in both cell types. CyP1A1 and CyP1B1 were up-regulated in vSMCs$^{+/+}$, but not vSMCs$^{-/-}$, following BaP (3μM) challenge. Incubation with ellipticine (Ellip) or α-naphthoflavone (α-NF), inhibitors of P450 and weak ligands of the AhR, prior to BaP challenge interfered with *c-Ha-ras* up-regulation by BaP. These data implicate cellular metabolism of BaP by constitutively expressed CyP1B1 in the redox regulation of *c-Ha-ras* in vSMCs. (Supported by NIH Grant ES 04849 and ES 09106.)

1286 PROTEIN INTERACTIONS WITH THE ELECTROPHILE RESPONSE ELEMENT INDUCED BY BENZO(a)PYRENE AND RELATED OXIDANTS.

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The toxicity and carcinogenicity of benzo(a)pyrene (BaP) may involve epigenetic events that culminate in the deregulation of gene expression. Previous investigations in this laborarory have demonstrated the involvement of a 5'-regulatory sequence within the c-Ha-ras promoter, termed the Electrophile Response Element (EpRE), that associates specifically with two vascular smooth muscle cell (vSMC) nuclear proteins of Mr 80 and 82 kDa. These protein/DNA associations are inducible by various reactive oxygen species (ROS) in different promoters and cell types, suggesting their participation in the regulation of genes harboring this element. More recently, our efforts have been directed at defining the nature of EpRE/protein(s) associations critical to gene regulation. Six EpRE-like oligonucleotide constructs were tested for their ability to bind EpRE binding proteins using electrophoretic mobility shift assays. The constructs were designed to evaluate the role of flanking sequences relative to the well-defined EpRE core region (5'RTGAYNNNGC3') in stabilizing protein/DNA interactions. Our results showed that a TMA box 5' to the core region, like that in the mouse GST-Ya ARE (5'TAATGGTGACAAAGCAACTT3'), is required for maximum binding. Consequently, the GST-Ya ARE sequence was used to construct a site-specific DNA-affinity column in order to isolate EpRE/ARE-associating proteins. Nuclear extracts from BaP-challenged vSMC were loaded directly onto the GST-Ya affinity column and four proteins of Mr 62, 60, 50, and 30 kDa were found to associate specifically. Cross-linking experiments with a BrdU-substituted GST-Ya oligonucleotide probe identified three specific complexes of Mr 80, 60, and 55 kDa. We conclude that the protein(s) specifically eluted by GST-Ya DNA affinity chromatography may participate in ROS-mediated deregulation of growth-related genes. (This work was supported by ES grants 048489 and E09106.)

1287 NEGATIVE REGULATION OF THE RAT GST-Ya GENE IN VASCULAR SMOOTH MUSCLE CELLS.

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Previous studies in this laboratory have established that dysregulation of growth-related genes by benzo(a)pyrene (BaP) in vascular smooth muscle cell (vSMCs) involves functional interactions between electrophile and aryl hydrocarbon receptor (AhR) signaling. Our previous transfection studies have suggested that a negative regulatory mechanism may be operative within the rat GST-Ya promoter in both vSMCs and HepG2 cells. In the present study, electrophoretic mobility shift assays were conducted to define protein/DNA interactions which influence the ability of the electrophile response element (EpRE) to function as a negative regulatory sequence in vSMCs. Our results showed that the C/EBP and AP-1 consensus sequences, along with the NF-E2, CREB/ATF and AhR binding sites, compete to varying degrees with the proteins that interact with EpRE. In contrast, Ets and NF-kB sequences did not effectively compete with EpRE binding proteins. These data suggest that multiple proteins interact with the rat GST-Ya promoter to influence EpRE protein binding. Supershift experiments showed that C/EBP-beta, but not -alpha, bind to both EpRE and AhRE. This finding is consistent with the presence of C/EBP sites within those consensus sequences. Surpringly, the AhR was detected in the protein complex binding to the EpRE in the absence of an AhR binding site. These data suggest that C/EBP-beta and the AhR participate in the negative regulation of rat GST-Ya gene in vSMCs. (Supported in part by NIH Grant ES 04849 and ES 09106.)

1288 TYROSINE KINASE INHIBITORS ACTIVATE THE ANTIOXIDANT/ELECTROPHILE RESPONSIVE ELEMENT IN NEUROBLASTOMA CELLS.

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NAD(P)H:quinone oxidoreductase (QR) detoxifies xenobiotics and inhibits free radical production by catalyzing the two-electron reduction of quinones and other hydrophobic compounds. The antioxidant/electrophile responsive element (ARE/EpRE) is located in the 5'-flanking region of the gene encoding QR. We hypothesize that tert-butyl-hydroquinone (tBHQ) activates the hQR-ARE/EpRE by inhibiting tryosine kinase activity. Human neuroblastoma (IMR-32) and human hepatoma (HepG2) cells were transiently transfected with a TATA-Inr minimal promoter luciferase reporter construct that contained a forty-one base pair DNA insert containing the ARE/EpRE from the hQR promoter. Treatment of IMR-32 cells with tBHQ (10μM) resulted in a 23-fold increase in hQR- ARE/EpRE luciferase expression. In contrast, tBHQ (200μM) had little effect on the hQR-ARE/EpRE-luciferase expression in HepG2 cells. β-Napthoflavone and the oxidants, hydrogen peroxide and tert-butylhydroperoxide, did not activate the hQR-ARE in the IMR-32 cells, suggesting that oxidative stress may not be involved in ARE/EpRE activation in these cells. Tyrphostin AG957, erbstatin analog and lavendustin A, share a common hydroquinone based structure with tBHQ. HQR-ARE/EpRE-luciferase expression, QR protein and QR activity were increased by treatment with these tyrosine kinase inhibitors, whereas lavendustin B, the inactive analog to lavendustin A, had no effect on QR in IMR-32 cells. These data suggest that tyrosine kinase inhibition is involved in ARE/EpRE activation by tBHQ in IMR-32 cells. (Supported by ES08089 and BWF New Investigator Award.)

1289 IDENTIFICATION OF ANTIOXIDANT/ELECTROPHILE RESPONSIVE ELEMENT (ARE/EpRE)-DRIVEN GENES IN HUMAN NEUROBLASTOMA CELLS.

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Pretreatment of neuroblastoma cells with tert-butyl hydroquinone (tBHQ) has been shown to protect against glutamate-induced toxicity. This protection was correlated to increased NAD(P)H:quinone oxidoreductase (QR) activity. Recent studies have shown that QR is not solely responsible for blocking cytotoxicity since over expression of QR did not confer protection to the cells from dopamine induced toxicity. Hydrogen peroxide formation and increased oxidative stress is the mechanism of cell death for both glutamate and dopamine in these cells. We have postulated that a coordinate regulation of multiple genes rather than a single gene product is responsible for neuroprotection, and that this coordinate regulation is mediated via the ARE/EpRE. Few human genes have been shown to have functional ARE/EpREs. Our laboratory has demonstrated that IMR-32 human neuroblastoma cells are highly responsive to treatment with tBHQ resulting in robust transcriptional activation of the ARE/EpRE. Suppression subtractive hybridization has been performed on these cells using Clontech's PCR Select™. We have produced a differential cDNA library in which QR is ~ 10^7 more prevalent in the tBHQ treated population than in the control. Two other genes isolated from the differential library were tested and one was found to be differentially expressed. Sequencing of 48 random clones has produced 15 different known genes and 7 unknown sequences. Because many types of cell death may involve the formation of oxidants, coordinate regulation of genes identified as containing the ARE/EpRE may lead to a new strategy to combat neurodegenerative disease. (Supported by ES08089 and BWF New Investigator Award.)

1290 PI3-KINASE IS A CRITICAL ACTIVATOR OF THE ANTIOXIDANT/ELECTROPHILE RESPONSIVE ELEMENT.

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There is evidence that phosphatidylinositol 3-kinase (PI 3- kinase) promotes cell survival both in cell culture and in primary neuronal cultures by providing antiapoptotic signals. Protein kinase B (Akt2), a downstream target of PI 3-kinase, has been shown to be a key serine/threonine kinase required for inhibition of apoptosis. The antioxidant/electrophile responsive element (ARE/EpRE) is an enhancer element located in the 5'-flanking region of many protective genes, including human NAD(P)H:quinone oxidoreductase (QR); however, the signal transduction cascade for the ARE/EpRE is yet undefined. The purpose of these studies was to determine if PI 3-kinase is involved in the upstream signaling cascade leading to ARE/EpRE activation. IMR-32 cells were transiently transfected with a TATA-Inr minimal promoter: luciferase reporter construct containing a 41 base pair ARE/EpRE insert from the hQR promoter. HQR-ARE/EpRE-luciferase expression was increased 22-fold by tert-butylhydroquinone (tBHQ) in these cells. Pretreatment (15 minutes) of the IMR-32 cells with an inhibitor of PI 3-kinase, LY294002, resulted in an 80% inhibition of the tBHQ-mediated activation. A corresponding inhibition was observed for the endogenous QR activity. These data suggest that PI 3-kinase is a critical component of the signaling pathway leading to ARE/EpRE activation in neuroblastoma cells. (Supported by NIEHS 08089 and BWF New Investigator In Toxicology Award.)

1291 DIFFERENTIAL INDUCTION OF THE ANTIOXIDANT/ ELECTROPHILE RESPONSIVE ELEMENT BY HEAVY METALS IN NEUROBLASTOMA CELLS.

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NAD(P)H:quinone oxidoreductase (QR) detoxifies xenobiotics and inhibits free radical production by catalyzing the two-electron reduction of quinones and other hydrophobic compounds. The antioxidant/electrophile responsive element (ARE/EpRE) is located in the 5'-flanking region of the gene encoding human QR (hQR). The ARE/EpRE has been shown to be inducible by cadmium, zinc and arsenate in HepG2 cells. Induction of QR activity has been shown in a neuroblastoma cell line in response to cadmium, manganese and lanthanum treatment. The purpose of these studies was to evaluate the transcriptional activation of the hQR-ARE/EpRE in IMR-32 human neuroblastoma cells in response to treatment with a variety of heavy metals. IMR-32 cells were transiently transfected with a TATA-Inr minimal promoter luciferase reporter construct that contained a forty-one base pair DNA insert containing the ARE/EpRE from the hQR promoter. Dose-dependent increases in transcriptional activation were seen for cadmium (19.8-fold), mercury (14.1-fold), arsenite (5.7-fold), lead (5.3-fold), iron (5.4-fold), and cobalt (3.7-fold). Other metals including, zinc, manganese and copper caused a minimal activation of the ARE/EpRE-luciferase expression (<3-fold). In addition, cadmium and mercury were the most potent inducers of QR protein and QR activity. The mechanism by which cadmium and mercuric maximally activate the ARE/EpRE in these neuroblastoma cells remains to be determined. (Supported by ES08089 and BWF New Investigator Award.)

1292 CADMIUM-MEDIATED ACTIVATION OF THE METAL RESPONSE ELEMENT IN HUMAN NEUROBLASTOMA LACKING FUNCTIONAL METAL RESPONSE ELEMENT-BINDING TRANSCRIPTION FACTOR-1.

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Metallothioneins (MTs) constitute a conserved family of cysteine-rich heavy metal binding proteins. MT-I and MT-II gene transcription is dramatically increased by heavy metals. Metal response elements (MREs) are essential for this induction, and these elements are present in multiple copies in the proximal promoters of mammalian MT genes. MTF-1 (MRE-binding transcription factor-1) binds specifically to MREs and transactivates MT gene expression in response to Zn, Cd and oxidative stress. Transcriptional activation of mouse mMT-I has been extensively studied in a variety of cell types, however there is little data on regulation of the mMT-I promoter in cells of neural origin. Thus, the purpose of this investigation was to determine if the mMT-I promoter is transcriptionally activated by metals and oxidative stress in IMR-32 human neuroblastoma cells. Cd activated a mMT-1 promoter (-150 bp) luciferase reporter 20- to 25-fold in transient transfections and Cd-mediated transcriptional activation of mMT-1 promoter was MRE-dependent. In contrast, tert-butylhydroquinone and Zn had little effect on luciferase expression. IMR-32 whole cell extracts from cells treated with Zn in vivo did not contain MRE binding activity detectable by gel shift assay and incubation of the IMR-32 extracts with Zn in vitro did not generate a MTF-1/MRE-s complex suggesting that IMR-32 cells lack functional MTF-1. Overexpression of mMTF-1 in transiently transfected IMR-32 cells regenerated a Zn-mediated induction of the MREd5-luciferase reporter without affecting Cd activation. Since no other transition metals tested activated the MRE, this effect appeared to be Cd-specific. These data demonstrate that, in IMR-32 human neuroblastoma cells, Zn and Cd can use independent mechanisms for activation of the mMT-I promoter and Cd-mediated MRE activation is independent of MTF-1 and Zn. (Supported by ES08089 and BWF New Investigator Award.)

1293 ESTROGEN CATECHOLS ACTIVATE THE ANTIOXIDANT/ELECTROPHILE RESPONSIVE ELEMENT IN IMR-32 HUMAN NEUROBLASTOMA CELLS.

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It has been shown that estradiol and estrogen catechols protect neuronal cells from peroxide accumulation and glutamate-mediated toxicity. This protection required the presence of a 3'-hydroxy group on the A ring of estradiol and was independent of the estrogen receptor. Protection was attributed to the antioxidant properties of these estrogens. In addition, we hypothesize that the estrogen catechols can activate gene transcription through the ARE/EpRE. These studies were designed to determine if estradiol and its catechol metabolites activate the ARE/EpRE and ARE/EpRE-containing genes in IMR-32 human neuroblastoma cells. IMR-32 cells were transiently transfected with a TATA-Inr minimal promoter luciferase reporter construct that contained a forty-one base pair DNA insert containing the ARE/EpRE from the human NAD(P)H:quinone oxidoreductase (QR) promoter. Treatment of IMR-32 cells with 5 μM 2-hydroxyestradiol and 5 μM 4-hydroxyestradiol activated the QR-ARE/EpRE luciferase expression 6.0- and 6.8-fold, respectively, while no activation occurred with 5 μM estradiol. Correspondingly, 4-hydroxyestradiol increased endogenous QR activity 5.2-fold, however, 2-hydroxyestradiol had no effect on QR. As expected, estradiol did not increase endogenous QR activity. Thus, the neuroprotective effects of estrogens may not be due solely to their antioxidant properties, but may be a combination of these properties and increased ARE/EpRE-driven gene expression. (Supported by ES08089 and BWF New Investigator Award.)

1294 MOLECULAR GENETIC ANALYSIS OF HORMONE RECEPTOR GENES FROM THE JAPANESE MEDAKA (*ORYZIAS LATIPES*).

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Reproductive dysfunction in humans and wildlife due to chemical exposure will invariably be the result of altered expression of those genes involved in normal reproductive physiology. Using the model teleost Japanese medaka we are investigating the effects of reproductive toxicants on the expression of several genes directly involved in piscine reproduction. These genes include the estrogen receptor (ER), the androgen receptor (AR), and the progesterone receptor (PR). We report the isolation of partial complimentary DNA clones for the AR and PR genes and the isolation of multiple cDNA clones for the ER which represent distinct messenger RNA isoforms derived from a single medaka ER gene. The medaka ER gene is expressed in a tissue-, sex-, and age-dependent manner. The medaka AR and PR cDNA's were cloned from male testis and female ovary, respectively, using RT-PCR with degenerate oligonucleotide primers based on conserved sequences within vertebrate AR and PR genes. The partial medaka AR cDNA clone encodes a putative AR peptide 87% identical to the region of human AR protein from amino acids 618 to 749. The partial medaka PR cDNA clone encodes a putative peptide which is 77% identical to the human PR (from amino acids 833 to 909) but is 96% identical to a reported cichlid (*Tilapia mossambica*) glucocorticorticoid receptor. Both the medaka AR and PR genes are differentially expressed in male and female liver and gonads.

1295 REGULATION OF MOUSE MAJOR URINARY PROTEIN mRNA LEVELS IN C57B6 MOUSE LIVER BY DI(2-ETHYL HEXYL) PHTHALATE AND FOOD RESTRICTION.

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Mouse major urinary protein (MUP) mRNAs are the most abundant mRNAs in male mouse liver. MUP genes are transcriptionally regulated. Although the function of this group of genes has not been established, their abundance, tissue-specific expression, and developmental and multi-hormonal regulation suggest their biological importance. Di(2-ethylhexyl) phthalate (DEHP), a peroxisome proliferator and the most widely used plasticizer, can cause liver cancer in rodents. Food restriction, on the other hand, is proposed to slow down aging and reduce cancers. Using mRNA differential display, RNase protection assay, Northern analysis and quantitative RT-PCR, we found that the MUP mRNA levels in C57B6 mouse livers were down regulated by DEHP and food restriction. This suppression was both dose and time dependent. Mice fed 2% DEHP in the diet consumed 50-70% food of the control group; their liver MUP mRNA expression is almost completely shut down after 4 weeks of treatment. Although DEHP and food restriction have the same effect on the mouse liver MUP mRNA levels, DEHP caused liver hypertrophy, while food restriction significantly decreases the liver/body weight ratio. Since DEHP is proposed to regulate its target genes through the peroxisome proliferator activated receptor PPARα, and food restriction can activate PPARα through the hypothalamus-pituitary-adrenal axis, it is possible that PPARα is the convergence of the two pathways by which DEHP and food restriction regulate MUP gene expression. To elucidate the mechanisms involved, we are currently undertaking an analysis of the promoter of one MUP gene.

1296 EFFECT OF TRICHLOROETHYLENE AND CHLOROACETIC ACIDS ON THE METHYLATION OF THE GENES FOR *C-JUN* AND *C-MYC* IN LIVER AND TUMORS OF FEMALE B6C3F1 MICE.

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Trichloroethylene (TCE) and its metabolites, dichloroacetic acid (DCA) and trichloroacetic acid (TCA) are environmental contaminants and hepatocarcinogens in female B6C3F1 mice. Their mechanism appears to be non-genotoxic involving increase in cell proliferation. DNA methylation controls in part the expression of genes involved in cell proliferation including the immediate-early protooncogenes, *c-jun* and *c-myc*. We have previously reported that mouse liver tumors initiated with methylnitrosourea and promoted by DCA or TCA are DNA hypomethyl-ated (Tao *et al.*, Toxicol Sci. 43, 139-144,1998). Therefore, we evaluated the effect of these contaminants on the methylation status of *c-jun* and *c-myc*. Female B6C3F1 mice were administered 3.2 gm/liter DCA or 4.0gm/liter TCA in their drinking water or 1,000 mg/kg TCE by oral gavage in corn oil for 11 days. DNA was isolated from the liver and the methylation status of c-*jun* promoter and c-*myc* exon II was determined using the Hpa II restriction enzyme (cuts DNA only when internal cytosine of CCGG sites is not methylated) and Southern blot analysis. Decreased methylation of CCGG sites in the *c-jun* promoter and *c-myc* exon II was observed in liver from animals exposed to TCE, DCA or TCA for 11 days. Furthermore, we report that the DCA and TCA promoted liver tumors including adenocarcinomas promoted by TCA from the study cited above also contain decreased levels of methylation of c-*jun* and c-*myc*. Thus, subchronic exposure to DCA, TCA and TCE appears to produce a decrease in the methylation of *c-jun* and *c-myc* that is maintained during promotion and progression to cancer. (Supported in part by US EPA Grant No. R 825384-01-0.)

1297 EFFECT OF TRICHLOROETHYLENE ON DNA METHYLATION AND THE EXPRESSION OF EARLY-INTERMEDIATE PROTOONCOGENES IN THE LIVER OF B6C3F1 MICE.

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Trichloroethylene is a multimedia environmental pollution that is carcinogenic in mouse liver. The ability of trichloroethylene to modulate cell proliferation, DNA methylation and expression of immediate-early protooncogenes was evaluated. Female B6C3F1 mice were administered 1,000 mg/kg trichloroethylene by oral gavage and 5 days/week. The mice were euthanized after 1, 4, 11, 32 or 300 days of exposure. The liver/body weight ratio and the Proliferating Cell Nuclear Antigen (PCNA)-Labeling Index in the liver were increased by 4 days of treatment with trichloroethylene. The liver/body weight ratio but not the PCNA-Labeling Index remained increased by treatment of longer duration. DNA was isolated, hydrolyzed with perchloric acid and 5-methylcytosine and the four bases separated and quantified by HPLC. Total DNA methylation was expressed as the percentage of cytosine present as 5-methylcytosine in the DNA. The extent of 5-methylcytosine was decreased during the evaluated 4-32 days of trichloroethylene exposure. After a single dose of trichloroethylene, the expression of the mRNA of *c-jun* increased for 90 minutes and then decreased by 120 minutes; of *c-myc* continued to increase during the 120 minutes; and of *c-fos* was not detected. After 300 days of exposure, trichloroethylene still increased expression of the mRNA for c-*jun* and c-*myc*. Thus, while trichloroethylene only induced a transient increase in cell proliferation, it caused chronic DNA hypomethylation and increased expression of *c-jun* and *c-myc*. It is possible that these chronic effects of trichloroethylene could result in increase cell proliferation and promotion in initiated hepatocytes in contrast to the majority of cells. (Supported in part by US EPA Grant No. R 825384-01-0.)

1298 GENE EXPRESSION ANALYSES IN UROEPITHELIAL CELLS FROM AN ARSENATE EXPOSED WORKER POPULATION.

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Arsenate exposure is associated primarily with skin, lung, and bladder cancer in humans. The purpose of this study was to examine the feasability of using gene expression analyses as a biomarker of arsenate exposure by examining this endpoint in uroepithelial (UE) cells. Slovakia power plant workers can be exposed to relatively high airborne levels of arsenic during boiler cleaning processes resulting in urinary excretion of arsenic metabolites. UE cells were isolated from a small number of workers before they began the cleaning process and again after 5 days of exposure using a protocol developed for field lab conditions, and shipped to our laboratory for anlaysis. The cells were carried through RNA amplification and hybridized to reverse northern blots. Genes included for analysis were those involved in stress responses (HSPs 25,60,70 and metallothionein I and II); cell cycling (Cyc A ,Cyc B, p21, p53) and DNA damage (Gadd 45 and 153). Control genes included were actin, puc18 and G3PDH. Approximately 80% of the samples successfully amplified and hybridized with fold amplification ranging from 1.4 to 71. Expression levels for each gene have been determined. Analysis is being carried out to assess the magnitude of the association(s) between specific gene expression changes and exposure as estimated both by the concentration of arsenic metabolites in urine and by individual occupational breathing zone arsenic concentrations. (Supported by USDOE #DE-FC01-95EW 55084 and EPRI #WO3370-23.)

1299 NICKEL ENHANCES TELOMERIC SILENCING IN *Saccharomyces cerevisiae.*

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Certain nickel compounds including crystalline nickel sulfide (NiS) and subsulfide (Ni_3S_2) are potent human and animal carcinogens. Nickel induced the inactivation of the *gpt* reporter gene in a transgenic *gpt+* Chinese hamster cell line (G12), which is located near an heterochromatic region, by chromatin condensation and DNA methylation processes. To determine if nickel can cause gene silencing independently of DNA methylation, based only on the induction of changes in chromatin structure, we measured its influence on the TPE (telomeric position effect) levels in the yeast *Saccharomyces cerevisiae*. We demonstrate that nickel chloride repressed a telomeric marker gene (URA3) and caused a stable epigenetic switch. This phenomenon is dependent on the number of cell doubling prior to selection and also on the distance of the marker gene from the end of the chromosome. The level of TPE induction increased linearly by elevation of nickel concentration. Magnesium inhibited this effect, but it did not silence the reporter gene by itself. The level of silencing was also assessed following treatment with other transition metals: cobalt, copper and cadmium. In the sublethal range, cobalt induced similar effects to nickel, while copper and cadmium did not change the basal level of gene suppression, suggesting that nickel and cobalt are active in gene silencing while copper and cadmium are not.

1300 MODULATION OF SULFUR MUSTARD STRESS GENE INDUCTION BY ANTI-INFLAMMATORY AGENTS.

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Sulfur mustard (SM) exposure to human skin can produce delayed skin vesication by unknown mechanism(s). Recent studies have found that certain anti-inflammatory compounds have lessened or eliminated skin pathology occurring within the mouse ear 24 h after SM exposure. We were interested in the modulatory effects of three classes of anti-inflammatory compounds on specific stress gene responses produced by SM. Transformed HepG2 cells stably expressing chloramphenicol acetyl transferase (CAT) through a specific promoter or response element were used as model human cells for SM exposure (CAT Tox (L) Xenometrix, Boulder, CO). Cells were treated with 20mM indomethacin (INDO), 1mM dexamethasone (DEX) or 1mM olvanil (OV) for 1h prior to addition of 12.5 to 200μM SM. The medium mixture (100μL/96-plate well) was permitted to incubate at 37°C for 24h for CAT induction. Comparison of DEX treatment to SM alone showed a significant decrease in the activity of the promoter for 45kDa growth arrest and DNA damage protein (GADD45) and decreased activity of the response elements for the tumor suppressor gene p53 (p53RE), retinoic acid receptor (RARE) and cyclic AMP (CRE). INDO treatment decreased the promoters for 78kDa glucose regulated protein (GRP78) and GADD45 and decreased in the response elements for p53RE and CRE. OV treatment decreased metallothionein IIA, cytochrome P450 1AI, 153kDa growth arrest and DNA damage protein (GADD153), GADD45, and 70kDa heat shock protein (HSP70) promoters and decreased p53RE and CRE. OV produced a shift in nuclear factor kappa B requiring a higher SM concentration to produce a maximum response. These preliminary data suggest OV had the greatest effect to lower HD induced stress genes.

1301 ALDEHYDE DEHYDROGENASES: A GROWING GENE SUPERFAMILY.

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Aldehyde dehydrogenases (ALDHs) represent a superfamily of $NAD(P)^+$-dependent enzymes having similar primary structures that catalyze the oxidation of a wide spectrum of endogenous and exogenous aliphatic and aromatic aldehydes. Aldehydes are highly reactive molecules that are very important in numerous biologic and pharmacologic processes. Aldehyde-mediated effects vary from physiologic to cytotoxic, genotoxic, mutagenic and/or carcinogenic. The last five years, it has become evident that ALDHs represent a large superfamily. At the latest count, August 1988, a total of 164 ALDH cDNAs/genes have been described across various species including 4 in archaebacteria, 71 in bacteria, 18 in plants, 16 in fungi and 55 in animals. In addition, as of August 1998, a total of 2,330 ALDH-like expressed sequence tags (ESTs), or 0.13 % of the total EST database, have been generated by the Genome Projects including the Human. Evolutionary analysis of the eukaryotic ALDHs leads to the definition of 18 gene families and 37 subfamilies. The percent identities for two members of the same family and of the same subfamily are 40% and 60%, respectively. Of the 37 subfamilies, 13 and 4 have been mapped to the human genome and the mouse genome, respectively. This evolutionary-based scheme was used for the nomenclature system of the eukaryotic ALDHs. (Supported by NIH Grant EY11490 and NIAAA Training Grant 5T32AA07464.)

1302 THE CO-PLANAR PCB CONGENER 3,3',4,4',5-PEN TACHLOROBIPHENYL IS UTEROTROPIC IN PREPUBERTAL RATS.

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Co-planar (CP) PCB congeners, inactive in *in-vitro* neural preparations and the adult animal, elevate brain dopamine (DA) concentrations in rats following developmental exposure (TAP, 146, 1997). To elucidate the mechanisms responsible for these elevations we have examined the potential estrogenic actions of ip administration of the CP PCB congener 3,3',4,4',5-pentachlorobiphenyl (PentaCB) on uterine wet weight (UWW) in prepubertal female rats. Rats were injected ip on postnatal days (PND) 21-23 with corn oil (vehicle control), 17ß-estradiol (E_2, 20 *ug*/kg, PND 22 and 23), PentaCB (64 to 400 *ug*/kg, PND 21 and 22) only or in combination with the estrogen receptor blocking agent (ICI 182,780, 0.5 mg/kg, PND 21-23) and were sacrificed on PND 24. Uteri were removed, cleaned of connective tissue and weighed in a blinded manner. Compared to control animals, E_2 increased UWW by approximately 250% while PentaCB increased UWW in a dose-dependent manner with a maximal response of 150% of vehicle control. Co-administration of ICI-182,780 with PentaCB reduced UWW to levels similar to vehicle control values. These results demonstrate that PentaCB or its metabolites increase UWW via the uterine estrogen receptor, although PentaCB is approximately two order of magnitude less potent than E_2. Thus, the elevations in brain DA seen in offspring following exposure of the dam to CP PCB congeners during pregnancy may, in part, be due to exposure of the fetal brain to estrogen-agonists. (Supported in part by NIH grant (ESO4913) to RFS.)

1303 STIMULATION OF [^{3}H]ARACHIDONIC ACID RELEASE IN RAT CEREBELLAR GRANULE NEURONS BY POLYCHLORINATED BIPHENYLS: STRUCTURE-ACTIVITY RELATIONSHIPS AND POSSIBLE MECHANISM(S).

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Previously, we have demonstrated significant stimulation of [^{3}H]arachi-donic acid (AA) release by Aroclor 1254 and the *ortho*-substituted 2,2'-dichlorobiphenyl -(DCB), but not by non-*ortho* substituted 4,4'-DCB. This [^{3}H]AA release seems to be mediated by activation of phospholipase A_2 (PLA_2). The objective of this study is to extend structure-activity rela-tionship (SAR) among polychlorinated biphenyl (PCB) congeners, their metabolites, and also to delineate the possible mechanism(s) by which 2,2'-DCB stimulated the release of [^{3}H]AA. Cerebellar granule cells at seven days in culture, obtained from 6-7 day old rat pups, were labeled with [^{3}H]AA for 16-20 h and exposed to pharmacological agents and/or PCBs. In agreement with previous results, other *ortho* PCBs such as 2,2',5,5'-tetrachlorobiphenyl (TeCB), 2,2',3,5',6-pentachlorobiphenyl (PeCB), and 2,2',4,4',5,5'-hexachlorobiphenyl (HCB) significantly stimu-lated [^{3}H]AA release at 10-30 μM while 3,3',4,4'-TeCB and 3,3',4,4',5-PeCB were not effective even at 50 μM. 2,2',4,5,5'-Pentachloro-4-biphe-nylol is as active as its parent compound, 2,2',4,4',5,5' HCB suggesting that hydroxy-metabolites of *ortho*-PCBs are equally active. As various types of PLA_2 exist in brain, we characterized the effects of 2,2'-DCB. Pretreatment with EGTA/AM, cell permeable Ca^{2+} chelator, only parti-ally blocked the response. Also, dantrolene (50 μM) and xestospongin C (10 μM), blockers of intracellular Ca^{2+} release from endoplasmic reticu-lum, partially reduced 2,2'-DCB-induced [^{3}H]AA release. 4-Bromo-phenacylbromide (1-30 μM) did not attenuate the 2,2'-DCB-stimulated [^{3}H]AA release, indicating that secretory PLA_2 is not the primary path-way. On the other hand, methyl arachidonyl fluorophosphonate (5 μM) completely inhibited [^{3}H]AA release suggesting the involvement of cytosolic PLA_2s. These results confirm our previous SAR data and [^{3}H]AA release caused by *ortho* PCBs might be due to activation of cytosolic PLA_2 that is Ca^{2+}-dependent as well as Ca^{2+}-independent.

1304 MATERNAL EXPOSURE TO AROCLOR 1254 IMPAIRS SPATIAL LEARNING AND MEMORY IN MALE OFFSPRING.

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Developmental exposure to polychlorinated biphenyls (PCBs) has been associated with cognitive deficits in children. The current study examined the effects of gestational and lactational exposure to a commercial PCB mixture, Aroclor 1254 (A1254), on spatial learning and memory using the radial arm maze (RAM) and spatial discrimination-reversal learning (RL). Pregnant Long-Evans rats (10/dose) received 0 or 6 mg/kg A1254 (po in corn oil) from gestational day 6 to postnatal day 21. After they reached adulthood, one male and one female from each litter were tested on a working/reference memory task using a 12-arm RAM. Eight of the 12 arms were baited, with the pattern of baited arms remaining the same on every trial. Following RAM testing, the same animals were tested on a spatial discrimination-reversal learning (RL) task in standard 2-lever operant testing chambers. After a criterion of 85% correct on 2 consecutive days was achieved, five position reversals were given. Compared to controls, the A1254-exposed males made significantly more working memory errors (4.58 ± 0.19 and 5.45 ± 0.32 errors for control and A1254 males, respectively) and reference memory errors (3.88 ± 0.18 and 5.01 ± 0.40 errors for control and A1254 males, respectively) during acquisition of the RAM, and tended to take more trials to reach criterion on the first reversal of the spatial RL task.

In contrast, A1254-exposed females were not impaired relative to control females on either the RAM or spatial RL. These data suggest that perinatal exposure to A1254 may cause sex specific deficits in spatial learning and memory. (Supported by NIEHS ES07921 and EPA R825812.)

1305 COMPARATIVE EFFECTS OF POLYCHLORINATED BIPHENYLS ON NITRIC OXIDE SYNTHASES IN RAT BRAIN REGIONS DURING DEVELOPMENT.

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Polychlorinated biphenyls (PCBs) have been reported to affect learning and memory during development. Also, PCBs have been shown to inhibit long-term potentiation (LTP)which is a physiological mechanism for synaptic plasticity and neuronal growth. Nitric oxide synthases (NOSs) mediate the production of nitric oxide, a neurotransmitter that has important role in LTP as well as learning and memory. The objective of this study is to unravel the molecular mechanism for the PCB effects on learning during development. We have compared the effects of *ortho* PCB, 2,2'-dichlorobiphenyl (-DCB) with that of non-*ortho* PCB, 4,4'-DCB on cytosolic and membrane NOSs from cerebellum, hypothalamus and hippocampus of PND 7, PND 21, and adult male Long-Evans rats. Among the three brain regions in adults, basal levels of cytosolic and membrane NOSs were highest in cerebellum and lowest in hippocampus. However, hypothalamus was more sensitive to 2,2'-DCB (IC_{50}s for cytosolic NOS; 15 μM for hypothalamus, 30 μM for cerebellum, > 50 μM for hippocampus). The membrane NOS of all three brain regions was inhibited to a maximum of only 15-20%. During development, the basal levels of cytosolic and membrane NOSs were low in all the brain regions of PND7 animals but increased several folds in PND 21 and adults. In all three brain regions, NOSs in PND7 animals (IC_{50}s = > 50 μM) were less sensitive to 2,2'-DCB than those of PND21 and adults (IC50s=15-30 μM). 4,4'-DCB did not have any effect on either cytosolic or membrane NOS of all brain regions of young and adult animals. These results indicate: (1) non-coplanar PCB was active but not the coplanar PCB; (2) cytosolic NOS was more abundant than membrane NOS in all brain regions; (3) age- and brain region-specific expression of NOSs and their differential sensitivity to 2,2'-DCB may play a role in the neurotoxic effects of PCBs during development (NRC SRA award to RS; This work does not necessarily reflect EPA policy).)

1306 NONCOPLANAR PCBS CAN DISCRIMINATE BETWEEN GENETIC ISOFORMS OF MICROSOMAL IMMUNOPHILIN/ Ca^{2+} CHANNEL COMPLEXES.

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ortho-Substituted noncoplanar PCB congeners have been shown to interact with ryanodine-sensitive Ca^{2+} release channels (ryanodine receptors; RyR) localized within microsomal membranes and to alter cellular Ca^{2+} signaling. Both at the cellular and subcellular levels, the mechanism appears to require the integrity of a complex between the RyR channel protein and the major T-cell immunophilin FKBP12 (Wong *et al.*, 1999). Using the specific [^{3}H]ryanodine binding assay we have further examined the structure-activity relationship amongst 20 environmentally relevant noncoplanar PCBs toward two of the three genetic isoforms of RyR. A very stringent structural requirement was observed which revealed that three *ortho* chlorines were needed to produce full agonist activity towards both Ry1 and Ry2 isoforms. Significantly, Ry2, the major isoform in heart and brain, was found to be 2-5 fold more sensitive than Ry1 to PCB 41(2,3,4,2'; EC_{50}= 710 nM), PCB 132 (2,3,4,2',3',6'; EC_{50}= 370nM), PCB 138 (2,3,4,2',4',5'; EC_{50} =1.6 μ M), and PCB 170 (2,3,4,5,2',3', 4'; EC_{50}=745nM). By contrast, Ry1, the major form in skeletal muscle and the minor form in neurons, was significantly more sensitive to PCB 95 (2,3,6,2',5'; EC_{50}=350nM) and 149 (2,3,6,2',4',5'; EC_{50}=750nM) than Ry2. These data show that non-coplanar PCBs can discriminate between genetic isoforms of the major Ca^{2+} induced Ca^{2+} release channels found in excitable cells. (NIH ES05002 and ES05707)

1307 DOWN-REGULATION OF GLUCOCORTICOID RECEPTOR BY POLYCHLORINATED BIPHENYL (PCB) AROCLOR-1254 IN THE RAT.

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Our laboratory has previously shown that PCB down-regulates type II glucocorticoid receptor (GR) in a variety of tissues in the rats at the level of transcription, leading to a reduction of GR protein. The current study examines the modes of action for PCB on GR regulation. Since Aroclor-1254 is known to induce hypothyroidism, we investigated whether the GR down-regulation was related to thyroid hormone deficiency. Sprague-Dawley rats were rendered hypothyroid with propylthiouracil (PTU,10 mg/L in drinking water) for 16 days and tissue GR levels were evaluated. Despite the profound reduction of circulating T_4 level in the PTU-treated rats, GR mRNA in hippocampus and pituitary were not significantly different from controls, indicating that the PCB-induced GR down-regulation was not mediated by hypo-thyroidism. To evaluate whether PCB might alter GR through feedback regulation of the HPA axis, Aroclor-1254 (24 mg/kg, *p.o.*) was given to rats for 16 days and serum corticosterone (CORT) and ACTH were measured. Aroclor-1254 decreased GR mRNA and protein but did not significantly alter the circulating CORT or ACTH, suggesting that PCB may exert a direct effect to regulate GR. To confirm this hypothesis, the effect of PCB on GR was examined in rat pituitary cell cultures. Cells were exposed to Aroclor-1254 (100 ppm) for 24 h while controls were treated with or without DMSO. DMSO did not alter cell viability or GR mRNA level. In contrast, Aroclor-1254 markedly reduced GR mRNA, by 45% compared to DMSO-control. Thus, these data suggest that Aroclor-1254 alters GR regulation by a direct mechanism. (This abstract does not reflect US EPA policy.)

1308 DIFFERENTIAL EFFECTS OF AROCLOR 1254 MIXTURES WITH TWO LOT NUMBERS: INTRACELLULAR CALCIUM BUFFERING AND PROTEIN KINASE C TRANSLOCATION IN RAT BRAIN.

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Aroclor 1254 is a widely studied commercial polychlorinated biphenyl (PCB) mixture which, by definition, contains 54% chlorine by weight. Recent reports indicate substantial differences in the congener composi-tion among Aroclor lots and hence, their biological effects. The current study was designed to compare the effects of two lots of Aroclor 1254 (Lot #s 6024 and 124-191; AccuStandard). These two lots were analyzed for all 209 PCB congeners, dibenzofurans and any dioxins. For testing their biological activity, previously established techniques on intracell-ular Ca^{2+} buffering and protein kinase C (PKC) translocation were used. Results indicate that *ortho* and non-*ortho* congeners in these two lots varied in their percent contribution. Among all congeners, the percentage of non-*ortho* congeners (PCBs 77, 81, 126, and 169) were higher in Lot # 6024 (3.07% of total) versus Lot # 124-191 (0.02% of total). Dioxins were not detected in these two lots (<2 ppb). However, dibenzofurans were also higher in Lot # 6024 (38.7 ppm) versus Lot # 124-191 (11.3 ppm). When these two Aroclors were tested on Ca^{2+} buffering and PKC translocation, the effects were significantly different. While Lot # 124-191 was more potent on PKC translocation than Lot # 6024, the effects were almost similar on Ca^{2+} buffering. These effects could not be attributed to the differences in the percentage of non-*ortho* congeners or dibenzofurans, since they were inactive on these two parameters. However, these differential effects could be due to differences in the composition of *ortho*- congeners in these two mixtures. Because of these differential effects with different lot numbers, the composition of Aroclor mixtures used in investigations should be disclosed.

1309 DIFFERENTIAL EFFECTS OF AROCLOR 1254 WITH TWO LOT NUMBERS: HEPATIC ENZYME INDUCTION AND CIRCULATING THYROID HORMONE LEVELS.

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Toxicity studies with Aroclor 1254 mixtures have given different results.

Aroclor 1254 is defined by the weight percentage of chlorine. However, the polychlorinated biphenyl (PCB) congener composition varies from lot to lot. Two lots which have been used in toxicity studies, #124-191 and #6024 (AccuStandard), were analyzed for their congener composition. Lot #6024 has approximately ten times the dioxin toxic equivalents (TEQ) of Lot #124-191. The purpose of this study is to determine if the difference in the TEQ for the two lots explains the different responses seen on a weight basis. Male Long Evans rats (70 days old) were treated with 0 to 1000 mg/kg po of each lot. Hepatic EROD, MROD, PROD and serum T3/T4 concentrations were determined. On a weight basis, Lot #6024 led to greater responses for EROD, MROD, PROD and T3/T4. The differences in TEQ explained the values for EROD and MROD but did not account for the lower response of Lot #124-191 for PROD or T3/T4. The PROD effects are not due to dioxin-like congeners. The T3/T4 effects are due to multiple mechanisms. These results illustrate that the differences in the TEQ explains the differences in the dioxin-like effects, but the non-dioxin-like congeners cause other effects that are not associated with the Ah receptor. Thus, overall toxicity cannot be entirely predicted based on the TEQ values, and in the future it is vital that the lot number and congener composition are reported in studies conducted with Aroclor 1254. (This abstract does not necessarily represent EPA policy. DEB supported by NIEHS T32-ES07126 and EPA, CT902908.)

1310 PCB95 ALTERS METABOLISM IN PC12 CELLS WITHOUT DIRECTLY AFFECTING RESPIRATION IN ISOLATED RAT BRAIN MITOCHONDRIA.

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Previously we reported that certain *ortho*-substituted PCB congeners, including PCB95, selectively alter Ca^{2+} signaling in rat pheochromocytoma cells (PC12) through an immunophilin-dependent ryanodine-sensitive pathway. We further investigated the effect of PCB95 on PC12 cells by using a pH-sensitive fluorescence dye (BCECF) and the photoimaging technique. PC12 cells loaded with BCECF were individually monitored for changes in cytosolic pH by measuring emission ratio at 530nm with excitation at 490nm/439nm. Fluorescence was calibrated by perfusing the cells with pH4 buffer followed by pH9 buffer. Perfusion of 5μ M PCB95 in the cell chamber which was shown to significantly mobilize Ca^{2+} from ryanodine-sensitive stores (Wong *et al.*, 1998), initiated intracellular acidification that coincided with altered Ca^{2+} signaling and persisted for >7min (at 7min, Δ pH = – 0.33± 0.04, n = 15 cells). Pre-incubation of cells with FK506 eliminated PCB95-induced acidification and Ca^{2+} transients. In order to further discriminate the mechanism by which PCB95 alters cellular metabolism, we measured the rate of O_2 consumption and coupling of mitochondria isolated from rat brain, using O_2 polarography. At all stages of respiration, the presence of PCB95 or PCB126 in the mixing chamber did not cause any significant changes in O_2 consumption rate. Moreover, calculation on ADP/O_2 ratio, in the presence of either PCB95 or PCB126, demonstrated a tight coupling of the isolated mitochondria. These results are consistent with a highly selective receptor mediated interaction between non-coplanar PCBs and microsomal FKBP12/RYR initiates alterations in neuronal cellular metabolism. (NIH ES05002 and ES05707)

1311 INTERACTION AMONG SIGNAL TRANSDUCTION PATHWAYS IN ACTIVATION OF RAT NEUTROPHILS BY POLYCHLORINATED BIPHENYLS.

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Stimulation of rat polymorphonuclear neutrophils (PMNs) by polychlorinated biphenyls (PCBs) requires a number of intracellular signals including increased intracellular free calcium, activation of phospholipase A_2 (PLA_2) and activation of tyrosine kinase. The sequence of activation of these pathways and their interdependence are unknown. The purpose of these studies was to examine the interaction among several pathways involved in activation of PMNs by PCBs. Isolated, rat PMNs were labeled with ^{3}H-arachidonic acid (^{3}H-AA), and activation of PLA_2 was determined from release of radioactivity into the medium. Exposure to the PCB mixture Aroclor 1242 induced release of ^{3}H-AA, and pretreatment with genistein, an inhibitor of tyrosine kinase, caused a small but significant decrease in this release. Daidzein, a genistein analog with no activity to inhibit tyrosine kinase, had no effect on ^{3}H-AA release. An inhibitor of mitogen-activated protein kinase (MAPK), SB203580 did not affect Aroclor-induced PLA_2 activity; however, PD98059, which inhibits MAPK kinase (MEK), decreased ^{3}H-AA release to about the same extent as genistein. Staurosporine, an inhibitor of PKC, did not inhibit PCB-induced release of ^{3}H-AA. To examine interaction between calcium signalling and PLA_2, the effects of TMB-8, a blocker of intracellular Ca^{2+} release, and two different calmodulin inhibitors, W7 and trifluoroperazine (TFP), were determined. Aroclor-induced release of ^{3}H-AA was inhibited by TMB-8 and by TFP but not by W7. Moreover, TFP by itself induced a release of ^{3}H-AA release, an increase in intracellular calcium and functional changes similar to Aroclor 1242. These results suggest that tyrosine phosphorylation and the MEK pathway, as well as release of intracellular calcium, play a role in PCB-induced activation of PLA_2. (Supported by ESO4911.)

1312 ALTERATIONS IN INTRACELLULAR FREE CALCIUM CONCENTRATION INDUCED BY POLYCHLORINATED BIPHENYLS IN RAT NEUTROPHILS.

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Polychlorinated biphenyls (PCBs) stimulate polymorphonuclear neutrophils (PMNs) acting on different subcellular targets. The objective of this study was to characterize the effects of PCBs on Ca^{2+} homeostasis in rat PMNs. Aroclor 1242, a technical PCB mixture, induced a dose- and time-dependent increase in the concentration of intracellular free Ca^{2+} ($[Ca^{2+}]_i$) in Fura 2/AM-labeled PMNs. A significant increase in $[Ca^{2+}]_i$ was observed only after 4 min, reaching a plateau after 13-15 min. The effects of PCBs on $[Ca^{2+}]_i$ were structure-dependent: 2,4'-dichlorobiphenyl, an ortho-substituted congener, increased $[Ca^{2+}]_i$ with a similar time course as Aroclor 1242, whereas 3,3',4,4'-tetrachlorobiphenyl, a coplanar congener, was inactive. The dose-response curve for 2,4'-dichlorobiphenyl revealed a biphasic response in which increases in $[Ca^{2+}]_i$ were observed at 10-100 nM and 10 μM but not with 1 μM. The Aroclor 1242-induced increase in $[Ca^{2+}]_i$ was not dependent on extracellular Ca^{2+}, implicating an intracellular Ca^{2+} pool as the source released by PCBs. The PMN stimulus, fmlp, causes a rapid increase in $[Ca^{2+}]_i$ from inositol trisphosphate-sensitive pools, and this increase was abrogated by Aroclor 1242 in a time-dependent manner. These results suggested a shared intracellular calcium store for fmlp and Aroclor 1242. In summary, the PCB-induced increase in $[Ca^{2+}]_i$ in PMNs is dose-, time- and structure-dependent, and the Ca^{2+} pool released may be the same store as that released by fmlp (Supported by ESO4911.)

1313 CYTOTOXICITY AND AROMATASE (CYP19) ACTIVITY MODULATION BY ORGANOCHLORINES IN HUMAN PLACENTAL JEG-3 AND JAR CHORIOCARCINOMA CELLS.

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The human placental JEG-3 and JAR choriocarcinoma cell lines have been used extensively as models for placental endocrine functions. These cell lines have been used to a limited extent to measure the effect of xenobiotics on the modulation of aromatase (CYP19) enzyme activity. Widely varying compound- and concentration-dependent (1.0 nM to 10 μ M) effects were observed for twenty-one, environmentally persistent organochlorine (OC) compounds and their hydroxyl (HO-) and methyl sulfonyl- ($MeSO_2$-) containing metabolites, on the cytotoxicity, cell proliferation and aromatase activity. In JEG-3 cells, polychlorinated biphenyl (PCB) 126 (3,3' ,4,4' ,5-Cl_5) and benzo[*a*]pyrene (B[*a*]P), PCB-52 (2,2' ,5,5' -Cl_4), and -70 (2,3' ,4' ,5-Cl_4), tris(4-chlorophenyl)methane (TCPMe), TCPMeOH, 3- and 4-$MeSO_2$-PCB metabolite compounds of PCB-52, -70, -87 (2,2' ,3' ,4' ,5-Cl_5) and -CB101 (2,2' ,4' ,5,5' -Cl_5), and the metabolite compound 4-OH-CB30 (2' ,4' ,6' -Cl_3), induced concomitant decreases in the aromatase activity, protein level and DNA content, and increases in the percent lactate dehydrogenase leakage at dosing concentrations as low as 1.0 nM . This similarity to 1,4-naphthaquinone indicated an OC-mediated modulation of cytotoxicity and cell proliferation, and therefore an indirect modulation of the aromatase activity. The effects were dependent on the cell density, and were largely negated by the presence of 10% fetal calf serum. Binding of the apoptotic cell-specific APO2.7, DNA fragmentation and trypan blue staining after OC treatment suggested an apoptotic mechanism of cytotoxicity. The JEG-3 and JAR cell lines are too unstable for use as *in vitro* biomarkers to evaluate the potential, xenobiotic-mediated modulation of aromatase activity. These cell

lines may prove useful for examining the capacity of xenobiotics to modulate placental toxicities.

1314 DIFFERENTIAL EFFECTS OF PCB CONGENERS ON RAT THYROID FUNCTION.

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Circulating levels of thyroid hormones can be reduced by a number of compounds, including polychlorinated biphenyls (PCBs). Numerous studies have been conducted using mixtures of PCB congeners known as Aroclors, but little is known about the effects of individual congeners. The objective of this study was to determine the effects of PCB congeners on serum levels of thyroxine (T_4) and triiodothyronine (T_3). PCB congeners are commonly divided into three general categories. The "2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) type" are those congeners that have affinity for the aryl hydrocarbon receptor (AhR) and induce cytochrome P450 1A1 (CYP1A1); the "phenobarbital type" are those congeners that induce CYP2B1, and the "mixed type" are those cogeners capable of induction of both CYP1A1 and CYP2B1. Four PCB congeners were selected for use in this study: PCB 126, a "TCDD type", PCBs 95 and 99, which are "PB types", and PCB 118, a "mixed type". Arocolors 1242 and 1254 and TCDD were also evaluated. Each compound was administered at a minimum of four dose levels *via* gavage to male Sprague-Dawley rats for seven days. Blood was obtained *via* the retro orbital sinus on days -3, 3, and at necropsy on day 7. On day 7, serum T_4 levels were reduced to 5-10% of control values in rats administered Aroclor 1254 and PCBs 99 and 118. Reductions up to 50% of control values occurred for rats administered each of the remaining compounds. Serum T_3 levels were reduced to 30% of control values in rats administered Aroclor 1254. The remaining compounds caused only slight or no reductions in serum T_3. Results of this study suggest that PCB congeners may induce the majority of their effects on thyroid function *via* pathways not mediated by the AhR. (Supported by NIH grant ES-07079 and EPA Grant R826297.)

1315 ALTERATIONS OF STEROID HORMONE METABOLISM IN JUVENILE CD-1 MICE BY PCB153.

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Several classes of xenobiotics, including the polychlorinated biphenyls (PCBs), are known to perturb the metabolism of steroid hormones by induction and/or repression of cytochrome P450 isozymes responsible for the hydroxylation of these compounds. Modulation by PCBs of CYP isoforms which hydroxylate testosterone has been investigated primarily in the rat. Less studied has been the effects of individual PCB congeners on steroid metabolism in the mouse, and the effects of these toxicants on estradiol as well as testosterone metabolism. In this study, juvenile (30 day old) male CD-1 mice were dosed with PCB 153 (a non-coplanar, phenobarbital-type P450 inducer) in their diet for 7 days at 5 mg/kg/day. This PCB congener was shown to be a potent inducer of Cyp2b-10 protein and a weak inducer of Cyp3a protein. PCB treatment induced the hydroxylase activity associated with the 2-hydroxy metabolite of estradiol, while concurrently repressing the 16-α estradiol hydroxylase. *In vitro* testosterone hydroxylation using hepatic microsomes from PCB treated mice revealed an increase in 6-α and 16-β hydroxylase activities. In addition, seminal vesicle weights of the PCB treated mice were significantly lower than those of control mice. Taken together, these data suggest effects of PCBs on steroid hormone metabolism in the mouse which are different from those published for the rat, and that altered metabolism of testosterone may have *in vivo* consequences. Continuing studies will determine the effects of PCB congeners which have differing P450 induction profiles on steroid metabolism and reproductive parameters in male and female mice.

1316 CATECHOL METABOLITES OF POLYCHLORINATED BIPHENYLS (PCBS) INHIBIT THE CATECHOL-O-METHYL TRANSFERASE-MEDIATED METABOLISM OF CATECHOL ESTROGENS.

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The catechol metabolites of estradiol are potent signalling molecules and are hypothesized to be central to estrogen-linked carcinogenesis. Methylation by catechol-O-methyltransferase (COMT) is the principal means of catechol estrogen (CE) deactivation in the liver and other tissues. The present studies were conducted to determine the effects of PCBs and catechol PCB metabolites on the COMT-mediated catabolism of 4- and 2-hydroxyestradiol (4-OHE_2 and 2-OHE_2, respectively) in vitro and in vivo. Liver homogenates of female SD rats treated with Araclor 1254 for 21 days (5 mg/kg/day) showed a 30% and 40% reduction of COMT activity towards 2-OHE_2 and 4-OHE_2, respectively. Incubation of [^{3}H]-β-estradiol with these same liver homogenates, followed by HPLC analysis demonstrated an elevation of CEs and a nearly complete reduction in levels of methylated catechol estrogens. In classical enzyme kinetics studies, the catechol PCBs were demonstrated to be good substrates for COMT, with K_ms approximately equivalent to those of the CEs. Catechol PCBs were also mixed -type inhibitors of the O-methylation of CEs. The data suggest that PCBs significantly alter the metabolism of catechol estrogens in vivo and that this effect may be mediated by catechol PCB metabolites. Further, methyltransferase inhibition by PCB catechols may contribute to PCB-mediated endocrine effects and liver carcinogenesis.

1317 POLYCHLORINATED BIPHENYLS (PCBs) MEASURED IN A GRADE SCHOOL FOLLOWING A FLUORESCENT LIGHT BALLAST FAILURE.

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A series of field investigations was performed following an incident in which a fluorescent light fixture failed resulting in overheating, leaking, and release of fumes from a PCB-containing ballast in a private elementary school that was built in 1961. Investigations performed shortly after removal of the failed ballast revealed trace levels of Aroclor 1254 (0.1 to 0.5 ug per 400 cm^2) in 16 of 19 rooms. No measurable airborne PCBs were observed using NIOSH Method 5503, although the detection limits were high ranging from 28,000-42,000 ng/m^3. Ballasts throughout the school were then removed and checked for PCB content and leakage. In total, 91 of 121 ballasts were identified as containing PCB fluids, 30 of the 91 PCB ballasts were leaky to some degree, and 11 of the 19 rooms in the school had at least one leaky PCB ballast. Following the incident, all PCB ballasts were replaced with non-PCB ballasts, and all accessible surfaces of the building and furniture were cleaned thoroughly with detergent and rinsed. Studies six months later using more sensitive sampling and analysis techniques (polyurethane foam, high volume sampler/EPA Method 8080; detection limit 0.5 ug/m^3) revealed the presence of airborne PCBs with patterns resembling Aroclors 1248 and 1254 at total PCB concentrations ranging from 170 to 368 ng/m^3. The source(s) of these airborne PCBs were not related to outdoor air or concurrent electrical equipment in the building. These findings appear to be consistent with other published reports examining the distribution and occurrence of PCBs in public buildings and homes with PCB-containing electrical equipment.

1318 90-DAY INHALATION TOXICITY OF 1,1-DIFLUORO-1,2,2-TRICHLOROETHANE (HCFC-122) IN RATS.

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The toxicity of HCFC-122 was evaluated by exposing Crl:CD® (SD) BR male and female rats to 0, 25, 250, or 750 ppm for 90 days. Body weights and food consumption were measured weekly. Clinical pathology evaluations were conducted after 37 and 85 days on test. Morphological, hormonal, and hepatic biochemical evaluations were conducted after 90 days. Female rats exposed to 750 ppm had significantly lower mean body weights and body weight gains during the exposure period. Compound-related increases in liver weights were present in the 250 and 750 ppm groups. Microscopically, these liver weight changes were associated with minimal hepatocellular hypertrophy, centrilobular apoptosis, and increased pigment. Hypertrophy was typically characterized by minimal increases in cytoplasmic area and loss or decrease in the normal basophilic stippling of hepatocytes. In males, centrilobular hepatocytes often had clear granular swelling and, in some animals, variably-sized, clear vacuoles. Similar cytoplasmic granularity has been noted in association with peroxisome proliferation. The presence of apoptosis in the affected groups may represent a controlled mechanism to eliminate excess cells, rather than a manifestation of toxic injury to hepatocytes. Hepatic beta-oxidation rates were increased in all dose groups, with 219%, 375%, and 334% of control values at 25, 250, and 750 ppm, respectively. The no-observed-adverse-effect level (NOAEL) for 90 days inhalation exposure to HCFC-122 in male and female rats was not determined based on the hepatic biochemical results at 25 ppm.

1319 IN VITRO METABOLISM OF BETA-CHLOROPRENE.

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Beta-chloroprene (2-chloro-1,3-butadiene, CD) is the monomer used in the production of polychloroprene (neoprene). The development of tumors in laboratory rodents exposed to CD by inhalation raises concern for humans potentially exposed during production and handling of this chemical. By analogy with compounds of similar structure (e.g., 1,3-butadiene, isoprene) metabolism of CD is probably a key determinant of toxicity. The purpose of the current study is to elucidate the metabolic fate of CD and determine if there are differences between rodents and humans with regard to the types and amounts of toxic metabolites formed. Liver microsomes from B6C3F1 mice and Fisher 344 rats were incubated at initial headspace concentrations of CD ranging from 10 to 200 ppm. CD in the vial headspace was quantitated by GC/FID, and its disappearance displayed saturable Michaelis-Menten behavior. The values for Vmax (nmol/min/mg protein) were 11.2 (rat) and 4.7 (mouse) with corresponding values for Km (uM in the aqueous medium) of 9.5 (rat) and 12.1 (mouse). The values for Vmax/Km (0.09 for rat and 0.38 for mice) indicated that mice were ~ 4 times faster at metabolizing CD than rats. Further experiments are underway to quantitate CD metabolism in human liver microsomes as well as identify the metabolites formed by each of the species. (Sponsored by the Scientific Oversights Committee of the International Institute of Synthetic Rubber Producers.)

1320 TOXICOKINETICS OF TRICHLOROETHYLENE (TCE) AND ITS METABOLITES IN THE BLOOD AND TISSUES OF RATS.

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TCE, a frequent contaminant of drinking water, is of concern as a potential human carcinogen. Current risk estimates are based upon results of chronic, high-dose cancer bioassays in rodents. Physiological models can be used to contrast target organ doses of toxic moieties in man and in rodents receiving the same administered dose. There are very few tissue time-course data, however, with which to construct models and to validate their predictions. The objective of this work was to clearly delineate the systemic disposition of TCE and its key metabolites. Male S-D rats were gavaged with 1, 5, 25, 50, 100 or 200 mg TCE/kg bw as an aqueous emulsion. Serial micro blood samples were taken via an indwelling arterial cannula. Other groups of rats were sacrificed periodically and tissues taken. Concentrations of TCE, trichloroethanol (TCEOH), trichloroacetic acid (TCA) and dichloroacetic acid (DCA) were analyzed by headspace gas chromatography, using a new esterification technique developed in the laboratory. TCE was rapidly absorbed and eliminated from the blood and tissues. TCEOH levels diminished quite rapidly, but TCA remained elevated in tissues for an extended period. Only trace amounts of DCA were seen at initial time-points. The toxicokinetics of the other TCE metabolites was dose-dependent. (Supported in part by DOE Grant #DE-FG01-912EW506.)

1321 MEASUREMENT OF TRICHLOROETHYLENE (TCE) AND ITS MAJOR METABOLITES IN BIOLOGICAL SAMPLES.

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A rapid and simple, yet sensitive method was developed for simultaneous measurement of TCE and its major metabolites (trichloroacetic acid (TCA): dichloroacetic acid (DCA); and trichloroethanol (TCEOH)) in blood and tissues. Most techniques used to date to quantify TCE and its polar metabolites are time-consuming and complex. Volatilization of TCE and instability of DCA have been problematic. The basic approach has been to derivative the polar metabolites, so they will volatize and can be analyzed by gas chromatography (GC). The method presented here involves transfer of 5-100 µl of blood or tissue homogenate to 20-ml vial containing 200 µl of esterizer (distilled water: conc sulfuric acid: methanol; 6:5:1; v:v:v). The vials are capped with PTFE-lined rubber septa, crimped tightly, vortexed and placed into a HS-101 headspace autosampler. They are heated for 30 min at 110°C, before being vented into a 10' x 1/8" stainless steel column packed with 10% OV-17 on a Supelco support. TCE, TCA, DCA and TCEOH are well resolved. The limit of detection for each compound is ~100 pg/l (ppt). This technique should be useful for toxicokinetic studies of TCE, in which large numbers of samples must be analyzed. (Supported in part by DOE Grant #DE-FG01-912EW506.)

1322 THE IMPACT OF EXPERIMENTAL UNCERTAINTY AND INTERINDIVIDUAL VARIABILITY ON *IN VITRO* TO *IN VIVO* EXTRAPOLATION FOR TRICHLOROETHYLENE METABOLISM IN HUMANS.

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To examine the extent to which experimental uncertainty and human interindividual variability separately impacted the ability of physiologically based pharmacokinetic (PBPK) model to predict metabolism and blood concentration of trichloroethylene (TCE) in the human, two distinct modules were developed and fitted into a current PBPK model (*Risk Anal.* 13:71, 1993) for TCE. The Monte Carlo (MC) module sampled values from the range determined from the distribution of measured parameters, while the "drop-in" module was constructed to extrapolate *in vivo* metabolic rates from microsomal protein and hepatocyte recovery experiments and kinetic parameters (Km and Vmax) determined from *in vitro* experiments with human microsomes (*Toxicol. Appl. Pharmacol.* 142:311, 1997) and isolated human hepatocytes (*Toxicol. Appl. Pharmacol.* 152:XXX, 1998). The modified PBPK model was constructed to allow multiple PBPK simulations for various (within the range of experimentally determined standard deviations) headspace TCE concentrations, microsomal protein contents, partition coefficients hepatocytes/air, Vmax and Km in microsomes and hepatocytes, and the number of hepatocytes in the liver. Experimental uncertainties were estimated by repeated determinations of samples from the same individuals. The interindividual variability was determined by calculating the mean and standard deviation for the sample population. Our analyses have identified the measured parameters that contributed most to the overall uncertainty of the PBPK model output. This study demonstrates that the experimental errors from both *in vitro* systems added little to the uncertainty of the simulated values when compared with overwhelming and intrinsic differences in TCE metabolism as expressed in liver preparations from different human donors. (Partially supported by the Department of the Air Force Contract No. F41624-96-C-9010.)

1323 EXTENT AND TIMELINESS OF TISSUE REPAIR MODULATES THE DOSE-RELATED INCREASE IN TOXICITY OF CHLOROFORM.

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Under a cooperative agreement with ATSDR we are studying toxic effects of chemical mixtures. Earlier studies led us to hypothesize that the rate and extent of liver tissue repair response to a given dose of hepatotoxicants determines the end result of toxicity (death or recovery) regardless of the mechanisms by which injury is inflicted. To understand the effects of mixture we are studying the effects of model hepatotoxicants alone and in combination. Present studies were designed to test our hypothesis with chloroform, a well-known environmental pollutant. Male S-D rats were injected with a ten-fold dose range of chloroform (0.05, 0.125, 0.25 and 0.5 ml/kg or 74, 184, 368 and 736 mg/kg) and hepatotoxicity and tissue repair were studied during a time course of 0 to 96 hr. The highest dose (736 mg/kg) of chloroform led to 40% mortality by 72 hr. Liver injury as assessed by plasma alanine aminotransferase (ALT) elevation showed a dose-response over a ten-fold dose range. A significant and progressive increase in plasma ALT was observed in the highest dose (736 mg/kg) treated group after 24 hr. Tissue regeneration response as measured by [^{3}H]-thymidine incorporation into hepatocellular nuclear DNA peaked at 36 hr in rats treated with lower two doses of chloroform (74 and 184 mg/kg). Further increase in chloroform dose to 368 mg/kg resulted in an earlier increase in [^{3}H]-thymidine incorporation at 24 hr, which still peaked at 36 hr. However, with highest dose of chloroform (736 mg/kg), tissue repair was delayed and attenuated allowing for unrestrained progression of liver injury. These results support the concept that in addition to the magnitude of tissue repair response, the time at which this occurs is critical in restraining the progression of injury. Measuring tissue repair and injury as simultaneous biological responses to toxic agents might increase the usefulness of dose-response paradigms in predictive toxicology and risk assessment. (Supported by ATSDR Grant U61/ATU681482.)

1324 VEHICLE, VOLUME AND TIME DEPENDENT EFFECTS OF CHLOROFORM ($CHCl_3$) INDUCED HEPATOTOXICITY.

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Digestible oils have been used frequently as vehicles for oral administration of volatile lipophilic chemicals. Previous results from our laboratory have shown that both the hepatotoxicity and nephrotoxicity of $CHCl_3$ were modulated by gavage vehicle with delivery in corn oil resulting in substantially greater toxicity than aqueous vehicle delivery. As a large gavage volume (10 ml/kg) was used in these studies, and gavage volume is also known to modulate toxicity, our objective in the present study was to examine the influence of vehicle at a lower gavage volume on the hepatotoxicity of Male F-344 rats (~70 days) received by oral gavage 0, 0.25, and 0.50 ml $CHCl_3$ /kg (N=6) in both corn oil and aqueous (10 % Alkamuls) based vehicles at a gavage volume of 5 ml/kg. Hepatotoxicity was assessed at 24, 48 and 72 hr post gavage. Little or no apparent change was evident in body and relative liver weight at any $CHCl_3$ dosage administered in either vehicle over 72 hr. Serum enzymes sorbitol dehydrogenase (SDH), and alanine (ALT) and aspartate (AST) aminotransferase showed that a dose–dependent increase in $CHCl_3$ hepatotoxicity occured following delivery in either vehicle. At the lower dosage, serum levels of AST, ALT and SDH were highest at 24 hr with recovery evident by 48 hr. In contrast, at the higher dosage, serum SDH, ALT and AST were similar at 24 and 48 hr with apparent recovery delayed until 72 hr. Statistically significant differences between oil and aqueous–based delivery of $CHCl_3$ were not detected at either $CHCl_3$ dose level at any time. This stands in contrast to the large differences seen in $CHCl_3$ toxicity between oil and aqueous vehicles at a large gavage volume. In conclusion, $CHCl_3$ toxicity observed in oil and aqueous vehicles appears to be modulated by gavage volume. (This abstract does not necessarily reflect EPA policy.)

1325 FURTHER REFINEMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC/PHARMACODYNAMIC MODEL FOR THE TOXICOLOGIC INTERACTION BETWEEN KEPONE AND CARBON TETRACHLORIDE.

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The earlier PBPK/PD model for Kepone/CCl_4 interaction from our laboratory (El-Masri et al, 1996) was refined further by incorporating: (1) the liver zonal concept advanced by Andersen et al (1997); (2) the liver zonal distribution of P450 2E1 and P450 2B1/2, enzymes likely responsible for generating free radicals from CCl_4; (3) the concept of energy metabolism as related to hepatocellular regeneration (Soni et al, 1993); (4) the new categorization of liver histopathological changes resulting from studies in our own laboratory. The reason for these refinements is for a better capacity of predicting lethality as well as hepatocellular changes over a range of doses. In PBPK modeling, we simulated liver zonal bioactivation of CCl_4 mediated by cytochrome P-450 2E1 and P-450 2B1/2 with the incorporation of zonal expression and inductions of these P-450 isoenzymes. In PBPD modeling, the simulated liver dynamic process was composed of two stages: hepatocellular injury and hepatocellular regeneration. The construction of PBPD modeling was based on the liver histopathological study and PCNA study conducted in our lab. In histopathological study, hepatocytes were categorized as normal, injured, balloon changed and necrotic cells. In PCNA study, mitotic cell and G2 cell were counted as proliferative cells. This integrated PBPK/PD model can be used to predict liver histopathological change in rats treated with Kepone/CCl_4 at different doses of CCl_4. When coupled with Monte Carlo simulation, this model can be used to predict lethality. In Kepone/CCl_4 treated rats, at CCl_4 dose levels of 0.26, 0.52, 1.04 mmol/kg, mortality by 48 hours was 19.7%, 69.5%, 96.1% respectively in simulation involving 1,000 rats. In comparison, we observed 6.6%, 74.1%, 100% lethality from experiments in 9 to 27 rats. (Supported by NIEHS Superfund Basic Research Program Project P42 ES05949.)

1326 OLDER RATS ARE RESILIENT TO THE HEPATOTOXICITY OF CCl_4 AND CHLORDECONE + CCl_4.

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While a considerable age-dependent variation in the incidence of chemical-induced hepatotoxicity is known, the reasons for this variation remain to be fully explored. The objective of the current studies was to investigate the effects of CCl_4 alone and chlordecone + CCl_4 in 24 month old rats. Male S-D adult (60 day) and aged (24 months) rats were challenged with 2.5 ml CCl_4/kg alone or were maintained on chlordecone (CD, 10 ppm) diet for 15 days and challenged with CCl_4 (100 μ l/kg, ip). In aged rats no mortality was observed at high dose of CCl_4 as against 30 % lethality in adult rats. Exposure to CD + CCl_4 resulted in 100 % mortality in adult rats, whereas, no mortality was observed in aged rats. Liver injury was assessed by plasma enzymes (ALT and SDH) and by histopathology at 0, 6, 24 and 48 h. In high dose treated rats comparable increase in plasma enzymes at 6 and 24 h was observed in both age groups. At 48 h a decline in plasma enzymes was noted in aged rats, whereas in adult rats these enzymes remained elevated. Both plasma enzymes showed a significant progressive elevation in adult rats exposed to CD + CCl_4. Interestingly, aged rats exposed to CD + CCl_4 showed marginal and transient increase in plasma enzymes. Histological studies supported the plasma enzyme observations. Hepatocellular regeneration as measured by [^{3}H]-thymidine incorporation into DNA was suppressed in adult rats. Interestingly, in aged rats undergoing either high dose CCl_4 toxicity or interactive toxicity, S-phase stimulation occurred early at 24 h. Further studies are needed to establish the role of tissue repair in aged rats in the protection against hepatotoxicity These initial studies suggest that the liver of aged rats has greater plasticity for repair after toxic injury, compared to adult rats. (Supported by Louisiana Board of Regents Support Fund.)

1327 ARSENIC STIMULATES BLADDER EPITHELIAL CELL PROLIFERATION.

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Epidemiological studies have demonstrated a strong correlation between increased arsenic concentrations in drinking water and the incidence of bladder cancer in human. Additionally, it has been reported that dimethylarsinic, one of the metabolites of inorganic arsenicals, promotes nitrosamine - induced rat urinary bladder carcinogenesis. We have used in vivo and in vitro models to analyze the effect of inorganic arsenic (As3+) on bladder epithelial cells. Rats treated with arsenic in the drinking water (0.02%) had high levels in the urine of total arsenic represented by DMA, MMA and As3+, and demonstrated an increase in bladder epithelial cell proliferation in 4 weeks. The proliferative changes were documented by HE staining, PCNA immunostaining and electron microscopy. Consistent with the in vivo data, arsenic induced an increase in [3-H] thymidine incorporation in several bladder epithelial cell lines, a marker for DNA synthesis and cell proliferative response. Furthermore, arsenic stimulated the activation of transcription factors associated with modulation of the growth factor expression and control of the cell cycle of bladder epithelial cells, including AP-1. Taken together, these studies suggest that arsenic-induced bladder cancer may be associated with the ability of arsenic to directly stimulate bladder epithelial cell growth. Cell proliferation alone can provide an explanation of carcinogenesis. Arsenic can promote cell proliferation of previously initiated cells or the rapid cell proliferation can be prerequisite for increased mutational rate.

1328 PERTURBATION OF MOUSE LEYDIG CELL PROLIFERATION BY ESTROGENS.

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Estrogen is a testicular carcinogen in animal models. Leydig cell tumors in human are estrogen secreting and/or estrogen sensitive. Recently, estrogenic chemicals have been implicated in the etiology of testicular cancer. The roles estrogenic chemicals play in the etiology of testicular cancer are not clear, as estrogens typically induce testicular atrophy in man when administered at pharmacological doses. In this study, we have conducted a cell proliferation

assay using the normalized mouse Leydig cell line, TM3. Cells were subcultured and plated for 24 hours prior to treatment, after which their growth medium was replaced with a non-hormone supplemented serum-free medium. Treatments consisted of DES (diethylstibestrol), 17β -estradiol, or 17α -estradiol in 1 of 7 (1*pg*-1μ g/ml) different concentrations, along with vehicle (ethanol) treatment of the controls. All treatments resulted in a bi-phasic stimulation of the cell proliferation. Results for DES ranged from 14.16%-248.23 % growth compared to controls assumed as 100%. Peak proliferation was noted at 72 hours with 1*pg*/ml and 100*ng*/ml DES treatments (186.87 and 248.23, respectively). Severe cytotoxicity was noted with 1μ g/ml DES treatment (14.16%). Results for 17β -estradiol ranged from 101.49-125.48% growth with peak values occurring at 1*pg*/ml and 100*ng*/ml. Results for 17α -estradiol ranged from 77.33-136.0% growth with peak values occurring at 1*pg*/ml and 100*ng*/ml. Cytotoxicity was noted for both 17β and 17α -estradiol at 1μ g/ml. These data presented here are both novel and intriguing, as an apparent tri-phasic response has been demonstrated. We conclude that the normalized mouse Leydig cell line TM3 can be both up and down regulated through estrogen exposure. Estrogen-increased cell proliferation may allow errors to occur during replication and this may be a factor responsible for estrogen-induced cancer.

1329 PROLIFERATIVE EFFECTS IN RESPIRATORY TISSUES OF THE SPRAGUE-DAWLEY RAT FOLLOWING STYRENE INHALATION EXPOSURE.

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Styrene is an industrial chemical widely used in the plastics industry. Styrene 7,8-oxide, the primary metabolite of styrene, is thought to be responsible for the carcinogenic and toxic effects associated with styrene exposure. Animal studies have shown that styrene is a respiratory toxicant and that styrene oxide causes proliferation in various tissues. The mechanism of carcinogenicity may therefore involve an increase in proliferation. The purpose of this study was to measure the levels of styrene oxide in lung, nasal mucosa and blood following a 50 ppm inhalation exposure and to determine if there was an increase in proliferating cell nuclear antigen (PCNA), a marker of proliferation, in these tissues immediately following, and 24 hours after exposure. Immediately following exposure, the level of styrene oxide in the blood was 20.9 ± 3.1 ng/g tissue while in the lung and nasal mucosa it was 62.8 ± 6.6 and 135.7 ± 18.7 ng/g tissue, respectively. There was no significant change in the level of PCNA in the lung at either time point following exposure. In the respiratory nasal mucosa, there was a significant increase in PCNA immediately following and 24 hours after exposure. In the olfactory nasal mucosa, there was a significant increase in PCNA immediately following exposure, but PCNA levels returned to control values 24 hours later. This indicates that, compared to the lung, the higher concentration of styrene oxide in the nasal mucosa may have a proliferative effect. Also, within the nasal mucosa, the olfactory and respiratory epithelia both proliferate following styrene exposure, but 24 hours after exposure proliferation is still occurring in the respiratory epithelia, while the olfactory epithelia has recovered.

1330 ALPHA ASSOCAITED DECRESAED P53 BYSTANDER EFFECTS AND ENHANCED CELLULAR PROLIFERATION IN HUMAN LUNG FIBROBLASTS.

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In our study, exposure of the cells to only 1 cGy of α particles resulted in markedly elevated levels in p53 and p21 protein levels, as seen by western blotting. However, based on our previous morphometric analyses, we estimate that ~7% of the cells in our study actually receive one or more nuclear hits by the α particles. We therefore investigated the possibility that a transmissible factor(s) may be induced by α particles that could contribute to the greater than expected p53 and p21Waf-1 elevations observed in the above experiment. We irradiated confluent HFL1 with 1 cGy of α particles and immediately and 4 hrs after irradiation we transferred their supernatants onto unirradiated HFL1. Four hrs later, we analyzed the cells for their p53 and p21Waf-1 contents by Western analysis. The supernatants from α -irradiated cells consistently caused *decreases* in the basal levels of p53 and p21Waf-1 in unirradiated cells, and more pronounced decreases in the cell cycle regulating proteins were obtained with supernatants that were harvested from cells at 4 hrs after exposure than immediately after exposure. Additionally, cells that have been treated with supernatants from α -irradiated cells show diminutions in p53 and p21Waf-1 protein elevations after subsequent exposure to α particles. Further experiments also indicated that cells preexposed to supernatants from a irradiated cells are compromised in their abilty to increase p53 levels on a subsequent exposure to α irradiation. We also describe new evidence that suggests exposure to low dose α particles can enhance cell proliferationas seen by clonogenecity assays. Such hyperproliferation likely is a step in the complex process that can culminate in bronchogenic carcinoma. Further, the decreased p53 bystander effect may have marked ramifications, such as, the transmittal of genomic defects to cell progeny due to checkpoint loss, replication of unrepaired DNA and, the failure of cells with excessive DNA damage to undergo p53-mediated apoptosis, and even, perhaps, a role in the yet to be explained phenomenon of genomic instability. (Funded by U.S.DOE.)

1331 A P53-INDEPENDENT METHYLMERCURY-INDUCED G_2/M ARREST.

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We evaluated the role of p53 in the cell cycle inhibition induced by methylmercury (CH_3HgOH, 0-6 μM, 24 hrs) using wild type, heterozygous and p53-deficient mouse embryonic fibroblasts at passage 5 to 8. Following methylmercury exposure, cells were harvested and stained with 4', 6-diamidino-2-phenylindole (DAPI) to establish a DNA content-based cell cycle profile using a Coulter Elite flow cytometer. Consistent with a G_1-phase regulatory role for p53, untreated wild type cells accumulated in the G_1 phase at a greater fraction than p53-deficient cells. Methylmercury-exposed wild type cells showed a dose-dependent increase in the G_2/M phase population and a subsequent decrease in the percentage of cells in G_1, consistent with predicted effects of methylmercury on mitotic spindle function. In contrast, methylmercury-exposed p53-deficient cells demonstrated a decrease in the S phase cell fraction and increases in the hyperdiploid and sub-G_0 population suggesting heightened chromatid segregation errors and induction of apoptotic/necrotic cell death. The heterozygous cells exhibited an intermediate response to methylmercury. The results of the present study are consistent with earlier studies on p53-dependent G_1 arrest and methylmercury-induced mitotic spindle dysfunction, and suggest that the G_2/M phase inhibition after methylmercury exposure may be p53-independent. Further investigations on the fate of the growth-inhibited cells, expression of cell cycle control genes, and consequences on genetic stability will help understand the complex role of p53 in cell proliferation in the context of methylmercury toxicity. (This project is supported by USEPA R825358 and NIEHS P42ES-04696.)

1332 HUMAN MAMMARY EPITHELIAL CELLS EXHIBIT A DIFFERENTIAL p53-MEDIATED RESPONSE FOLLOWING EXPOSURE TO IONIZING OR UV RADIATION.

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The long term objective of our research is to provide a better understanding of how breast epithelial cells respond to DNA-damaging agents. The tumor suppressor protein, p53 plays a critical role as a transcriptional activator of downstream target genes involved in the cellular response to DNA damaging agents. We examined the cell cycle checkpoint response of human mammary epithelial cells (HMEC) and their isogenic fibroblast counterparts to ionizing (IR) and ultraviolet radiation (UV), two genotoxic agents whose DNA damage response pathways involve p53. Using flow cytometric analysis, we found that HMEC, which contain wild-type p53 sequence, do not exhibit a G1 arrest in response to IR (4 Gy), but show an intact G2 checkpoint. Western analyses were performed to examine the expression of p53 and its downstream target associated with G1 arrest, $p21^{WAF1}$. Supportive evidence obtained from these experiments revealed that there was neither an increase in p53 nor $p21^{WAF1}$ proteins in HMEC exposed to IR. In contrast, isogenic mammary fibroblasts arrest at the G1 checkpoint and induce the p53 and $p21^{WAF1}$ proteins following IR. By comparison, HMEC exposed to UV (5 J/m^2) displayed a cell cycle arrest and induced the expression of p53 and $p21^{WAF1}$. Our results show that the cellular response to DNA damage depends on both the type of damage introduced into the DNA and the specific cell type. Studies are currently underway to determine the phosphorylation status of p53 in HMEC after exposure to either IR or UV. In addition, we are performing luciferase assays to determine whether p53 in irradiated HMEC is in a transcriptionally active form. Thus, we are extending the initial investigations to experiments that will yield important mechanistic information about the p53-mediated response in irradiated HMEC.

1333 COORDINATE INHIBITION OF CELL CYCLE PROGRESSION AND TCDD ACTIVATION OF *CYP1A1* BY 12-0-TETRA- DECANOYLPHORBOL-13-ACETATE.

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The relationship between the effects of 12-0-tetradecanoyl-phorbol-13-acetate (TPA) on cell cycle progression and the transcriptional activation of *CYP1A1* by 2,3,7,8-tetrachlorodi- benzo-*p*-dioxin (TCDD) was examined in the human breast epithelial cell line MCF10A-Neo. Exposure of MCF10A-Neo cells to TPA induced a concentration-dependent (IC_{50} ~ 0.5 nM) G_1 arrest and inhibition of *CYP1A1/1B1* activation by TCDD. Both effects occurred rapidly but were transient and lasted less than 24 hr. The kinetics of cell cycle arrest/reentry paralleled the loss/recovery of responsiveness to TCDD. Cultures treated with TPA 24 hr prior to a second TPA challenge neither arrested nor lost their responsiveness to TCDD following the second TPA exposure. Within one hr of TPA treatment cultures produced a cytostatic conditioned media capable of partially suppressing *CYP1A1* activation by TCDD. Preabsorption of conditioned media with an antibody to TGF-β inhibited its cytostatic activity by ~50%. Addition of exogenous activated TGF-β duplicated the cytostatic and suppressive effects attributed to TGF-β in conditioned media. However, whereas direct treatment of cultures with anti-TGF-β prior to TPA addition inhibited the cytostatic activity of TPA by 50%, it did not inhibit suppression of *CYP1A1* activation. Thus, in MCF10A-Neo cells TPA activates protein kinase C-dependent processes which cause autocrine production of cytostatic cytokines (one of which is TGF-β) capable of weakly inhibiting TCDD activation of *CYP1A1*. TPA also activates a second non-related process which strongly inhibits *CYP1A1* activation by TCDD. (Research was supported by NIH grant CA34469.)

1334 BIOLOGICALLY-BASED DOSE-RESPONSE MODELING OF MULTISTAGE CARCINOGENESIS: DEVELOPMENT OF A MODIFIED MVK MODEL WITH EXPERIMENTAL DATA FROM SYRIAN HAMSTER EMBRYO (SHE) CELLS.

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To further incorporate biologic information into quantitative cancer modeling, the two-stage MVK model has been modified for use with SHE cell neoplastic progression. There are four phenotypic stages included in this model, normal, immortal, anchorage-independent growth, and tumorigenic. Cells in each stage have distinct division, death and mutation rates, and mutation is assumed to occur during cell division. Model development and related experiments are focused on studying the abilities of lead, arsenic, chromium and a mixture of these three metals to induce progression of SHE cells from one phenotype to the next. The first phase of experimental work involved cytotoxicity studies and the exploration of methods for determining the division, death, and mutation rates. Toxicity of the three metals and the metal mixture has been assessed using the MTT assay. Results from cytotoxicity studies show that a mixture of the three metals are less-than-additive, suggesting some type of interaction at the level of cell killing. Cell division and death rates have been assessed using flow cytometric analysis for inclusion in the model. Cell division rates were measured using bromodeoxyuridine (BrdU) incorporation with propidium iodide staining, which allows for the calculation of potential doubling time, which is a cell cycling time that takes growth but not cell loss into account. Flow cytometry methods for measuring cell death in SHE cells is currently under development. Laboratory measurements of metal and metal mixture induced changes in key cell parameters are used to verify the development of this modified multi-stage carcinogenesis model. (This work was supported by ATSDR (Cooperative Agreement U61/ATU 881475) and NIEHS Superfund Basic Research Program (P42 ES05949).)

1335 CELL AUTONOMY AND THE RESPONSE OF THE HEPATOCYTE TO PARTIAL HEPATECTOMY.

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The adult liver is a quiescent organ, but following 2/3 partial hepatectomy mature hepatocytes undergo DNA synthesis and cell division to replace lost parenchyma. This response depends initially upon cues external to the hepatocyte, namely the loss of liver mass, but proceeds to a stage at which DNA synthesis is initiated in the hepatocyte, a process that is intrinsic to the cell or cell autonomous. Exploring the stage at which control of hepatocyte proliferation switches from non-cell autonomous to cell autonomous was an objective of this study. uPA (urokinase-type plasminogen activator) transgenic mice were used to address this issue. Diseased liver of uPA transgenic mice was repopulated with hepatocytes of two different somatic genotypes, rat and mouse, which display different responses to 2/3 partial hepatectomy in the respective host species. In rats, peak DNA synthesis occurs around 24 hours post-hepatectomy. In mice, peak DNA synthesis is delayed approximately 12 hours, occurring around 36 hours post-hepatectomy. Immunodeficient transgenic uPA mice with chimeric livers composed of rat and mouse hepatocytes were subjected to 2/3 partial hepatectomy. At different times post-hepatectomy, BrdU was administered to mice one hour prior to sacrifice, and the number of rat and mouse hepatocytes undergoing DNA synthesis in the chimeric livers was quantitated and compared. In particular, we wished to determine whether peak DNA synthesis in rat hepatocytes follows the response of the rat donor species or the mouse host species. Our results suggest that donor rat hepatocytes initiate DNA synthesis earlier than endogenous mouse hepatocytes in the chimeric liver, and at the expected time for rat hepatocytes in a rat. That is, entry of hepatocytes into the S phase of the cell cycle appears to be regulated in a cell autonomous manner. This system of generating chimeric livers can also be used to study differences in metabolism and/or toxicity of chemical compounds between two different species.

1336 INHIBITED GROWTH OF HUMAN HT29 COLON CARCINOMA CELLS BY DETOXIFICATION ENZYME INDUCERS IS REVERSED BY N-ACETYL CYSTEINE.

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Differentiation of the human colon adenocarcinoma cell line HT29 with sodium butyrate (NaB) results in increased activity of the detoxification enzymes glutathione S-transferase (GST) and NAD(P)H:quinone reductase (N:QR). These events are also accompanied by a decrease in intracellular glutathione (GSH) concentrations and a shift to a more oxidized redox potential, as calculated from GSH and GSSG concentrations, using the Nernst equation. To determine whether compounds that induce GST and N:QR activity also affect carcinoma cell proliferation, HT29 cells were treated with 5 to 25 μM benzyl isothiocyanate (BIT) and 20 to100 μM dimethyl fumarate (DMF). In a concentration-dependent manner, BIT and DMF decreased thymidine incorporation to less than 25% of controls. These decreases in cell proliferation were reversible by pretreatment with 1 to 5 mM N-acetyl cysteine (NAC). In contrast, NaB-mediated decreases in cell proliferation to less than 25% of controls were not reversible by NAC. In fact, cell growth was further diminished by NAC, dose-dependently, to less than 5% of controls at 2.5 mM. These findings indicate that although BIT, DMF and NaB are capable of inibiting carcinoma cell proliferation and share a common property of detoxification enzyme induction, their effects on cell growth differ in their response to NAC. (Support: GM-028248, ES-09047.)

1337 THE ACTIVATED AH RECEPTOR (AHR) BINDS TO HYPOPHOSPHORYLATED RETINOBLASTOMA (RB) PROTEIN AND REINFORCES THE REPRESSION OF E2F-DEPENDENT EXPRESSION.

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Polyhalogenated aromatic hydrocarbons, of which 2,3,7,8-tetrachloro-*p*-dioxin (TCDD) is the prototype compound, elicit a variety of toxic, teratogenic, and carcinogenic responses in exposed animals and in humans. One of the most puzzling aspects of the biological impact of TCDD is that it causes a plethora of apparently unrelated effects, including craniofacial anomalies and hydronephrosis during rodent embryogenesis, and hepatic carcinoma and pulmonary and skin tumors in adult mice. TCDD also causes disturbances of lipid metabolism and has immunotoxic, reproductive and endocrine disruption effects, some of which appear to be present also in exposed humans. The effects of TCDD at the cellular level are just as diverse, and often contradictory, affecting cell cycle regulation differently in different cell lines. For example, TCDD induces keratinocyte proliferation and terminal differentiation, thymocyte apoptosis, and inhibition of the estrogen-dependent proliferation of human breast cancer cells. All the

effects of TCDD are mediated by the AHR, which prompted us to examine possible mechanisms that would connect the Ah receptor with cell cycle regulation. Using yeast two-hybrid assays, we find that AHR and RB can form heterodimers. To characterize the heterodimers we used in vitro pull-down techniques and found that both the LXCXE and the Q-rich AHR domains can form complexes with hypophosphorylated RB forms independently. Co-immunoprecipitation experiments with in vivo whole cell lysates show that AHR and RB coprecipitate using ether anti-AHR or anti-RB antibodies from lysates of TCDD-treated MCF-7 cells, but not from lysates of cells treated with DMSO vehicle. Using an E2F-dependent luciferase reporter, we find that ectopic expression of AHR and RB synergize to repress E2F-dependent expression. These results suggest that the Ah receptor may play a cell cycle regulatory role when cells are exposed to environmental toxicants. (Supported by NIH P30 ES06096 and R01 ES06273.)

1338 MECHANISM OF ESTROGEN-INDUCED E2F1 GENE EXPRESSION: ROLE OF ESTROGEN RECEPTOR/Sp1 INTERACTIONS WITH NF-YA.

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Treatment of MCF-7 human breast cancer cells with 10 nM 17β -estradiol (E2) induced E2F1 mRNA levels (3-fold) and immunoreactive protein (6-fold). Factors regulating basal and E2-inducible expression of E2F1 were investigated by transient transfection studies in MCF-7 human breast cancer cells using a series of deletion constructs ($pE2F1_{a-j}$) containing E2F1 gene promoter inserts linked to a luciferase (luc) reporter gene. Basal and inducible gene expression required a combination of one or more GC-rich sites (-169 to -116) and two downstream CCAAT elements (-122 to -54). Gel mobility shift assays using competitive oligonucleotides and transcription factor antibody supershift assays showed that Sp1 protein interacted with the GC-rich sites and NF-YA bound the CCAAT elements. $pE2F1_i$ (-146/-54) contains a single upstream GC-rich motif and two downstream CCAAT elements, and in transient transfection studies with wild-type $pE2F1_i$ and constructs with mutations in the GC-rich and CCAAT elements, only the wild-type plasmid was E2-responsive. Gel mobility shift analysis of proteins binding to the [^{32}P]-146/-54 oligonucleotide showed that ER protein enhanced Sp1 - DNA binding and Sp1 protein enhanced NF-YA - DNA interactions. This represents a unique complex ternary protein - DNA interaction required for E2-induced transactivation of E2F1. (Welch Foundation and NIH CA76636)

1339 DIETHYLSTILBESTROL (DES) INDUCES APOPTOSIS AND AFFECTS CELL CYCLE IN A MURINE THYMOCYTE-LIKE CELL LINE RL-3.

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It has previously been reported that DES, a synthetic nonsteroidal compound possessing estrogenic activity causes thymic atrophy both in exposed mice and in cultured fetal thymus organs. By using a murine thymocyte-like cell line RL-3 derived from a radiation-induced leukemia, we have further investigated the effects of DES in order to understand the mechanisms for thymic atrophy. RL-3 cells are $Thy1.2^+HSA^-CD45^+CD4^+CD8^+$, $CD3^{low}$, TCR^{low} and $c\text{-}kit^-CD44^-CD25^{hi}$. This cellular phenotype is similar to that of the majority of immature thymocytes in vivo. Exposure of the RL-3 cell line to between 20 and 40 μ M DES resulted in a significant reduction in cell number after 12 - 24 hours of culture. DES also affected cell cycle in RL-3 cells. After 6 - 12 hour of culture, the percentage as well as absolute number of cells in the G_2/M phase of cell cycle was increased. By using both the fluorescent TUNEL assay and electrophoresis of DNA, we also found that DES induced apoptosis. Although DES exposed RL-3 cultures did not manifest a significant difference in apoptosis for cells in S and G_2/M, there was a tendency to observe the increase of apoptosis in $G_{0/1}$ phase cells only after 12 hours but not at 6 hour exposure to DES. Analysis for apoptosis or cell cycle related gene expression by quantitative RT-PCR, showed that DES induced NOS mRNA in RL-3 cells after 6 - 12 hour of culture. DES also affected 14-3-3σ, p27, and bcl-2 gene expression. We did not find alterations of the expression of mRNAs for Fas, p21, p53, CDC2, CDK2a, CDK2L, CDK4, and cyclin A, D1, D2, D3 after DES exposure. (Supported by NIEHS grants ES07216 and ES04862.)

1340 INHIBITION OF THE MAPK PATHWAY BLOCKS QUINONE-THIOETHER MEDIATED CYTOTOXICITY, BUT NOT GROWTH ARREST, IN LLC-PK1 CELLS.

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2-Br-*bis*-(glutathion-S-yl)hydroquinone (BBGHQ) is a potent nephrotoxicant causing severe necrosis of renal proximal tubules *in vivo*. In a renal tubular epithelial cell model (LLC-PK1), BBGHQ causes DNA damage, growth arrest, and cell death. We now report that BBGHQ causes a dose- and time-dependent S-phase delay in LLC-PK1 cells. This cell cycle delay appears to be mediated by p21/WAF1, the cyclin kinase inhibitor, since it is strongly induced by BBGHQ. The transcriptional activity of p53 is also increased by BBGHQ, indicating that p21-mediated growth arrest is probably p53-dependent. BBGHQ also rapidly (<15 min) activates the mitogen-activated protein kinase (MAPK) pathway as evidenced by increases in hyperphosphorylated extracellular signal-regulated protein kinases ERK1 and ERK2. Activation is maximal by 1 hr and remains elevated for at least 5 hr. Other subfamilies of MAPK, including c-Jun N-terminal kinases (JNK) and p38 kinase, are also stimulated by BBGHQ. However, the activation of both JNKs and p38 occurs later (2 hr) than ERK activation. Pretreatment of cells with inhibitors of ERKs or p38, PD98059 and SB202190, substantially attenuates BBGHQ induced cytotoxicity by 70% and 40% respectively. In contrast, PD98059 and SB202190 do not appear to block the cell cycle arrest in response to BBGHQ, even though both agents slightly decrease the induction of p21. Finally, both catalase and deferoxamine, which decrease the generation of reactive oxygen species, block the cytotoxicity and cell cycle arrest caused by BBGHQ, presumably because both block MAPK activation and p21 induction. In summary, the data suggest that in LLC-PK1 cells, BBGHQ causes cytotoxicity and growth arrest via independent pathways. Cytotoxicity is associated with induction of MAPKs, while growth arrest is associated with p53-dependent p21 induction. The relationship between these two pathways is being further investigated. (ES 07359)

1341 DECOUPLING OF BREVETOXIN'S FOUR DISTINCT PHYSIOLOGICAL EFFECTS ON VOLTAGE-GATED SODUIM CHANNELS BY SITE-DIRECTED TOXIN DERIVATIZATION.

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The polyether brevetoxins (PbTx) are a series of toxins produced by the marine dinoflagellate *Gymnodinium breve*. This dinoflagellate is the organism responsible for Florida's massive red tides, and also causes neurotoxic shellfish poisoning in humans and numerous epizootics of manatees, birds, fish, and other wildlife. The toxins are composed of a series of 10-11 ether rings exhibiting *trans/syn* orientation. All natural brevetoxins, and there are 10 different natural derivatives, induce four separate and distinguishable physiological effects when applied to voltage-gated sodium channels under patch clamp conditions: a shift of the activation potential to more negative values, a prolonging of mean open time, an inhibition of inactivation, and induction of subconductance states. Of 26 derivatives previously examined for their affinity at Site 5 associated with the voltage gated sodium channel, 3 have been subsequently found to differ in one or more of the above-identified physiological effects. 1-desoxy-PbTx-3 inhibits inactivation and shifts the activation potential to more negative values but has no effect on prolonging mean open time nor does it reveal subconductance states. Truncated-PbTx-3 (deficient in four sequential rings comprising a spacer region) only shifts the activation potential to more negative values. 2,3 dihydro-PbTx-3 possesses all four effects of native toxin, but reveals an additional 3 subconductance states for a total of 5 conductance states. These results assist in providing a molecular view of the portions of the brevetoxin molecule responsible for each of the four activities present in native toxin. The A-ring, or "head end" of the toxin appears involved in inhibiting inactivation, in promoting the visualization of subconductance states, and in prolonging mean open time. The mere act of binding causes the shift in activation potential and involves the H-K ring region distal from the activity region in the A-ring. Further, the length of the spacer region appears important in delivering toxin loci into environments within the channel to allow for full expression of toxin action.

1342 BREVETOXINS INDUCE A NEUROTOXIC RESPONSE IN CULTURED RAT CEREBELLAR GRANULE NEURONS THAT IS MEDIATED BY NMDA RECEPTORS.

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Brevetoxins (designated PbTx-1 to -10) are potent lipid-soluble polyether compounds which are known to bind to and modulate voltage-gated sodium channel activity. These toxins have been implicated in massive fish kills and human intoxications during 'red tide' blooms of the marine dinoflagellate *Ptychodiscus brevis*. To investigate whether brevetoxins produce direct CNS neurotoxic effects, cultured rat cerebellar granule neurons were exposed to brevetoxins in Locke's buffer for 2 hrs at 20° C. Neuronal injury was quantified by assaying lactate dehydrogenase activity in the exposure buffer and in conditioned growth media collected 22 hrs post-brevetoxin exposure. Brevetoxins produced acute neuronal injury and death in neurons with a rank order potency similar to their previously determined affinities for binding to recognition sites on the sodium channel. The neurotoxic response could be prevented by co-application of the sodium channel antagonist tetrodotoxin. Brevetoxin toxicity was also prevented by the co-application of the competitive and non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists D-AP5 and dizocilpine, ketamine, dextromethorphan and dextrorphan, respectively. NMDA receptor antagonists afforded neuroprotection at potencies comparable to those measured previously for protection against glutamate-induced excitotoxic responses. These data indicate that brevetoxin-induced injury in cultured rat cerebellar granule neurons is mediated by NMDA receptors that are activated indirectly as a consequence of PbTx-induced sodium channel activation. (Supported by funds from the College of Veterinary Medicine, Univ. of Georgia.)

1343 OXIDIZED CEREBROSPINAL FLUID LIPOPROTEINS CAUSE MICROTUBULE DISRUPTION AND CYTOTOXICITY IN NEURO 2A CELLS.

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Several neurotoxicants (among them heavy metals and organic solvents) cause changes in the prooxidant status of neurons. Reactive oxygen species have been postulated to be mediators of a "final common pathway" in several mechanisms of neurotoxicity. The nervous system, with its high oxygen consumption and enrichment of polyunsaturated fatty acids is particularly vulnerable to lipid peroxidation. Cerebrospinal fluid (CSF) lipoproteins are a likely vehicle for products of lipid peroxidation. However, little is known about the effect of oxidized CSF lipoproteins on neurons. We therefore oxidized human CSF lipoproteins and analyzed their effects on Neuro 2A cells. Oxidation of CSF lipoproteins resulted in covalent modifications of apolipoproteins E and AI and loss of polyunsaturated fatty acids, but caused no changes in the ultrastructure of the lipoproteins as analyzed by electron microscopy. We have previously shown that 4-hydroxynonenal, a major product of lipid peroxidation, causes microtubule disruption in Neuro 2A cells (Neely et al., 1998, Toxicologist:547a). Like 4-hydroxynonenal, oxidized CSF lipoprotein caused microtubule disruption in Neuro 2A cells within 1 hour of exposure. When the cells were exposed for longer periods, the oxidized lipoproteins eventually induced cell death. We conclude that oxidized CSF lipoproteins can cause microtubule disruption and neuronal cell death. The mechanism(s) for these effects are the focus of our investigation. (NIH grants AG00774 and ES02611, American Foundation for Aging Research.)

1344 γ-DIKETONE NEUROPATHY: I. QUALITATIVE NEUROPATHIC ASSESSMENT.

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Exposure to 2,5-hexanedione (HD), the neurotoxic metabolite of n-hexane and other hexacarbons, causes a central-peripheral distal axonopathy characterized by axonal swelling and atrophy. To explore the spatiotemporal relationship of these morphologic changes to dosing rate and behavior, we conducted a comprehensive morphometric and molecular study of HD-induced peripheral neuropathy (see companion abstracts- Lehning et al., Chiu et al.). Rats were intoxicated at 3 daily dosing rates (175, 250 and 400 mg/kg/d) and were sacrificed at unaffected to severe behavioral end-points. Lumbar spinal nerves (5th), proximal and distal sciatic nerves and distal posterior tibial nerves were removed from perfused animals and were subjected to blinded qualitative morphologic evaluation. Also, swollen and atrophic axons were counted within selected nerve fields (x1000). All dosing rates produced axonal atrophy and swellings in peripheral nerve regions. However, swellings were very rare in the 400 mg/kg/d treatment group whereas, regardless of dosing rate, atrophied axons were abundant. For the 175 and 250 mg/kg/d groups, the incidence of atrophy increased as a linear function of distal distance along the nerve axis. Axonal swellings in these groups were initially present in tibial nerve (day 42) and then moved into sciatic regions as treatment continued. Axonal degeneration was not a prevalent feature of any HD dosing rate. In addition to these axonal changes, the 175 and 250 mg/kg/d groups displayed late-onset macrophage infiltration and bands of Bungner development. Results provide a basis for quantitative morphometric analyses and indicate that axonal atrophy is a significant component of HD neuropathy. Expression of swellings appears to be related to the length of neurotoxicant exposure. (Supported by NIEHS grant RO1 ES07912.)

1345 γ-DIKETONE NEUROPATHY: II. MORPHOMETRIC ANALYSES OF AXONAL ATROPHY AND SWELLING.

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We hypothesized axonal atrophy is a consistent neuropathic feature of γ-diketone neuropathy which is manifest regardless of dose rate. In contrast, expression of axonal swelling is an epiphenomenon related to subchronic low-level exposure (LoPachin and Lehning, *NeuroToxicology 18:7-22, 1997*). To investigate this hypothesis, rats were exposed by gavage to 3 dosing rates (175, 250 or 400 mg/kg/d) of 2,5-hexanedione (HD) up to the endpoint of severe hindlimb weakness (99, 69 and 22 days, respectively). Throughout exposure, morphometric techniques were used to quantify axonal atrophy and swelling in 4 proximodistal locations of the sciatic nerve. Axonal atrophy was a prominent response to all HD dose rates. Atrophy initially manifested distally (tibial nerve) and spread proximally to lumbar spinal nerves with continued dosing. For all dose rates and nerve regions, atrophy was characterized by progressive decreases in size which culminated in a 50-67% loss in median axon area in the majority of myelinated axons. Significant changes in axonal shape also accompanied atrophy. The number and size of axonal swellings correlated inversely to dose rate but directly to length of exposure, i.e., as dose rate decreased and length of exposure increased, axonal swellings were larger and more frequent. Regardless, axonal swellings were very infrequent in nerves of rats exposed to 400 mg HD/kg/d (<0.5%) and never constituted >10% of the population of myelinated axons in all nerves of rats from the other dose rates. These results indicate expression of axonal swellings is rare and is dependent upon subchronic low-level exposure. Thus, swellings might not have mechanistic or functional importance. In contrast, axonal atrophy is a consistent and prominent neuropathic response to HD exposure which can manifest in the absence of axonal swelling. This suggests axonal atrophy is a relevant mechanistic component of γ-diketone neuropathy. Companion abstracts (Fox et al., Chiu et al.) present the effects of HD dose rate on qualitative neuropathology and neurofilament subunit content. (Supported by NIEHS Grant RO1 ES07912.)

1346 γ-DIKETONE NEUROPATHY: III. NEUROFILAMENT SUBUNIT CONTENT.

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Quantitative morphometric analysis (see companion abstract; Lehning et al.) indicates axonal atrophy is a consistent component of γ-diketone peripheral neuropathy. To determine the molecular mechanism of atrophy, rats were exposed to 2,5-hexanedione (HD; 250 mg/kg /day) by oral gavage and development of neurotoxicity was assessed daily by gross behavioral indices. At endpoint, proximal sciatic and distal tibial nerve segments were removed, stripped of endoneurium and homogenized. Aliquots of nerve homogenates containing equal proteins were separated by electrophoresis, transferred to membranes and probed with monoclonal antibodies against neurofilament subunits (NF-L, NF-H) and β-tubulin. Immunoreactive bands were visualized and quantified in a densitometric scanner. Respective NF-L and NF-H contents in nerve regions from moderately affected, HD-treated rats were significantly reduced; i.e., in proximal sciatic, NF-L decreased to

50% of control, while in distal tibial nerve, NF-L content decreased to 34% of control. Smaller reductions were observed for NF-H in both peripheral nerve segments. Loss of neurofilament protein was generally greater in tibial than in proximal sciatic nerve. Decreased NF subunit content appears to be specific since no significant changes in β-tubulin were detected in nerve regions. Abnormal high MW NF-L and –H positive bands were not consistently observed. When present, these bands did not correlate with proximodistal reductions in native proteins. Thus, loss of NF subunits and inconsistent expression of high MW species do not support cross-linked proteins as a mechanistic feature of γ-diketone neuropathy. Moreover, reduced axonal NF content might underlie development of atrophy in HD-treated animals. (Supported by NIEHS grant RO1 ES07912.)

1347 ROLE OF CALCINEURIN AND IMMUNOPHILINS IN THE DECREASE OF BRAIN ENERGY METABOLISM CAUSED BY IMMUNOSUPPRESSANTS.

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Neurotoxicity is one of the major side effects of the immunosuppressants cyclosporine (CsA) and tacrolimus (FK506) after organ transplantation. Recently we demonstrated that CsA- and FK506-induced reduction of high energy phosphate concentrations is the leading cause for *in vitro* toxicity in rat brain slices. Since binding to immunophilins and calcineurin is required for immunosuppressive activity of both drugs, it was our objective to study whether or not calcineurin and/ or immunophilins are involved in the reduction of high energy phosphate concentrations in brain. We used ^{31}P-MRS of perfused rat brain slices to evaluate the energy state during perfusion with immunosuppressants. CsA and FK506 led to significant decreases of high energy phosphates (phosphocreatine, PCr to -27± 4%; nucleoside triphosphates, NTP to -22± 6%; P<0.01; n=3) at concentrations as low as 500 μ g/L. Although calcineurin is not involved in its immunosuppressive mechanism of action, the new macrolide immunosuppressant sirolimus (rapamycin) also decreased brain energy metabolism (PCr:-17± 2% and NTP: -10± 5%; P<0.01; n=3). However, the sirolimus derivative SDZ RAD (40-O-(2-hydroxyethyl)-rapamycin) did not significantly affect high energy phosphate concentrations (PCr: +3± 6%; NTP: +3± 5%; n=3). Since SDZ RAD and sirolimus are both substrates of the same immunophilin, FKBP, our results suggest that immunophilins are not involved in the reduction of brain energy metabolism by sirolimus. We conclude that reduction of high energy phosphate concentrations in the brain caused by cyclosporine, tacrolimus and sirolimus involves a calcineurin- and immunophilin-independent pathway.

1348 GLUTATHIONE AND ANTIOXIDANTS PROTECT AGAINST THE *IN VITRO* TOXICITY OF METHYL IODIDE TO CEREBELLAR GRANULE CELLS.

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Methyl iodide (MeI) induces toxicity in several organs including the central nervous system. The aim of this work was to ascertain if glutathione (GSH) and certain antioxidants could afford neuroprotection. Primary cultures of rat cerebellar granule cells were prepared from post-natal day 8 rats and maintained in a serum free medium plus 25mM KCl for 8 days prior to exposure. Cells were exposed to MeI (0-10mM) for 5 min and cytotoxicity assessed over 48h using the Live/Dead assay (Molecular Probes). For the neuroprotection studies, cells were preincubated with L-buthionine sulphoximine (BSO, 0.5mM) or the isopropylester of GSH (IP-GSH, 1mM) for 1h prior to exposure to MeI for 5min at 1 or 2mM respectively, and then maintained in the presence or absence of the GSH modulating agent for 24h. Cells were also exposed to 1.6mM MeI for 5min in the presence of vitamin E (10μ M), butylated hydroxytoluene (25μ M) or desferrioxamine (1mM) and viability determined after culture for 24h in the presence of antioxidant. MeI produced a concentration dependant loss of cerebellar cell viability, with an EC_{50} of 1.6mM at 24h. BSO potentiated the toxicity of MeI, while IP-GSH and all three antioxidants afforded protection. These studies indicate that conjugation with GSH is a detoxification pathway and that GSH is neuroprotective. Conjugation with GSH results in depletion of this critical cellular antioxidant. Thus provision of additional GSH or exogenous antioxidants protects the cells from the ensuing oxidative stress.

1349 EFFECTS OF ACUTE IBOGAINE ADMINISTRATION ON THE PERFORMANCE OF COMPLEX OPERANT TASKS IN RATS.

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Ibogaine is a psychoactive compound that has been reported to ameliorate withdrawal symptoms in humans and to interrupt the addiction process. However, a 100 mg/kg i.p. dose has previously been shown to produce consistent neurodegeneration of cerebellar Purkinje cells in rats. In the present study, the behavioral effects of ibogaine were assessed using performance on a complex battery of food-reinforced operant tasks. One group of rats (n=6) performed a temporal response differentiation (TRD) task, which requires subjects to press and hold a lever in the depressed position for a 10-14 second "window." The second group of rats (n=6) performed a conditioned position responding (CPR) task to assess auditory, visual, and position discrimination. The TRD and CPR tasks were both paired with a progressive ratio (PR) task to assess aspects of motivation in the same test session. Administration of a single dose of 100 mg/kg ibogaine hydrochloride (i.p.) produced a statistically significant decrease in the number of reinforcers earned and accuracy in the TRD task, without significantly changing the overall rate of responding. This effect recovered one day after ibogaine administration. In the CPR task, ibogaine significantly decreased both the rate and the accuracy of responding. The effect on CPR accuracy recovered one day after ibogaine administration and the effect on response rate recovered after two days. The PR task was differentially sensitive to ibogaine administration depending on whether it was paired with TRD or CPR. These results demonstrate that certain acute behavioral effects of ibogaine are reversible, and suggest that further studies correlating the acute neurochemical and/or neuropathological effects of ibogaine administration with its behavioral effects are necessary.

1350 CYTOTOXICITY OF 2-HALOPROPIONIC ACIDS TO CEREBELLAR GRANULE CELLS IN CULTURE.

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Daily administration to rats of L-2-chloropropionic acid (L-2-CPA) 2-bromo- (2-BPA) and to a lesser extent 2-iodo- (2-IPA) but not 2-fluoro-propionic acid (2-FPA) produces selective cerebellar granule cell necrosis. Evidence suggests that the mechanism of neurotoxicity may involve cerebellar granule cell N-methyl-D-aspartate (NMDA) receptors as dizocilpine affords protection against L-2-CPA neurotoxicity *in vivo*. The aim of this study was to determine the toxicity of these 2-haloacids in primary cultures of rat cerebellar granule cells and whether the toxicity was NMDA receptor mediated. Primary cultures of rat cerebellar granule cells were prepared from post-natal day 8 rats and maintained in a serum free medium plus 25mM KCl for 8 days prior to exposure. Cells were continuously exposed to the 2-haloacids over 24, 48 or 72h and cytotoxicity assessed using the Live/Dead assay (Molecular Probes). All compounds produced a time and concentration dependant loss of cerebellar granule cell viability. With L-2-CPA the EC_{50} values were >10mM, 6.2 ± 1.1mM and 3.7 ± 0.8mM for 24, 48 and 72h respectively. Dizocilpine (3μ M) afforded complete protection at 24h but only partial protection at ≥ 3mM when exposure was for 48 or 72h. The EC_{50} for 2-BPA, 2-IPA and 2-FPA for 24h exposure were 0.5 ± 0.1mM, 0.3 ± 0.1mM and 1.7 ± 0.1mM respectively. The addition of dizocilpine with these analogues did not afford protection. These studies indicate that 2-halopropionic acids are toxic to rat cerebellar granule cells *in vitro*, and that the initial component of this toxicity with L-2-CPA occurs via an NMDA receptor dependant mechanism.

1351 PHARMACOLOGICAL MODULATION OF BEHAVIORAL CONVULSIONS INDUCED BY TRIMETHYLOLPROPANE PHOSPHATE (TMPP).

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TMPP {4-ethyl-1-phospha -2,6,7-trioxa-bicyclo-[2.2.2]- octane-1-oxide} is a potent bicyclophosphate convulsant in rats, hypothesized to act through binding to the picrotoxinin receptor site of the GABAA ionophore complex. Because TMPP can be produced through the partial pyrolysis of certain syn-

thetic lubricants or polyurethane foams, there is concern for accidental human exposure. Last year, we reported that the frequency and duration of EEG paroxysms induced in Fischer-344 rats by subconvulsive (0.20 mg/kg ip) administrations of TMPP could be effectively controlled with ethosuximide (ESM)+CGP 35348 > ESM > diazepam (DZP)+CGP 35348 > DZP > CGP 35348 > Phenobarbital (PB)+CGP 35348 > PB pretreatment. In the present study, young adult Fischer-344 rats were treated with PB (5-40 mg/kg), DZP (1-10 mg/kg), DNQX (5-20 mg/kg), scopolamine (SCOP; 1-5 mg/kg), tiagabine (TGB; 1-10 mg/kg) or MK-801 (0.5-3.0 mg/kg) 30 min prior to ip injection with a convulsive dose of TMPP (0.6 mg/kg). Rats were rated for convulsive activity for 2 hr post-injection. Time from TMPP injection to observation of subclinical seizures, generalized seizures and lethality, as well as frequency and duration of convulsive activity was rated for each pretreatment group. Convulsions were suppressed by DZP > PB > TGB > MK-801 > DNQX > SCOP.

1352 PAROXYSMAL DISCHARGES INDUCED BY TRIMETHYLOLPROPANE PHOSPHATE (TMPP) IN RAT HIPPOCAMPAL PYRAMIDAL CELLS.

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The actions of TMPP, a potent convulsant, were tested on hippocampal CA1 pyrmaidal cells using intracellular electrophysiological methods *in vitro*. Eighty percent of neurons tested exhibited spontaneous activities (0.03-4.5 Hz). Bath applications of TMPP (0.1-100 *u*M) caused membrane hyperpolarization (7±2 mV, n=7) associated with an increase in input resistance (51±8%, n=7) in 41% (7 of 17) of neurons tested, and membrane depolarization (3.7±0.5 mV, n=5) associated with an increase in input resistance (18±3%, n=5) in 29% (5 of 17) of neurons tested. TMPP (0.1-100 *u*M) induced short epileptiform discharges followed by epileptiform burst afterdepolarizations in 59% (10 of 17) of neurons tested. TMPP (0.1-100 *u*M) also increased the spontaneous activities (101±23% increase, n=7) in 41% (7 of 17) of neurons tested and decreased spontaneous activities (56±8% decrease, n=9) in 53% (9 of 17) of neurons tested. The epileptiform bursts and increases in spontaneous activities induced by TMPP were blocked by muscimol (0.5-10 *u*M; n=4), a GABAA agonist, diazepam (0.5-10 *u*M), a GABAA-benzodiazepine receptor agonist (n=4), and baclofen (10 *u*M), a GABAB receptor agonist. Bath application of muscimol (0.5-10 *u*M; n=4), diazepam (0.5-10 *u*M; n=3), or baclofen (1-10 *u*M) abolished the spontaneous activities of CA1 neurons. TMPP (2-10 *u*M) reversed the actions of muscimol and diazepam but not baclofen. TMPP (0.1-100 *u*M) did not induce any changes in threshold, duration and amplitude of the action potentials (APs) evoked by direct depolarizing current injection but increased the latency to onset of the APs. (n=6). In summary, TMPP has excitatory and inhibitory actions on CA1 pyramidal cells. The actions of TMPP may be due to its interaction with GABAA-benzodiazepine receptors.

1353 IN VIVO ASSESSMENT OF PERIPHERAL BENZODIAZEPINE RECEPTOR LEVELS IN A BABOON BRAIN FOLLOWING PERMANENT CAROTID ARTERY OCCLUSION.

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Peripheral benzodiazepine receptors (PBR), normally found in low concentrations in the brain, are increased in response to ischemia, chemically induced neurotoxicity, and in some neurodegenerative diseases. The PBR response in baboon brain (*Papio anubis*) following permanent ligation of the left common carotid artery (LCCA) was characterized by positron emission tomography (PET). [^{15}O]-H_2O PET was used to estimate regional cerebral blood flow 22 days after LCCA occlusion. PBR levels were subsequently assessed by [^{11}C]-PK11195 PET. Reductions of blood flow were measured in left frontal (12.1%), parietal (12.8%) and occipital (18.7%) cortical areas compared to the contralateral, intact hemisphere. Despite the reduction in cerebral blood flow, [^{11}C]-PK11195 binding was increased by 1.2%, 11.5% and 7.9% in frontal, temporal and parietal regions, respectively. Eight days later, the animal was euthanized and the brain was rapidly removed and frozen. Coronal sections of the occipital lobe were processed for [^{3}H]-PK11195 autoradiography. Preliminary evidence suggest PBR levels are increased by as much as 114% in the dorsal occipital gray matter. Smaller or no change was observed in the ventral regions. This study indicates that [^{11}C]-PK11195 PET may be a useful tool for assessing the *in vivo* PBR response to neuronal damage. (Supported by NIEHS grant #ES07062 to TRG.)

1354 DEVELOPMENT OF IN VITRO SCREENING ASSAYS FOR POTENTIALLY NEUROTOXIC POLYCYCLIC AROMATIC HYDROCARBONS IN C6 RAT GLIOMA CELLS.

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The C6 rat glioma cell line has properties of a bipotential glial progenitor cell with both astroglial and oligodendroglial characteristics. As such, C6 cells are a model for glial toxicity in the developing brain. Very little is known about the potential neurotoxicity of polycyclic aromatic hydrocarbons (PAHs). In the present study we examined the effects of of three PAHs: benzo(a)pyrene, chrysene, and anthracene. Two assays were used to assess cytotoxicity: trypan blue dye exclusion and amino acid incorporation. C6 cells were treated for 4 and 7 days with 0.03 to 30 μM of each compound. With both assays and at all dosages of each compound, no significant effect was observed when compared to controls. The relative insensitivity of the above assays, even after prolonged exposure at high doses, indicates that cytotoxicity is an inadequate measure of potential PAH toxicity in glia. We have previously observed alterations in intracellular Ca^{2+} and translocation of protein kinase C as a result of exposure of C6 cells to low levels of halogenated aromatic hydrocarbons. Such assays may be more useful for the detection of potential neurotoxicants. (Supported by ATSDR #U61/ATU684505-01.)

1355 FIXED-INTERVAL PERFORMANCE FOLLOWING DEVELOPMENTAL EXPOSURE TO A1254, A COMMERCIAL POLYCHLORINATED BIPHENYL MIXTURE.

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Lilienthal et al. (1990) reported that adult male rats, developmentally exposed to the commercial polychlorinated biphenyl (PCB) mixture (Clophen A30), displayed dose-dependent reductions in the post-reinforcement pause (PRP) and the index of curvature (IOC) on a fixed-interval 30-sec (FI 30-sec) schedule of food reinforcement. These results suggested that the temporal patterning of FI responding was disrupted by exposure to this PCB mixture. We tested whether a disruption in FI patterning could be achieved following perinatal exposure to another PCB mixture, Aroclor 1254 (A1254). Primiparous Long Evans rats were exposed to 0 or 6 mg/kg/day A1254 via oral gavage from Gestation Day 6 (GD6) through Postnatal Day 21 (PND21). At approximately PND90, male and female offspring (n=8/gender/treatment) were food deprived (85% free-feeding weight) and then trained to lever press for food reinforcers. Once the response was reliably emitted, a FI 30-sec schedule of reinforcement was introduced and remained in effect for 31 sessions. Under this schedule, reinforcement was available after 30 sec had elapsed since the previous reinforcement. There were no treatment-related effects on any aspect of FI 30-sec performance (IOC, PRP, or response rate). On the 32nd session the schedule requirement increased to FI 5-min for 69 additional sessions. For all animals, independent of treatment, there was a decrease in rate and IOC, and an increase in PRP, when the schedule was switched to FI 5-min. After approximately 30 sessions, the IOC gradually recovered to the asymptotic levels obtained under FI 30-sec, while responding persisted at a lower rate. These results, however, did not reveal a dose-related effect on any performance measure. These data are inconsistent with previous findings and suggest that A1254 may not affect fixed-interval performance in the same manner as did Clophen A30.

1356 PERFORMANCE ON A SPATIAL MEMORY TASK IN ADULT RATS FOLLOWING PERINATAL EXPOSURE TO AROCLOR 1254.

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Performance deficits in learning and memory tasks have been associated with PCB exposure in children and in animal models. The current work was designed to determine the effects in adult offspring of maternal exposure to Aroclor 1254 (A1254; Lot#124-191; AccuStandard), a commercial PCB

mixture that contains dibenzofuran contaminants. Primiparous rats (22-24/dose) received 0 (corn oil) or 6 mg/kg A1254 (po) from gestation day six through postnatal day 21 (PND21). We assessed one male and female per litter beginning approximately 90 days after birth on a reference memory task and a working memory task in a Morris-type water maze. Animals were tested in two blocks, approximately 6 months apart. Animals were first trained on a acquisition task where testing consisted of two trials per day for four weeks (5 days/week). Latency to reach the platform was the dependent variable. Following asymptotic performance in the fourth week, animals (first block only) were tested for five days on a repeated acquisition task; two trials per day, with the platform location changed every day (working memory). The latency difference between the two trials was used as the dependent variable. There were no treatment related effects on any dependent measures in reference or working memory tasks. These data suggest that maternal A1254 exposure does not affect spatial memory as assessed in the water maze.

1357 EFFECTS OF AROCLOR 1254 PRE-TREATMENT ON GABA INDUCED CHLORIDE INFLUX CULTURED CORTICAL NEURONS: A FLUORESCENCE IMAGING STUDY.

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GABA receptors are modulated by many endogenous ligands, drugs, and chemicals including, pregnane steroids, hormones (e.g. T3 & T4), barbiturates, benzodiazepines, and chlorinated insecticides. Polychlorinated biphenyls (PCBs) are developmentally neurotoxic to humans and may act through a variety of mechanisms, including thyroid hormone level disruption, sex steroid mimicry, and Ca homeostasis impairment. We sought to determine whether PCBs may also interact at the GABA receptor by examining muscimol–induced [Cl changes in mature cortical neurons using fluorescence imaging. Rat P0 cortical neurons cultured for 5-19 days in vitro (DIV) on a feeder–layer of glia in DMEM containing 10% horse serum were loaded with the permeant form of a Cl sensitive dye, MEQ. Muscimol–induced Cl influx in cortical cultures underwent time dependent maturation. At DIV 5–6, muscimol (25 μ %ÀM) stimulated net Cl efflux from cells, whereas at DIV 7–14, muscimol stimulated Cl influx (equivalent to 6–8 mM net accumulation inside the cell following 1 min of continuous application). Mature responses exhibited classical GABA pharmacology, being dose–dependent (0–50 μ %M muscimol), potentiated by the neurosteroid allopregnanolone (50 nÿþM), and blocked by the non competitive antagonist, lindane (50 μ %ÀM). To begin to investigate whether PCBs interfere with GABA receptor function, Aroclor 1254 (Ultra Scientific; 20 μ %M in 3 mL 0.1% DMSO) was added to the cultures for 13.5 hr. Exposures of this concentration and duration did not result in overt cytotoxicity as assessed with propidium iodide staining. In functional studies from 7–8 DIV cells, muscimol–induced Cl influx in PCB–exposed cells was significantly ($P<0.05$) less than the response in control cells. In addition, the response frequency distribution of PCB–treated cultures was shifted to lower values. These results suggest that Aroclor 1254 interferes with GABA–stimulated Cl influx in cortical cells. Studies are underway to determine concentration– and time–responses as well as whether the receptor per se or intracellular chloride regulation (or both) is the target.

1358 DEVELOPMENTAL EXPOSURE TO AROCLOR 1254 PRODUCES A PERSISTENT DECREMENT IN THE MAGNITUDE OF LONG-TERM POTENTIATION IN THE DENTATE GYRUS *IN VIVO*.

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Developmental exposure to polychlorinated biphenyls (PCBs) has been associated with cognitive deficits in children. A model of synaptic plasticity, long-term potentiation (LTP) is believed to represent memory at the synaptic level and has been extensively studied in the hippocampus. We examined the consequences of developmental exposure to a complex PCB mixture, Aroclor 1254 (A1254), on physiology and plasticity in the dentate gyrus of the adult rat *in vivo*. Pregnant dams were exposed to 6 mg/kg/day of A1254 from GD6 to PN21. This dosage of A1254 is associated with low-frequency hearing loss and reduced thyroid hormone concentrations in offspring during the early postnatal period. Between 5-7 months of age male offspring were anesthetized with urethane and stimulating and recording electrodes were placed in the perforant path and dentate gyrus. In the first experiment, input/output (I/O) functions were collected before and after a single series of high frequency (400 Hz) trains delivered at a moderate stimulus intensity (300 μA). The second experiment was performed on similarly exposed animals, but multiple train sets were delivered in an ascending series of train intensities (25-300 μA) to determine the effects of A1254 exposure on LTP threshold. Systematic A1254-induced alterations in baseline synaptic transmission as assessed by I/O functions were not observed in either experiment prior to the delivery of LTP-inducing trains. Posttrain I/O functions, however, revealed a reduced magnitude of LTP of the population spike (PS) in A1254-exposed animals relative to controls in both experiments. Effects on LTP of the EPSP slope were in the same direction but more variable and differences did not reach statistical significance. The mechanisms whereby this A1254 exposure leads to impairment in LTP are not known. However, disruption of the neurobiological processes that support hippocampal plasticity may contribute to the cognitive deficits observed following developmental exposure to A1254.

1359 THE EFFECTS OF DEVELOPMENTAL EXPOSURE TO POLYCHLORINATED BIPHENYLS ON D_1 AND D_2 DOPAMINE RECEPTORS IN JUVENILE AND ADULT RATS.

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Polychlorinated biphenyls (PCBs) are a class of halogenated aromatic hydrocarbons that have been shown to cause neurological dysfunction in humans and laboratory animals. PCBs have been shown to inhibit tyrosine hydroxylase and L-aromatic amino acid decarboxylase, two enzymes involved in the synthesis of dopamine. This inhibition may lead to a decrease in dopamine concentration. This project explores the possibility that dopamine receptors may up regulate in order to compensate for the postulated decrease in dopamine concentration. Pregnant Sprague-Dawley rats were administered 4mg/kg/day Arochlor 1254 in safflower oil p.o. beginning on gestation day 6 and ending on post-natal day 22. Pups from each group were sacrificed at post-natal days 15, 23, 31, or 70, and brains were removed and frozen for subsequent receptor analysis. B_{max} and K_d for D_1 and D_2 receptors were determined in membrane preparations from the corpus striatum for each group using ^{3}H-SCH 23390 and ^{3}H-spiperone, respectively. Receptor binding analysis indicated no apparent change in B_{max} and K_d values for ages 23, 31 and 70 days. However, there were differences in the age 15 day animals. A slight decrease was noted in the D_1 receptor B_{max1} but the K_d was unchanged. The D_2 receptor B_{max} and K_d values were both decreased. The treatment with PCBs did not appear to disrupt the pattern of receptor development, and did not appear to result in up regulation. (Supported by EPA R 825296.)

1360 SPONTANEOUS CATECHOLAMINE RELEASE AS MEASURED BY SINGLE CELL AMPEROMETRY: DISRUPTION BY TETRANDRINE AND 2, 2',4, 4'-TETRA CHLOROBIPHENYL.

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The plant alkaloid tetrandrine (TET) as well as the non-coplanar PCB-congener 2,2',4,4'-tetrachlorobiphenyl (2,4-TCB) increase spontaneous release of catecholamines from bovine chromaffin cell cultures (Wiegand et al., JPET, 1996; Messeri et. al, Arch. Toxicol.,1997). To quantify their effects on the single cell level we used amperometry of catecholamine quanta released spontaneously from single cells . We treated chromaffine cell cultures with both neurotoxicants by incubation with concentrations ranging from 10-100 μ M for time periods between 1 and 5 h. After the treatments the cells remained completely unstimulated and carbon-fibre microelectrodes measured the currents due to the oxidation of catecholamines spontaneously released from single cells as reviewed by Travis and Wightman (Annu. Rev. Biophys. Biomol. Struct.. 1998). Cells from untreated cultures served as controls. TET as well as 2,4-TCB increased the spontaneous catecholamine output by enhancing the frequency of spontaneously released catecholamine quanta from chromaffin cells in concentration ranges of 10-100 and 10-50 μ M, respectively. In addition the analysis of quantal signals revealed a statistically significant change of signal shape parameters which seemed to contribute to the catecholamine output. From these experiments we conclude that TET as well as 2,4-TCB disrupt neurohormonal release by interference with molecular mechanisms of the vesicular exocytotis. (This work is part of the thesis of Z H at the Heinrich-Heine-University Medical Faculty.)

1361 5-HYDROXYTRYPTOPHAN TOXICOSIS IN DOGS.

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5-Hydroxytryptophan (5-HTP) is a nutritional supplement that has gained popularity within the alternative medical community because of its reported ability to treat conditions such as depression, chronic headaches and insomnia in humans. 5-HTP apparently exerts its effects by increasing the levels of serotonin in the body. Serotonin is a central nervous system neurotransmitter, has a stimulatory effect on smooth muscle of the gastrointestinal tract, and is a promoter of platelet aggregation. The objective of this study was to describe the clinical syndrome, toxic doses, and treatment methods associated with accidental 5-HTP ingestion in dogs. Searches of the database at the ASPCA National Animal Poison Control Center identified 15 cases of accidental ingestion of 5-HTP by dogs. Dosages of 5-HTP ingested ranged from 2.5 mg/kg to 557 mg/kg, with 27 mg/kg being the lowest dose at which clinical signs occurred. Clinical signs developed in 13 dogs (86.7%), with the onset of signs ranging from 10 minutes to 2 hours post-ingestion. The most common clinical signs included diarrhea (33%), tremors (33%), seizures (33%), hyperthermia (33%), mydriasis (27%), and ataxia (20%). In all cases where follow-up data were available (10/15), the dogs fully recovered within 24 hours with supportive treatment including anticonvulsants, IV fluids, and thermoregulation. The clinical syndrome seen in these dogs shares similarities with "serotonin syndrome" described in humans following ingestion of tryptophan or monoamine oxidase inhibitors.

1362 AN EXAMINATION OF THE BIOCHEMICAL AND BEHAVIORAL EFFECTS OF MPTP IN TWO SNAKE SPECIES.

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The synthetic neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces a Parkinsonian-like syndrome in humans and non-human primates, and also causes similar movement disorders in rodents, fish, amphibians and lizards. In this study, the behavioral and biochemical effects of MPTP were assessed in the black ratsnake *Elaphe o. obsoleta* and the banded watersnake *Nerodia f. fasciata*. We report that MPTP induces in *E. o. obsoleta* a depletion of norepinephrine, dopamine and serotonin in fore, mid and hindbrain regions and also impedes righting ability. In *N. f. fasciata*, norepinephrine and dopamine were also depleted by MPTP in all three brain regions, but serotonin was only significantly reduced in the forebrain and righting ability was not affected. This study demonstrates a behavioral and biochemical sensitivity to MPTP in *E. o. obsoleta* that differs from that in *N. f. fasciata*. This novel reptilian model is beneficial for assessing central dopaminergic and serotonergic involvement in motor control in the lower vertebrates.

1363 ACUTE AND LONG-TERM EFFECTS OF MPP+ IN CELL LINES COEXPRESSING PLASMA MEMBRANE AND VESICULAR DOPAMINE TRANSPORTERS.

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The plasma membrane dopamine transporter (DAT) and the vesicular monoamine transporter (VMAT2) have been proposed to mediate the subcellular localization and ultimate toxicity of the active metabolite of MPTP, 1-methyl-4-phenylpyridinium (MPP^+). We have generated stable neuroblastoma cell lines (SK-N-MC) expressing either DAT or both DAT and VMAT2 to examine the effects of transporter expression on toxicity. Western blotting and immunofluorescence studies confirmed the expression of the transporter proteins. Basal levels of plasma membrane [^{3}H] dopamine uptake in either untransfected or VMAT2-expressing cell lines were 100 fmol/hr/10^6 cells and were unaffected by 10 μM nomifensine. Specific plasma membrane uptake was increased by 75-150 fold in cell lines expressing either DAT or DAT and VMAT2. Specific vesicular [^{3}H] dopamine uptake in digitonin-permeabilized VMAT2 cells was 500 fmol/hr/10^6 cells. Acute metabolic responses of these cell lines to MPP^+ were followed using a microphysiometer to measure proton excretion. Within 4 min. of application, MPP^+ (100 μM) caused a significant increase in proton excretion in DAT-expressing cells, which was attenuated by pretreatment DAT uptake inhibitors. The observed response was also attenuated in DAT/VMAT2 cells. Delayed toxicity was assessed by measuring the intercalation of the fluorescent probe, ethidium homodimer, into the DNA of dead cells. MPP^+ (10-500 μM) produced dose-dependent cell death in DAT-expressing cell lines during the 48 hr period after application. The rate of cell death (V_{max}) at an MPP^+ concentration of 10 μM was at least 40-fold higher than that in untransfected cells. In a cell line expressing DAT and VMAT2 (@ 500 μM) cell death was significantly attenuated compared to cells expressing DAT alone (V_{max} 110 and 240% of control). Together these data support the hypothesis that DAT acts as a gateway for MPP^+ entry into the cell and that intracellular compartmentalization of MPP^+ by VMAT2 can circumvent toxicity. (Supported by NIEHS 09248.)

1364 METHAMPHETAMINE NEUROTOXICITY : PROTECTIVE ROLE OF SELENIUM AND INVOLVEMENT OF S-ADENOSYLMETHIONINE.

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Methamphetamine (METH) is a drug of abuse known to cause dopaminergic neurotoxicity in rodents, non-human primates and humans. The present study was designed to evaluate if METH-induced dopaminergic toxicity can be protected by pre- and post-treatment with an antioxidant, selenium (Se) and how it correlates with changes in S-adenosylmethionine (SAM). Adult female C57BL/J6 mice were dosed with Se (0.5 mg/kg) in drinking water. One week later, animals were treated with 4 X 10 mg/kg METH, i.p. Se treatment was continued for the next week before mice were sacrificed, trunk blood was collected and the brains were quickly removed and dissected into caudate nucleus (CN), frontal cortex (FC) and hippocampus (HIP) for neurochemical analyses. Concentrations of dopamine (DA), serotonin (5-HT) and their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) were measured using HPLC/EC. SAM levels were measured in these brain regions as well as in blood using HPLC/PD. METH treatment produced a significant depletion of DA and its metabolites (DOPAC & HVA) and a significant increase in SAM in CN. Pretreatment with Se prevented the depletion of DA and its metabolites and increase in the SAM level in CN resulting from the METH treatment. In contrast, SAM level was decreased in the blood of animals treated either with METH or Se but in the case of animals treated with METH and supplemented with Se, blood SAM levels were equivalent to control. These data suggest that SAM is involved in the manifestations of METH neurotoxicity and Se has a possible protective role against METH-induced neurotoxicity.

1365 INTRASTRIATAL, INTRACORTICAL, AND INTRAHIPPO-CAMPAL ADMINISTRATION OF GLUTATHIONE AND N-ACETYLCYSTEINE CONJUGATES OF α-MEHTYLDOPAMINE PRODUCES SEROTONERGIC NEUROTOXICITY.

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The serotonergic neurotoxicity of 3,4-(±)-methylenedioxyamphet-amine (MDA) and 3,4-(±)-methylenedioxymethamphetamine (MDMA) is dependent upon the systemic generation of a neurotoxic metabolite(s), the identity of which remains unclear. α-Methyldopamine (α-MeDA) is a major metabolite of both MDA and MDMA, and following oxidation to the *ortho*-quinone undergoes conjugation with glutathione (GSH). The thioether conjugates of α-MeDA maintain the ability to redox cycle and generate reactive oxygen species. We now report that direct intrastriatal or intracortical administration of 5-(glutathion-S-yl)-α-MeDA (4 X 200 nmol, 4 X 400 nmol), 2,5-*bis*-(glutathion-S-yl)-α-MeDA (4 X 150 nmol, 4 X 300 nmol), and 5-(N-acetylcystein-S-yl)-α-MeDA (4 X 7 nmol, 4 X 20 nmol) causes significant decreases in striatal and cortical serotonin (5-HT) concentrations, 7 days following the last injection. Interestingly, intrastriatal injection of 5-(glutathion-S-yl)-α-MeDA or 2,5-*bis*-(glutathion-S-yl)-α-MeDA, but not 5-(N-acetylcystein-S-yl)-α-MeDA, causes decreases in 5-HT concentrations in the ipsilateral cortex. A similar pattern of changes is seen in the ipsilateral striatum, when the thioether conjugates of α-MeDA are injected into the cortex. Intrahippocampal injection of the thioether conjugates of α-MeDA also pro-

duces significant decreases in hippocampal 5-HT concentrations. The effects of the α-MeDA thioethers are confined to 5-HT nerve terminal fields, since no significant changes in monoamine neurotransmitter levels are detected in brain regions enriched in 5-HT cell bodies (midbrain/diencephalon/telencephalon and pons/medulla). In addition, the effects of the conjugates are selective to the serotonergic system, since no significant changes are seen in dopamine and norepinepherine concentrations. The results indicate that thioether conjugates of α-MeDA may contribute to the serotonergic neurotoxicity of MDA and MDMA. (DA 10832)

1366 REPEATED EXPOSURE RESULTS IN THE LOSS OF THE NEUROPROTECTIVE PROPERTIES OF RESTRAINT-INDUCED HYPOTHERMIA IN THE SUBSTITUTED AMPHETAMINE MODEL OF STRIATAL DOPAMINERGIC NEUROTOXICITY.

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Substituted amphetamines (SA) are well-characterized striatal dopaminergic (DA) neurotoxicants in mouse and restraint stress throughout the period of dosing prevents this neurotoxicity. The mechanism of this neuroprotection is unclear but appears linked to the ability of restraint to reduce SA-induced hyperthermia. We investigated whether with repeated exposure restraint remains neuro-protective. Female C57BL6J mice received 4 s.c. inj (0.2 mls every 2 hrs) of saline (SAL) or d-methylenedioxymethamphetamine (MDMA) at a dosage of 0 or 20 mg/kg, respectively on the 0, 1 (acute restraint) or 5th (repeated restraint) day of restraint (~8 hrs duration in plastic 50 ml centrifuge tubes) and rectal temperatures were monitored. On day 1-4 mice in the repeated restraint condition received SAL. Striatal tissue was collected at 72 hrs and levels of DA, its metabolites, tyrosine hydroxylase (TH) and GFAP (an astrocyte-localized protein known to increase in response to neural injury) were used to evaluate neurotoxicity. In concert with our previous findings MDMA induced hyperthermia and caused striatal dopaminergic neurotoxicity as indicated by significant depletions of DA and TH as well as elevations in GFAP; acute restraint blocked this hyperthermia and was neuroprotective. In repeatedly restrained mice significant core temperature reductions were observed on each of the first 4 days of restraint but not during MDMA administration on the final day. Neuroprotection was not observed in repeatedly restrained mice. These data suggest that hypothermia induced by restraint is a critical factor in the neuroprotective effects of this stressor. The molecular and cellular changes associated with acute and chronic restraint stress will be the focus of future work.

1367 EFFECTS OF MULTIPLE DOSES OF *l*-EPHEDRINE ON DOPAMINE, DIHYDROXYPHENYL ACETIC ACID AND 5-HYDROXYINDOLEACETIC ACID IN BRAIN MICRODIALYSATE AND BODY TEMPERATURE.

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Extracellular dopamine levels and hyperthermia are important factors in the development of the neurotoxicity of substituted amphetamines. We reported earlier that multiple doses of 40 mg/kg *l*-ephedrine (*l*-E) given at 3 hr intervals failed to produce significant decreases in caudate/putamen (CPu) dopamine levels at 7 days. Lack of a robust 5-hyroxytryptamine release may be associated with the observation that *l*-E does not produce depletions in CPu dopamine (Tor-Agbidye *et al.* Soc. Neurosci. 1998). The aim of this study was to further explore the differences in the neurotoxic potential of *l*-E versus *d*-amphetamine. Male Sprague Dawley rats (4 months) were used. The effective *l*-E dose (4 X 25 mg/kg, i.p.) was determined (dose-range finding study) and given at 2 h intervals. We studied the effects of multiple doses of *l*-E on CPu extracellular dopamine, 5-hydroxyindoleacetic acid (5-HIIA), concentrations of ephedrine and its metabolite norephedrine, and temperature using *in vivo* microdialysis in conscious, freely moving rats. Microdialysis samples were collected at 20 min intervals for up to 10 h. Dopamine levels in the microdialysate reached a peak 1.3 h (ranging from 25 ± 7 to 55 ± 15 nM) after each dose of *l*-E. Dihydroxyphenylacetic acic (DOPAC) levels dropped after each injection of *l*-E (from 1921 ± 200 nM to 250 ± 36 nM) whereas 5-HIIA concentrations remained relatively constant through out the experimental period ranging from 383 ± 55 to 533 ± 94 nM. A significant increase in the microdialysate levels of *l*-E and norephedrine was observed within the period of the first three doses. The extracellular levels of *l*-E and norephedrine plateaued after the 3rd and 4th doses of drug. In summary, multiple doses of *l*-E produced peak dopamine and DOPAC concentrations that are similar to those observed after *d*-amphetamine challenges in our previous study. However, CPu dopamine levels were only decreased by 29 %. Perhaps factors in addition to extracellular dopamine levels and hyperthermia should be considered as candidates that contribute to the neurotoxic potential of amphetamine and substituted amphetamines. (Supported by the USFDA/NCTR and Oak Ridge Associated PostGraduate Research Fellowship, Oak Ridge, Tennessee.)

1368 COPPER SULFATE PRETREATMENT INHIBITS MPP -INDUCED STRIATAL LIPID PEROXIDATION (LPO) IN MICE.

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1-methyl-4-phenylpyridinium (MPP+) is the major metabolite of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) a drug which induces parkinson-like damage in several animal species. It is now accepted that MPP+ is responsible for the neurotoxic effects of MPTP in dopaminergic nigrostriatal pathway. Our group previously reported that MPTP decreases copper content (50%) in *corpus striatum*, suggesting an important role of copper in dopaminergic neurotoxicity of MPTP. In this study, we evaluated the possible effect of copper sulfate pretreatment on MPP+-induced striatal lipid peroxidation. Adult male C57-Black mice (25-30g) were injected i.p. with either copper sulfate (2.5 mg/kg) or saline. After either 30 min or 24 h, mice were injected into their right lateral ventricle with MPP+ (20μg/3μl) and 2 hours later they were killed to measure striatal lipid peroxidation (LPO). Results showed an increase of 116% in striatal LPO in mice from the MPP+ group at 24 hours as compared with control group, while a non-significant increase of 32% in LPO levels of the copper plus MPP+ group versus control values was observed. In contrast, the $CuSO_4$ pretreatment 30 minutes before the microinjection with MPP^+ was not able to decrease MPP+-induced LPO. These results suggest that the mechanism involved in the MPP^+-induced LPO is inhibited by copper administration only after activation of delayed processes. This work has been supported by CONACyT Grant 26504-M.

1369 METALLOTHIONEIN-I AND -II KNOCK-OUT MICE ARE NOT MORE SENSITIVE THAN CONTROL MICE TO 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE NEUROTOXICITY.

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Oxidative stress, resulting from either excess generation or reduced scavenging of free radicals, has been proposed to play a role in damaging striatal neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity. Metallothionein (MT) has been proposed to be a scavenger of oxygen-derived free radicals. It is hypothesized that MT protects against oxidative damage of the central nervous system by free radicals. In the present study we used MT-I and -II knockout (MT-null) mice to determine whether the absence of MT protein alters MPTP neurotoxicity. Control and MT-null mice were treated with MPTP at doses of 30, 40 and 50 mg/kg, ip for 11 or 35 days. There was a time- and dose-dependent increase in lethality, but no difference was observed between MPTP-treated control and MT-null mice. Seven days after the last dose, brains were removed, and the dopamine (DA) and homovanillic acid (HVA) contents were analyzed in striatum and midbrain by HPLC. Striatal DA content in MPTP-treated control mice was reduced by 53%, 74% and 84% after 30, 40 and 50 mg MPTP/kg respectively. Similar decreases were observed in MT-null mice treated with MPTP. MT-null mice were not more sensitive than control mice to MPTP neurotoxicity. The data suggests that MT-I and -II proteins do not play a major role in protecting against MPTP neurotoxicity. (Supported in part by NIH Grant ES-01142.)

1370 METALLOTHIONEIN EXPRESSION AND RADIATION PROTECTION IN PRIMARY HUMAN CNS CULTURES.

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Little is known about metallothionein (MT) synthesis in the human brain after exposure to ionizing radiation (IR) and the role of MT in IR-induced damage. In this study, MT protein synthesis and mRNA expression after exposure to IR were examined in human CNS cultures. The effects of pre-

induced MT by zinc (Zn) and cadmium (Cd) on IR-induced cytotoxity were also investigated. Primary human CNS cultures from fetal brain were grown as aggregates and monolayers, and were exposed to doses of 30-120 Gy γ - rays. Total MT proteins, and mRNA for MT-II and MT-III were measured by Cd-heme assay and by reverse transcription PCR. Cytotoxic effects of radiation on CNS cells were detected by LDH release and apoptotic cell death (ACD), using DNA gel electrophoresis and TUNEL techniques. Immunohistochemistry was used for staining of MT as well as astrocytic and neuronal markers. These results showed the induction of total MT protein and MT-II mRNA, but not MT-III mRNA, following exposure to IR. Exposure of human CNS cells to IR caused a marked increase in LDH release, ACD and cell loss evaluated by specific markers. All these effects could be ameliorated significantly after induction of MT synthesis with 5 μ M Cd or 100 μ M Zn. MT induction by Cd or Zn was confirmed by Cd heme assay and immunohistochemistry. In cultured mouse astrocytes, enhanced MT expression (by Cd) was associated with marked protection from IR-induced ACD. This suggests that the relative high resistance of astrocytes to IR (compared to that of neurons) may be due to an efficient MT induction mechanism. These results suggest that MT protein or MT-II mRNA is inducible in CNS cells by IR. Pre-induced MT can protect human CNS cells from IR-induced cytotoxic injury. Both findings are of importance in developing strategies to protect CNS tissue from oxidative damage. (Supported by funds from Medical Research Council of Canada.)

1371 EBSELEN (2PHENYL-1,2 BENZISOSELENAZOL-3-(2H) ONE) INDUCES HO-1 PROTEIN (HEME OXYGENASE-1) IN RAT HIPPOCAMPAL ASTROCYTES.

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Ebselen (2phenyl–1,2 benzisoselenazol-3(2H)-one) a seleno-organic compound, has been shown to have cytoprotective properties. This has been attributed to its ability to imitate the selenium dependent enzyme, glutathione peroxidase (GSH/Px). It has also been implicated in increasing intracellular glutathione (GSH). Ebselen has been shown to protect against ischemic injury and peroxidative damage in other cell types possibly by the induction of stress proteins. The presence of stress proteins has been used to demonstrate alterations in cellular activity which may result in cellular degeneration. The purpose of this experiment was to evaluate astrocytic viability after treatment with ebselen and to investigate the ability of ebselen to induce the expression of stress proteins in these cells. Rat hippocampal astrocytes grown in Dulbecco's modified Eagle's essential media were treated with 3,6,9,15 and 30μ M ebselen at subconfluency. Effects on cell viability were seen in treatment groups. A significant decrease in cell viability was seen at the highest dose. Treatment of astrocytes with 15μ M and 30μ M ebselen increased the expression of the stress protein HO-1 (heme oxygenase - 1) in a dose dependent manner as assessed by western blots. Induction of HO-1 is associated with oxidative damage resulting from heme, metals, hormones, sulfhydryl depleting agents and UV radiation. The expression of stress protein and decreased cell viability may indicate a toxic insult rather than initiating a cytoprotective effect as described in other cell types.

1372 ACUTE NEUROTOXIC EFFECTS OF INHALED TRICHLOROETHYLENE (TCE): LINKING APPLIED DOSE, ABSORBED DOSE (BLOOD), TARGET TISSUE DOSE (BRAIN), AND OUTCOME MEASURES.

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The ability to understand dose-response relationships is improved by considering sequentially the steps of applied dose, absorbed dose, target tissue dose, and adverse outcome. We have done this for acute changes in neurological function following TCE inhalation. A physiologically-based pharmacokinetic (PBPK) model was developed for *ad-lib* feed (350-450 g) and weight maintained (350 g) adult male Long-Evans rats. Rats were exposed by inhalation to TCE in concentrations of 0, 200, 500, 2,000, or 4,000 ppm. After durations ranging from 5-60 min, and up to 60 min after termination of exposure, rats were rapidly killed and blood, fat, liver and brain samples collected. Serial blood samples were collected from other rats with jugular vein cannulas during and after exposure. The concentration of TCE in tissues was measured using gas chromatography after solvent extraction, and compared with the predictions from the PBPK model. Blood [TCE] was higher in weight-maintained than ad-lib fed rats. Log_{10} [TCE] in blood showed a linear relationship to Log_{10} [TCE] in other tissues including brain (r^2 = 0.974). The [TCE] in brain at the time of recording accurately predicted TCE-induced reductions in the amplitude of pattern-elicited visual evoked potentials (PEP) (Boyes et al., SOT 1998), either during or after exposure. A declining exponential function fit the relationship between brain [TCE] and PEP amplitude ($r^2 = 0.908$). These results illustrate that the functional effects of many exposure scenarios can be understood in terms of target tissue dose. (This abstract does not necessarily reflect EPA policy.)

1373 ANILINE-INDUCED SPONGY CHANGE OF THE SPINAL WHITE MATTER IN RATS.

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To investigate the potent neurotoxicity of aniline, two sets of 42 male rats, 4-week-old, were singly treated with 1000mg/kg of aniline or olive oil by gavage. Rats were sacrificed at 3, 7, 11, 14, 21, 29 and 59 days after dosing. Brain, spinal cords, trigeminal nerve, sciatic and tibial nerves, and gastrocnemius were perfused *in situ* with fixatives and then trimmed. Paraffin or plastic sections were made for light and electron microscopic evaluation. In aniline-treated rats, one was dead within 24 hours after dosing. Most of the remaining rats showed ataxic paraplegia, after a transient sign of cyanosis. The ataxic paraplegia resolved up to 19 days postdose. Spongy change of spinal white matter was seen in all aniline-treated rats which were sacrificed at 7 days postdose and thereafter. The lesion was most severe in the outer tracts of lateral and ventral columns. Macrophages infiltrated in some distended periaxonal space and hypertrophied astroglia were also observed at 11 to 29 days after dosing. Minimal axonal changes were seen only in the severe cases. The spinal roots and trigeminal nerve were also severely affected. Affected nerve fibers were sporadically found in the upper and lower tracts, including mesencephalon, pons, medulla oblongata, and sciatic and tibial nerves. Spongy change was corresponded to the distention and destruction of myelin sheath, essentially due to the splitting of intraperiod line in myelin sheath. No abnormalities were found in neuronal cells. At 59 days postdose, there was a significant improvement in all changes described above. The spongy change in the spinal white matter could be induced by a single oral dose of aniline in rats.

1374 DOES THE VULNERABLE PERIOD FOR DEVELOPMENTAL NEUROTOXICITY OF NICOTINE EXTEND INTO ADOLESCENCE?

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Fetal nicotine exposure results in CNS cell loss, altered nicotinic cholinergic receptor (nAChR) expression, perturbed synaptic function, and elevated expression of genes putatively involved in apoptosis. In contrast, little work has been done on nicotine exposure during adolescence, despite the fact that most smokers begin their habit during that period. We administered nicotine continuously to adolescent rats using osmotic minipump implants, beginning on postnatal day (PN) 30 to PN47. In keeping with the effects of nicotine on nAChR in fetuses or adults, receptor expression was elevated in cerebral cortex and midbrain during and immediately after the end of the infusion period. However, in adolescent rats, unlike fetal rats, cortical receptor induction still remained prominent two weeks after discontinuing nicotine exposure. The effect was regionally-specific, as nAChR upregulation was shorter-lived in the midbrain. In contrast, fetal nicotine administration produced constitutive cortical protooncogene (c-fos) induction, whereas adolescent nicotine did not increase c-fos. Adolescent nicotine produced significant and persistent cell loss (decreased DNA) in the midbrain. We therefore suggest that the vulnerable period for adverse effects of nicotine on brain development extends into adolescence, when many behavioral and physiological patterns become consolidated. Supported by a grant from the Smokeless Tobacco Research Council.

1375 ESTABLISHMENT OF AN *IN VITRO* MODEL OF THE BLOOD-BRAIN BARRIER FOR DELINEATING THE TRANSPORT OF THIOETHER CONJUGATES INTO BRAIN.

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Systemic metabolism of 3,4-methylenedioxymetamphetamine (MDMA;

Ecstasy) and 3.4-methylenedioxyamphetamine (MDA) appears necessary to produce their serotonergic neurotoxicity, and thioether metabolites of α-methyldopamine (α-MeDA), a known metabolite of MDA and MDMA, may play a role in this toxicity. To assess mechanisms by which these metabolites gain access to the brain, we developed *in vitro* cultures of immortalised human cerebrovascular endothelial cells (IHCEC) as a model of the blood-brain barrier. Of five different extracellular matrices examined (collagen, fibronectin, gelatin, laminin, and Matrigelfi), gelatin gave the highest trans-endothelial cell electrical resistance (TEER; Ωcm2) and was used to coat filter inserts placed into larger wells for transport studies. The lumenal to basolateral transport of 5-(glutathion-*S*-yl)-α-MeDA (5-[GSyl]-α-MeDA; 100 μM) was monitored for 60 min, ~5% of 5-(GSyl)-α-MeDA was recovered intact in the basolateral compartment, whereas 78% of the substrate remained intact in the lumenal compartment. The residual 17% is likely metabolized by γ-glutamyl transpeptidase and dipeptidases since evidence of 5-(cystein-*S*-yl)-α-MeDA in both the lumenal and basolateral compartments was obtained by HPLC-coulometric electrode array analysis of the media. The barrier function of the IHCEC monolayers remains intact (no change in TEER) for the duration of the transport studies indicating maintenance of tight junctions. Transport of intact 5-(GSyl)-α-MeDA across the IHCEC monolayer is most likely due to the activity of an intact GSH transporter (DA 10832; Drug Metabolism, Merck & Co).

1376 THE CELLULAR NEUROTOXICITY TEST BATTERY USED IN THE ERGATT/CFN INTEGRATED TOXICITY TEST SCHEME (ECITTS).

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Neurotoxicity risk assessment is routinely based on animal studies. The availability of specific cellular test systems has made it possible to study the mechanisms of neurotoxic effects *in vitro*. This also offers economical and ethical advantages over large-scale *in vivo* studies. In the present study, the neurotoxicity of acrylamide, caffeine, diazepam, lindane, parathion and phenytoin was assessed in an *in vitro* test battery. The general cytotoxicity and neurite degeneration, as well as effects on intracellular Ca2+ homeostasis, protein synthesis, Ca2+ channel (VOCC) and acetylcholine receptor function, were investigated in human neuroblastoma (SH-SY5Y) cells after 72 hours exposure with the compounds. The most sensitive parameters were; neurite degeneration for acrylamide and parathion, VOCC function for caffeine, diazepam and lindane, and protein synthesis for phenytoin. The critical cellular neurotoxic concentration for each compound (i.e. lowest EC20 value) was integrated in a biokinetic model and the lowest effective *in vivo* doses (LOEDs) were estimated. The estimated LOEDs for acrylamide, caffeine and lindane correlated well with literature derived experimental LOEDs, implicating that the selected *in vitro* tests were appropriate for these compounds. However, the estimated LOEDs for diazepam, parathion and phenytoin were more than half a magnitude higher than the experimental LOEDs, possibly because no tests for benzodiazepine receptor function and choline esterase activity were included in the test battery. This result illustrates the importance of designing *in vitro* test batteries, in which endpoints, relevant for each class of compounds, are included.

1377 NOISE-INDUCED HEARING LOSS IN DIFFERENT CARBON MONOXIDE ENVIRONMENTS.

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Intense noise, especially its high frequency components, may cause hearing loss (HL). The noise-induced-HL (NIHL) can be enhanced by simultaneous carbon monoxide (CO) exposure. Carbon monoxide exists in many environments. For example, pure cigarette smoking may cause an alveolar CO level of about 300 ppm. Therefore, it is important to know the lowest CO-level at which the CO interacts with intense noise and makes the noise-induced auditory impairment worse. In the present study, high-frequency octave-band noise (9.6-19.2 kHz, 100 dB_{Ln}) and CO-levels of 1200 ppm, 700 ppm, 500 ppm, 300 ppm and 0 ppm were applied. The hearing loss was determined by recording of CAP (compound action potential) from the round window, 4 weeks after exposure. The octave-band noise alone (0-ppm-CO) induced an approximate 30-dB permanent hearing loss in rats at frequencies higher than 10 kHz. CO alone did not induce any hearing losses, even at 30 minutes following the exposure. The combined exposure of the noise and 1200-ppm-CO, however, caused a much greater permanent hearing loss, especially in high frequency region. The HL-enhancement phenomenon by CO was obvious. The enhancement of the noise-induced hearing loss lessened gradually with CO-level. The hearing loss to the combined exposure with 300-ppm-CO was similar to that to the noise alone (0-ppm-CO). The lowest CO-level, which shows interaction with intense noise, must be between 300 ppm and 500 ppm. In human subjects the lowest CO-level, showing interaction with intense noise, might be lower than the data obtained in the rats. (Supported in part by NIOSH grant #03481 and NIH grant ES08082.)

1378 ACUTE DISRUPTION OF COCHLEAR POTENTIALS BY POTASSIUM CYANIDE.

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Cyanide is present in various environmental settings and is also an occupational hazard for firefighters. Effects and mechanisms of cyanide (CN) toxicity are unclear in hearing loss. In this study, adult male Long Evans hooded rats were used to examine the effects of potassium cyanide (KCN). Interactions with noise on cochlear potentials, namely, compound action potential (CAP) and cochlear microphonic (CM) were studied. Subjects were injected with KCN (7mg/kg, ip) and compared with controls (saline, ip). CAP sensitivity is dependent upon the function of inner hair cells and spiral ganglion cells. The CM is generated predominantly from outer hair cells. CAP and CM were recorded pre and post administration of KCN. Hearing levels at three frequencies (2 kHz, 12 kHz and 40 kHz) were tested. Blood cyanide levels were measured following KCN administration for a period of 30 min. A temporary threshold shift in both CAP and CM was observed for a period of 5 to 15 min. following KCN administration. Concurrent exposure to KCN and noise (110 dB, broad band) for 2 hours demonstrated no threshold shift in either CAP or CM. (Supported in part by NIOSH grant OH 03481 and NIEHS grant ES 08082.)

1379 EFFECTS OF EXPOSURE DURATION ON POTENTIATION OF NOISE INDUCED HEARING LOSS BY CARBON MONOXIDE.

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Noise induced hearing loss (NIHL) is still the most common occupational disease in the U.S. Concurrent noise exposures with chemical asphyxiants such as carbon monoxide (CO) is common in work environments of firefighters, automobile mechanics and tunnel-toll booth workers. Potentiation of NIHL following acute exposure to noise (102-103 dB, broad band) and CO (1500 ppm) has been demonstrated previously. It is well known that noise exposure of varying duration is related to exposure intensities. The equal energy principle states that an increase in exposure intensity is compensated for by a corresponding decrease (halving) in exposure duration. This study investigates whether or not the NIHL resulting from combined exposures complies with the equal energy principle. Adult male Long Evans hooded rats were acutely exposed to noise alone (100dB_{Ln}, 9.6-19.2 kHz), CO alone (1500 ppm), noise & CO, and air (controls). Duration of exposure varied between 2 and 8 hours. Auditory sensitivity was measured four weeks post-exposure. End points measured were electrophysiological, namely, compound action potential (CAP) and cochlear microphonic (CM). CAP is a measure of cochlear output generated at the inner hair cell-type I spiral ganglion synapse, and, CM is generated largely by the outer hair cell. These potentials were recorded in response to pure tones between 2 and 40 kHz. NIHL was found to comply with the principle of equal energy. However, hearing loss resulting from combined exposure did not follow a similar pattern. (This study is supported in part by NIOSH grant #03481 and NIEHS grant ES 08082.)

1380 SUBCHRONIC TOXICITY OF PILOCARPINE IN RATS.

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Pilocarpine, a cholinergic parasympathetic agent with predominantly muscarinic action, is indicated as a treatment for symptoms of xerostomia in Sjogren's syndrome or head and neck cancer patients following radiation. A 90-day range-finding study was done to assess the toxicity, define an esti-

mated MTD, and assess the PK necessary to select doses for a carcinogenicity study. Male and female CD rats were assigned to 1 control group and 4 treated groups to assess toxicity (10/sex/group) and PK (61 animals /sex/group). Animals were dosed daily by oral gavage at 0 (control), 3, 9, 18, and 36 mg/kg/day; controls received the vehicle, reverse osmosis water. Detailed examinations, body weights, and food consumption were recorded weekly. Plasma pilocarpine levels were determined predose and at selected intervals through 180 minutes postdose on Day 1 and during Week 13. Toxicity animals were necropsied during Week 14; selected organs were weighed, and selected tissues were examined microscopically. Pilocarpine was rapidly absorbed and eliminated with no bioaccumulation during treatment. Clinical observations were consistent with the known muscarinic pharmacological activity, dose dependent, and persisted throughout treatment. Significant decreases in body weight, body weight gain, and food consumption were present for males given 9, 18, or 36 mg/kg. An MTD was identified in the range of 9 to 18 mg/kg for males and 18 mg/kg or below for females. In the absence of dose limiting pathological findings, doses of 3, 9, and 18 mg/kg for the carcinogenicity study were determined based on the relative ratios of AUC for rats and humans.

1381 ENHANCED ACUTE PULMONARY TOXICITY OF MERCURY VAPOR IN METALLOTHIONEIN-NULL MICE.

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To elucidate the protective role of metallothionein (MT) in the acute toxicity of mercury vapor, we examined the sensitivity of MT-null transgenic mice, a kind gift from A. Choo, to the exposure to mercury vapor. Ten-week-old male MT-null mice and wild-type control mice (15 mice for each group) were exposed to mercury vapor at 6.6 to 7.5 mg/mm^3 for 4 h each day for 3 consecutive days. Blood, lung, liver and kidney were removed from each mouse under diethylether anesthesia at 24 h after the last exposure to mercury vapor. 67 % of MT-null mice died during this study whereas all of wild-type mice survived. In the lung of MT-null mice, marked morphological changes such as congestion, atelectasis and hemorrhages in the alveoli were observed following mercury vapor exposure. In contrast, the wild-type mice exposed to mercury vapor showed a lesser degree of pulmonary damage. In the liver and kidney of MT-null mice and wild-type mice, no histopathological changes were observed following exposure to mercury vapor. The present study indicates that MT-null mice were more sensitive to mercury vapor-induced pulmonary damage and lethality, and that MT in the lung plays a protective role against acute pulmonary toxicity caused by mercury vapor.

1382 REDOX REGULATION OF COPPER-METALLOTHIONEIN INTERACTIONS.

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Metallothioneins (MTs) are cysteine-rich metal-binding proteins that exert cytoprotective effects during metal exposure and oxidative stress. The specific role of MTs in modulating Cu-dependent redox cycling remains unresolved. While MT is capable of binding up to 12 Cu^{1+} atoms/peptide, we hypothesize that oxidation of free thiols critical for metal binding can reduce MT/Cu interactions and potentiate Cu^{1+}-dependent redox cycling. We utilized a chemically-defined model system to study MT modulation of Cu-dependent redox cycling under physiologic reducing (Cu/ascorbate) and mild oxidizing (Cu/ascorbate + H_2O_2) conditions. In the presence of Cu and ascorbate, MT blocked Cu-dependent lipid oxidation and ascorbyl radical formation with a stoichiometry corresponding to Cu/MT ratios $\leq$ 12. In the presence of H_2O_2, the degree of protection by MT was less and biological oxidations and radical formation were inhibited only up to Cu/MT ratios of 6. Physical interaction of MT and Cu was measured by using low-temperature EPR of free Cu^{2+} in solution. The maximal amount of EPR-silent Cu^{1+} (presumably in complex with MT) corresponded to 12 molar equivalents of Cu/MT under reducing conditions, but only 9 in the presence of H_2O_2. Dithiodipyridine titration of reduced sulfhydryls on MT revealed a 50% decrease after H_2O_2 oxidation, which could be regenerated by the reducing agent, dihydrolipoic acid. H_2O_2 modulated the ability of MT to protect HL-60 cells from Cu-induced cell death in a manner that correlated with the ability of MT to mitigate Cu-redox cycling in cell-free systems. Thus, optimal binding of Cu to MT is achieved only under reducing conditions, however, a portion of this Cu appears releasable under oxidizing conditions. Release of free Cu from MT during oxidative stress could enhance the formation of reactive oxygen species and potentiate cellular damage. (Supported by ES-09387, EPA Star Grant Program, AICR 97B128, Johns Hopkins CAAT 97039, NCI Oncology Res. Faculty Develop. Prog., Intl. Neurological Science Fellowship F05NS10669 by NIH/NINDS and WHO.)

1383 METALLOTHIOEIN ISOFORM GENE EXPRESSION IN HUMAN PROXIMAL TUBULE CELLS EXPOSED TO ZINC, COPPER, CADMIUM, MERCURY, SILVER, AND LEAD.

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The organization of the human metallothionein (MT) gene family is more complex than the commonly utilized mouse and rat models, having a total of 10 functional and 7 non-functional isoforms. The objective of the study was to determine if the human MT expression is similar to or divergent from animal models. Human proximal tubule (HPT) cells were exposed to lethal and sublethal concentrations of Cd, Zn, Cu, Ag, Hg, and Pb, and the total MT protein content was determined. Large MT protein accumulations were evident in cells exposed to Cd, Zn, Cu, whereas no significant protein accumulation was found for the other metal exposures. In order to differentiate between the many MT isoforms, PCR primers were designed that would specifically amplify each individual mRNA isoform. MT-2A was expressed at the highest level; however, it was shown that the levels of mRNA did not increase significantly with metal treatments that induced total MT protein accumulation. This observation is different from results seen in mouse or the rat models. For the MT-1 isoforms, MT-1X and MT-1E were found to be major transcripts, whereas MT-1F and MT1A were found to be minor transcripts. The levels of mRNA for 1A and 1E were shown to increase significantly with metal treatments (Zn, Cd, Cu, but not Hg, Ag, or Pb) at concentrations that were toxic (MT-1A) and nontoxic (MT-1E).

1384 TRANSIENT NUCLEAR TRANSLOCATION OF METALLOTHIONEIN DURING MYOBLAST PROLIFERATION AND DIFFERENTIATION.

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Metallothionein (MT) may play a role in homeostasis of Zn and Cu and may have protective effects against heavy metal toxicity and active oxygen species. The requirement of Zn during G1/S phase transition of the cell cycle coincides with the cell cycle dependent expression of MT and its transient nuclear localization in certain proliferating cells. In order to investigate the changes in cellular localization of MT during proliferation and differentiation, two skeletal muscle cell lines were used: L6 and H9C2 myoblast. Both Insulin-like Growth Factor (IGF-I) and decrease in fetal bovine serum (FBS) to 1% stimulated the differentiation of myoblasts to myotubes within 8 days. MT was localized mainly in the cytoplasm in myoblasts but was translocated to the nucleus of newly formed myotubes during early differentiation. At later stage of differentiation, MT synthesis was decreased and was localized in the cytoplasm of myotubes. The changes in localization of MT were followed by immunofluorescence using a polyclonal MT-antibody and anti rabbit IgG labelled with FITC. In both cell lines, MT was localized in the nucleus at late G1/early S-phase after blocking cell growth with aphidicolin (an inhibitor of DNA polymerase-α) or deletion of the FBS. The slow progression of cell cycle leads to transfer of MT to the cytoplasm at the end of M-phase. Addition of rapamycin (serine/threonine p70 kinase inhibitor), PD98059 (an inhibitor of MAP kinase) or LY294092 (an inhibitor of PI3 kinase) blocked the translocation of MT in the nucleus of myoblast and myotubes. The results suggest that the increase of MT content and its translocation into the nucleus of myoblasts and myotubes during early differentiation may be due to a rapid mitogenic and myogenic response. It also suggests that various kinases and signal transduction cascades probably regulate these processes. (Supported by a grant from Medical Research Council of Canada.)

1385 ROLE OF METALLOTHIONEIN (MT) IN THE UPTAKE AND DISTRIBUTION OF ENVIRONMENTAL LEVELS OF CADMIUM IN MICE.

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Humans are exposed to environmental cadmium via food. Metallothionein (MT), a small, inducible, cysteine-rich, metal-binding protein is believed to be at least partially responsible for the retention and correspondingly long half-life of Cd in tissues. The study presented here was aimed at exploring the role of MT in the uptake, transport and distribution of Cd administered intravenously and via gavage at doses that do not induce the biosynthesis of MT. The experiments were conducted on MT (I and II) knockout (MTKO) and MT normal (MTN) mice. The gavage experiments yielded results which were – 1. independent of MT status of the mice: (a) Cd was rapidly absorbed and retained by duodenum (% total dose – MTN: 34±9; MTKO: 38±10), (b) early deposition favored liver over kidney (4h liver/kidney Cd – MTN: 4.6±0.6; MTKO: 5.1±0.5), and (c) Cd transported across the GI tract amounted to only ~1% of the total dose; 2. MT-dependent: (a) Cd distributed throughout the body was 2-3X higher in MTKO than in MTN mice (% total dose – MTKO: 3.1±1.7; MTN: 0.6±0.2) and (b) absence of MT correlated with a significant increase in liver deposition after 8h following administration (% total dose – MTN: 0.3±0.03; MTKO: 0.9±0.1). The following features were independent of both route of exposure and MT status: (a) Cd was cleared from blood extremely rapidly (b) the remaining carcass (muscle, pelt, etc.) was a major deposition site for transported Cd and (c) liver deposition of Cd was consistently higher than that of kidney. Size-exclusion chromatography of plasma after gavage or iv administration revealed albumin to be the major Cd-binding entity in both MTN and MTKO mice. Diafiltration and metal-affinity chromatography of duodenal extracts from gavage-dosed mice indicated that most of the Cd retained in this tissue was associated with low molecular weight (<3 kda) chelators. Thus at basal MT levels and low doses (0.1 μg) of Cd, MT along with small chelators function to limit intestinal absorption and direct Cd away from the liver towards the kidney. (Supported by NIEHS grant ES07398.)

1386 SURGICAL CHOLESTASIS DOES NOT PRODUCE ZINC (Zn) MOBILISATION FROM PLASMA TO LIVER IN RATS.

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The mobilisation of Zn from plasma to the liver and the role of hepatic metallothionein (MT) and MT-I and MT-II isoforms during cholestasis were investigated. Bile duct was surgically ligated in adult male Wistar rats (200 g) and were sacrificed under deep anaesthesia (ether) at 3, 6, 9, 12, 16, 20 and 24 h. Controls received no surgical treatment. Livers were homogenised (sacarose 0.25M) and centrifuged. Zn (atomic absorption spectrometry) serum and liver supernatants, hepatic MT (cadmium saturation method) and MT-I and MT-II isoforms (ion exchange chromatography) were measured in liver supernatants. Cholestasis increased 1.5-fold hepatic Zn content (9 h) and correlated with MT increases (3.2-fold) but, MT-I and MT-II ratio and serum Zn content remained unchanged, suggesting that cholestasis do not promote a Zn plasma/liver mobilisation. Cholestasis results are in contrast with the post-surgical stress response where hepatic Zn increases correlate with serum Zn decreases and changes in the liver MT-I/MT-II ratio. These results suggest that the source of Zn in the post-surgical stress is plasma whereas in cholestasis might be the stagnated bile. Differences between post-surgical and cholestasis responses might be due, in the former, to the activation of hormonal and cytokine receptors in the vascular phase of the hepatocyte inducing MT-II and initiating Zn capture whereas, in the latter, passive mobilisation of Zn from bile to the hepatocyte may take place in addition to surgical stress changes.

1387 METAL PRETREATMENT INDUCES METALLOTHIONEIN AND ALTERS HORMONE RESPONSIVENESS IN MAMMALIAN CELLS.

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Metallothioneins (MTs) are the major low molecular weight zinc-binding proteins in mammalian cells. It has been hypothesized that they play a role in the function of zinc-dependent signal transduction proteins and transcription factors. Glucocorticoids interact with a zinc-dependent glucocorticoid receptor (GR) to induce gene transcription via binding to the glucocorticoid response element (GRE). We studied, in a mouse mammary tumor cell line (2305), the ability of the synthetic glucocorticoid dexamethasone (DEX) to stimulate transcription of a chloramphenicol acetyl transferase (CAT) gene controlled by a GRE-containing mouse mammary tumor virus promoter. In cells pretreated with 20 to 100 μ M $ZnCl_2$, DEX-induced CAT activity correlated with zinc-induced MT levels. In contrast, pretreatment with 1 μM $CdCl_2$ significantly reduced CAT activity despite increased MT levels. This may reflect a direct effect of $CdCl_2$, or a functional difference between zinc-associated MT and cadmium-associated MT. Significantly more CAT activity was also observed in heat-shocked cells. Although heat shock alone (in the absence of DEX) did not induce MT protein, the possibility exists that both heat and zinc affect DEX-induced CAT activity through an MT-mediated mechanism. Changes in GR levels could not account for variations in CAT activity. These data indicate that hormonal signaling in mammalian cells can be altered by exposure to metal salts, and the effect is correlated with the level of MT and its associated metal ion. (Supported by the MRC of Canada.)

1388 METALLOTHIONEIN LEVELS IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES: UTILITY IN ENVIRONMENTAL EXPOSURE ASSESSMENT.

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MT levels of human peripheral blood lymphocytes were determined using a MT-specific antibody and flow cytometry. Treatment of human whole blood *ex vivo* with $CdCl_2$ was found to induce a concentration- and time-dependent increase in lymphocyte MT levels at concentrations as low as 0.3 μM and within a 12 h period. Interestingly, differences were observed in the magnitude of cadmium-induced MT levels in the lymphocytes of six (6) human test subjects. Two members of the study population exhibited $CdCl_2$-induced cellular MT levels that were up to twofold greater than the lymphocytes of other human subjects. While the lymphocytes of most test subjects exhibited a symmetric (unimodal) distribution of cadmium-induced MT-specific fluorescence, the cells of two individuals displayed a heterogeneous (non-uniform) distribution of MT levels. Dual-parameter flow cytometric analysis using phenotype-specific antibodies indicated that variations in the responsiveness of subpopulations of lymphocytes to $CdCl_2$ was responsible for the heterogeneous distribution of MT-specific cellular fluorescence. T-helper (CD4-positive) and T-suppressor/cytotoxic (CD8-positive) lymphocytes expressed higher cellular levels of MT than other lymphocyte subpopulations (i.e., B-lymphocytes, natural killer cells). Our results suggest that MT protein levels of peripheral blood lymphocytes, as determined by this flow cytometric method, may be used to assess human exposure to toxic metals and characterize various quantitative/qualitative aspects of the response of individuals to cadmium and possibly to other types of environmental stresses.

1389 METALLOTHIONEIN AND INNATE ACTIVATION OF PRIMARY HUMAN AND MOUSE MONOCYTES.

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Interactions with extracellular matrix may be important for the migration and location of immune cells in the tissue. In this study, we examined the adhesion of human intestinal mast cells to extracellular matrix proteins coated to plastic dishes. Mast cells were isolated from normal surgery tissue specimen and purified up to 99 % by positive selection using magnetic cell separation (MACSTM). 96-well plates were coated for 2 h at 37°C with 100 μl PBS containing 2 μg of different adhesion proteins. 5 x 103 mast cells/well were incubated for 90 min at 37°C in culture medium with or without addition of 100 ng/ml SCF. The dishes were washed three times and the quantity of adherent mast cells was determined by histamine measurement after cell lysis. Only few mast cells bound to collagen type I, III, IV, VI and XIV (3.0-8.1 %). Mast cells adhesion was more pronounced to laminin (12±6.7%) and to fibronectin (21.9±8.9%). Following stimulation with SCF mast cell adhesion increased from 8.1±6.2% to 16.0±8.8% on collagene type I, from 12±6.7% to 23.4±10.8% on laminin and from 21.9±8.9% to 44.7±17.3% on fibronectin. Independent of SCF stimulation, RT-PCR analysis revealed mRNA expression for ß1, ß3, a4 and a5-integrin receptor subunits. Conclusion: Human intestinal mast cells are able to adhere to fibronectin. SCF stimulates adhesion of mast cells to fibronectin and other matrix proteins about 2.5-fold.

1390 CARDIOVASCULAR SAFETY ASSESSMENT OF BMS-196854 (ONCOSTATIN M) IN CONSCIOUS CYNOMOLGUS MONKEYS.

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BMS-196854 (Oncostatin M) is a pleiotropic cytokine that was examined for its potential use in acute inflammatory diseases. Two (one male and one female) conscious, biotelemetry-instrumented cynomolgus monkeys were used in this cardiovascular safety study. Arterial pressure (systolic, diastolic, and mean) and heart rate were monitored following a 30 μg/kg dose of BMS-196854 administered as a 60-minute infusion or by bolus intravenous injection. A similar dose volume of vehicle was administered as a 60-minute infusion or by bolus intravenous injection for control purposes. No significant effects on blood pressure were observed during or following the 60-minute intravenous infusion. Immediately following bolus injection, blood pressure declined by approximately 20-30% from pretreatment values and was associated with reflex tachycardia. The decline in pressure was effectively treated with either levophed or dobutamine. The cardiovascular effects of 30 μg/kg of BMS-196854 when administered by bolus intravenous injection did not occur when administered as a 60-minute infusion in cynomolgus monkeys. The results of this study supported intravenous infusion of BMS-196854 in Phase I trials in humans.

1391 APPLICATION OF THE INTEGRATED TELEMETRY SYSTEM FOR CARDIOVASCULAR ASSESSMENT OF PHARMACOTHERAPIES TO TREAT COCAINE ABUSE.

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Potential pharmacotherapeutics for the treatment of cocaine addiction require an evaluation in the presence of cocaine to determine the likelihood of an adverse cardiovascular interaction. Adult male beagle dogs were instrumented with telemetric left ventricular and aortic pressure transducers, pulmonary artery flow probes and base-apex leads to provide measures of cocaine's major actions on hemodynamics, left ventricular function and electrocardiographics. Cocaine (0.3-3.0 mg/kg, i.v.) dose-dependently elevated mean arterial pressure, heart rate (HR), cardiac output, vascular resistance, left ventricular pressure, contractility and concomitantly increased myocardial oxygen demand while lengthening the P-R and QRS intervals. Additionally, the β_1-antagonist propanolol reduced resting HR and contractility and mitigated the tachycardiac and positive inotropic effects of cocaine. Comparison of these results with those obtained in sling-restrained animals demonstrate that the telemetered dog model provides an accurate depiction of the acute cardiovascular effects of cocaine, without the confounding effects of artificial restraint. (Supported by NIDA contract N01DA-6-8068.)

1392 FOUR-DAY CONTINUOUS INTRAVENOUS INFUSION TOXICITY STUDIES OF THE ENDOTHELIN ANTAGONIST CI-1020 IN BEAGLE DOGS AND CYNOMOLGUS MONKEYS.

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The butenolide CI-1020 is a potent and selective endothelin A receptor antagonist. As part of the preclinical toxicity program, CI-1020 was given for 4 days as a continuous intravenous infusion to beagle dogs at 0.144 to 480 mg/kg/day and to cynomolgus monkeys at 24 to 480 mg/kg/day. In dogs, emesis and miosis were observed at 24 to 480 mg/kg/day, aggressive and hyperreactive behavior at 72 to 240 mg/kg/day, tremors at 120 to 480 mg/kg/day, and hypoactivity at 240 to 480 mg/kg/day. All dogs at 480 mg/kg/day died on Day 2. In monkeys, emesis, hypoactivity, lordosis, and death occurred at 480 mg/kg/day. Decreases in hepatic microsomal cytochrome P450 and erythromycin N-demethylase occurred in dogs at 7.2 to 480 mg/kg/day and all CI-1020-treated monkeys. CI-1020 plasma concentrations increased disproportionately with dose in dogs and proportionately in monkeys. Plasma endothelin-1 concentrations increased 10 to 20 fold in dogs at 120 to 480 mg/kg/day and in monkeys at 480 mg/kg/day. Arteriopathy was observed in all dogs at 7.2 to 480 mg/kg/day, occurring primarily in coronary arteries, and characterized by focal or multifocal fibrinoid necrosis, hemorrhage, and mixed inflammatory cell infiltrate in the media and/or adventitia. A low incidence of arterial lesions was noted in other organs at 7.2 to 480 mg/kg/day. No pathologic findings were identified in monkeys. In conclusion, CI-1020 administered continuously for 4 days induced similar clinical signs and effects on hepatic microsomal enzymes and plasma endothelin-1 in dogs and monkeys. Arteriopathy was only observed in the dog.

1393 MATHEMATICAL CORRECTIONS FOR THE INVERSE RELATIONSHIP BETWEEN QT INTERVAL AND HEART RATE IN CONSCIOUS BEAGLE DOGS AND CYNOMOLGUS MONKEYS.

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Pharmacodynamic prolongation of ventricular repolarization (QT Interval) has the potential liability of inducing polymorphic ventricular tachycardia (Torsades de Pointes), ventricular fibrillation and death. An inverse relationship exists between changes in heart rate and the QT interval which poses a dilemma for studying drugs that affect heart rate. Of the various mathematical formulae that have been proposed as a correction for this problem, 12 of these were evaluated in the present study. Twelve one and two parameter linear regression models were tested in the following manner. Electrocardiograms were collected using lead I, II, III, aVR, aVL, and aVF configurations from restrained, nontreated beagle dogs (n=872) and cynomolgus monkeys (n=235). There were a total of 1,808 and 470 QT - RR intervals pairs (dog and monkey, respectively) evaluated with each formula. The precision of each formula was tested using an Akike Information Criteria (AIC). Based on the results for the one and two parameter models, there are several formulae that would provide good precision for the corrected QT interval over a wide range of heart rates. These include $a \bullet RR^{(1/3)}$, $a \bullet RR^{(1/4)}$, $a \bullet \log RR$ and any of the 2 parameter models. However the one parameter models, would provide the most practical correction only requiring a single measurement.

1394 CARDIOTOXICITY OF NERIIFOLIN (NSC1243975) IN BEAGLE DOGS.

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Neriifolin, a cardiac glycoside, has demonstrated antitumor activity. Dose levels for the anticipated cardiac glycoside effects such as increased cardiac contractility and arrhythmic activity were not known. This study was designed to determine if cardiac effects were observed at effective antitumor concentrations via IV bolus and 24 hour infusion. Systemic and left ventricular pressures, heart rates and ECG's were collected via implanted radiotelemetry. Abnormal cardiac electrical activity was minimal at the 25 μ g/kg level and increased in severity in a dose related fashion. The abnormal electrical activity included a wide range of digitalis-like findings including PQ interval prolongation, 1st and 2nd degree heart block of the Mobitz type I, ventricular premature depolarizations and sustained tachycardia. The onset of delayed arrhythmic activity observed in the 50μ g/kg animals was unusual. Arrhythmic therapies alleviated, albeit to a minimal extent, arrhythmias that occurred shortly after dosing, suggesting an initial digitalis-like effect. Inability of these treatments to have an effect upon delayed arrhythmias suggests a nondigitalis-like mechanism. Hemodynamic alterations were subtle at the 50 μ g/kg dose and reached physiological significance at the 100 and 200 μ g/kg doses. Cardiac contractility was elevated for an extended period in animals dosed at 100 μ g/kg. The persistence of increased contractility was consistent with the known activity of some cardiac glycosides. A more impressive increase in contractility may be anticipated in a failing heart. Significant heart rate alterations were evident. The magnitude of the hemodynamic alterations appeared to be dose related. The 200 μ g/kg dose was lethal. There was no reduction in the toxicity of neriifolin delivered over 24-hours versus IV bolus infusion and therapeutic doses could not be administered without severe cardiotoxicity. (Sponsored by the National Cancer Institute, Contract NO1-CM-37834.)

1395 BOLUS INTRAVENOUS INJECTIONS OF PHOSPHOROTHIOATE OLIGODEOXYNUCLEOTIDES CAUSE SEVERE HYPOTENSION BY ACTING AS ALPHA-1 ADRENERGIC RECEPTOR ANTAGONISTS.

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A phosphorothioate oligonucleotide (PS ODN) with sequence antisense to *c-myc* mRNA (5'-AACGTTGAGGGGCAT-3'; LR-3280) was injected into the right atrium of the heart of Rhesus monkeys as a 30-60 second bolus. A dose of 3.3 mg/kg into conscious monkeys produced no observable alterations in blood pressure while injection of 5.0 mg/kg produced profound hypotension with blood pressure as low as 27 mm Hg. Radioligand binding assays demonstrate LR-3280 competes with prazosin for the α_1 adrenergic receptor with an IC_{50} of 15μM which favors binding over serum albumin (IC_{50} = 54.7 μM). The LR-3280 concentrations required to inhibit vasoconstriction produced by epinephrine were estimated between 28 and 40 μM which is higher than free drug concentration of 27.4 μM produced by 3.3 mg/kg infusion. However, free drug concentration of 46.2 μM from 5.0 mg/kg of LR-3280 blocks sympathetic tone produced by epinephrine resulting in profound vasodilation followed by decreased cardiac output and blood pressure with increased heart rate. Hypotension was reversed by i.v. isotonic saline and prevented by admixing serum albumin to 5.0 mg/kg LR-3280 prior to injection confirming that free ODN concentration determines cardiovascular events. LR-3280 decreased *in vivo* response to phenylephrine suggesting a common adrenergic receptor site. Reducing the number of bases of the PS ODNs to 5 and 9 bases reduced the binding affinity *in vitro* and bolus infusion of 5.0 mg/kg of 9-mer no longer produced hypotension. In conclusion, the array of anionic charge is responsible for PS ODN induced hypotension following intravascular bolus injections.

1396 THE EFFECTS OF NANDROLONE ON MYOCARDIAL FUNCTION IN HAMSTERS: BIOINDICATORS OF TOXICITY.

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The anabolic-androgenic steroid class of drugs has been implicated in sudden myocardial death. This is supported by laboratory research demonstrating cardiovascular changes that could decrease cardiac health. The present research represents a portion of an on-going project to examine the effects of nandrolone decanoate (ND) on myocardial function. Golden Syrian hamsters (90 days old) were administered with 4 mg ND (100 mg/ml in sesame oil) subcutaneously once weekly for 24 weeks, during which time animals were weighed weekly and observed daily for gross adverse effects. Following the dosage period, subjects were anaesthetised with pentobarbitone (50 mg/Kg, intraperitoneally). Upon induction of anaesthesia, electrocardiograms (ECG) were obtained, using Chart 3.3.5 for MacLAB, for 2 min. Following ECG data collection and while the animal was anaesthetised, euthanasia by exsanguination (direct cardiac puncture) was performed. Blood was collected and subjected to centrifugation. The resultant serum was stored at -20°C for later analysis. The heart was then removed, ventricles dissected out, weighed, and homogenised. Following centrifugation of heart homogenate, the supernatant was collected and stored in glycerol at -20°C for analysis. Liver, kidneys, and testes were excised, weighed, and examined for gross abnormalities. Serum was used for the spectrophotometric analysis of creatine kinase activity, lactate dehydrogenase activity, and calcium. Ventricular homogenates were used for determination of creatine kinase activity. The results indicate that subjects dosed with ND exhibit less weight gain (20% increase in body weight) than controls (35%). Heart:body weight ratios were not significantly different between dosed (0.395±0.025) and control (0.36±0.02) subjects. However, testicular (1.38±0.245 vs. 2.95±0.276) weights were significantly lower in treated subjects. Kidney (1.24±0.032 vs. 0.79±0.017) and liver (5.34±0.243 vs. 4.06±0.0146) weights were significantly greater in treated subjects. There were no significant differences in serum or ventricular CK activity. However, serum calcium was significantly elevated in dosed subjects (4.47±0.512 vs. 2.137±0.149) as was serum LDH activity (317.8±41.7 vs. 112.4±17.2). Subsequent analysis of ECG data indicated no significant difference in PR intervals, QRS intervals, ST segments, nor in P, QRS, or T wave amplitudes. The data suggest that their is no direct toxic effect of ND on the myocardium of hamsters, as assessed by the parameters of this study. Therefore, if cardiovascular health is compromised by the anabolic steroid, it seems reasonable that it results from some indirect action which increases the risk of cardiovascular health, although further research is required to support this theory.

1397 COADMINISTRATION OF HERCEPTIN WITH ANTHRACYCLINE AND CYCLOPHOSPHAMIDE: NONCLINICAL INVESTIGATIONS INTO MECHANISM OF CARDIOTOXICITY.

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HER2 (erbB2), a member of the human epidermal growth factor family of receptor tyrosine kinases, is overexpressed in 25-30% of primary breast cancers. Although cardiotoxicity is a known side-effect of anthracycline (A) + cyclophosphamide (C) administration, in Phase III clinical trials with Herceptin (recombinant humanized monoclonal anti-HER2, trastuzumab) an increased incidence of signs and symptoms of cardiac dysfunction was observed in some women receiving the combination of Herceptin + AC, compared with women receiving AC only. Cardiac dysfunction was not predicted by the nonclinical safety studies; there was no evidence of Herceptin binding to cardiac tissue, and there was no evidence of direct toxicity in chronic studies conducted in primates. In addition, nonclinical pharmacokinetic studies demonstrated that Herceptin did not impact the disposition of doxorubicin or the combination of doxorubicin + cyclophosphamide. To elucidate the possible role of Herceptin in the pathogenesis of cardiac dysfunction, HER2 expression was evaluated in endomyocardial biopsies (EMB) obtained from 3 patients enrolled in Herceptin Phase III clinical trials, and compared with archived heart tissue from cardiomyopathic and normal hearts. Evidence of HER2 gene expression was not detected by in situ hybridization (ISH) in normal heart but was detected in 2/3 of the EMB specimens and in the archived cardiomyopathic heart tissue. In contrast, there was no evidence of gene expression by ISH for neu (the rodent homolog of HER2) in a rat model of doxorubicin-induced cardiomyopathy. The use of animal models is limited by the species-specificity of Herceptin for human and primate HER2, thus a surrogate antibody was identified for use in a rat model of doxorubicin-induced cardiomyopathy. Based on the results of these studies, the role of Herceptin in the observed cardiotoxicity is still unknown.

1398 KINETICS OF CALCIUM IN SPONTANEOUS CARDIAC CALCIFICATION IN DBA/2 MICE.

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Two types of spontaneous cardiac calcification occurred in DBA/2 mice: massive myocardial calcification below the right ventricular epicardium and small focal calcification with no-specific location. Incidence of the former was found to be high at a relatively young (near 100% at 8 to 10 weeks). An ultrastructural study revealed that as an incipient event, electron-dense granular deposition occurred in mitochondria and myofibriller degeneration was concurrently observed. X-ray microanalysis demonstrated that such electron-dense granular deposits were a complex of calcium (Ca) and phosphorous. To investigate the kinetics of Ca in DBA/2 mice, uptake of ^{45}Ca in the cardiac tissue was measured in 4- to 8-week-old mice, and whole-body and heart autoradiograms (ARG) were made at 6 weeks of age. BALB/c (low incidence) and C57BL (zero incidence) mice were used for comparison. Corresponding with gross and histologic cardiac lesions, ^{45}Ca uptake in the cardiac tissue was higher in the right ventricle, and an extremely high level of ^{45}Ca uptake in the right ventricle was observed in 2 of 10 7-week-old DBA/2 mice. Quantification of tissue concentration of ^{45}Ca, using the whole-body ARG, revealed that uptake of ^{45}Ca was at similar level among 3 strains in tissues excluding right ventricle. On the heart ARG, 2 types of ^{45}Ca uptake, massive and focal as mentioned above, were also observed. These results and prophylactic efficacy of Ca antagonists reported by other researchers suggest that rapid Ca influx and resulting Ca overload in the cardiomyocytes might lead to degeneration and necrosis of them. Why massive myocardial calcification occurred below the right ventricular epicardium is still obscure.

1399 RAT CARDIOVASCULAR DYSFUNCTION PRIOR TO DEATH DURING EXPOSURE TO CONCENTRATED AMBIENT AIR PARTICLES.

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Groups of 12 rats with or without monocrotaline treatment (MT)-induced pulmonary inflammation were exposed by inhalation to either filtered air (FA) or concentrated urban air particles (CAPs) for five hours per day on three consecutive days. Continuous electrocardiograms (EKGs) were recorded on three members of each group during exposures. CAPs concentrations were approximately 200, 600, and 150 μ g/m^3, respectively, on day 1, 2, and 3 of exposure. During the exposure period, one MT, FA-exposed (8.3 %) and three MT, CAPs-exposed (25%) animals expired. Continuous EKG was recorded on one of the MT rats which succumbed on the third day of CAPs exposure. Death occurred 90 minutes (min) into exposure and was preceded by an initially steady heart rate (HR) for 15 min followed by a linear reduction in HR from 340 to 290 beats per min over the next 60 min. Concomitant increases in PR interval, QRS duration, HR standard deviation, low frequency HR variability power, and low-to-high frequency ratio were observed. QT interval contemporaneously decreased accompanied by a decrease in T wave height, deepening of the S wave, and emergence of possible U waves. Numerous premature ventricular contractions and other dysrhythmias were noted prior to demise. To our knowledge, this is the first continuous recording of cardiac death apparently induced by inhalation of CAPs. A similar mode of death (bradycardia and dysrhythmia followed by asystole) was reported in MT animals instilled with residual oil fly-ash particles (Watkinson et al, *Tox Sci* 41:209). Canines exposed to CAPs in our laboratory have also exhibited HR slowing. Furthermore, analysis of human data obtained from implantable cardiac defibrillators has associated bradycardic events with $PM_{2.5}$ elevation. Cardiac conduction system dysfunction may be an important mechanism of death associated with fine particle exposure in the MT rat. (Supported By: ES00002, ES08129, HL05947, and HL54958.)

1400 ACUTE CARDIORESPIRATORY EFFECTS OF PYRIDOSTIGMINE BROMIDE AND N,N-DIETHYL-m-TOLUAMIDE (DEET) IN RATS.

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Acute co-administration of a reversible, peripherally acting cholinesterase inhibitor, pyridostigmine bromide (PB), and a personal insect repellent, N,N-diethyl-m-toluamide (DEET), resulted in potentiation of lethality. This study was designed to explore the mechanism(s) of that lethal interaction. Whole body plethysmography was used to measure respiratory activity in conscious freely-moving rats, while cardiovascular function was monitored simultaneously through an arterial catheter, following acute i.p. administration of PB (2 mg/kg) and/or DEET (300 or 500 mg/kg). PB (2 mg/kg) alone stimulated respiration and increased blood pressure. Arterial pH levels were decreased, while pO_2 and pCO_2 remained at control levels. Administration of DEET (300 mg/kg) alone increased tidal volume and reduced blood pressure. Blood gases and pH levels were unaltered. A higher dose of DEET (500 mg/kg) also decreased respiratory and heart rate. Co-administration of PB (2 mg/kg) and DEET (300 mg/kg) increased tidal volume, decreased arterial pH, and elevated pCO_2. Heart rate and blood pressure declined progressively after drug co-administration. Pretreatment with atropine methyl nitrate reduced the individual effects of PB or DEET, and significantly increased survival after co-exposure to both agents. While changes in respiratory function contributed to the lethal interaction, it was concluded that the primary cause of death was circulatory failure. The results are consistent with a hypothesis that DEET depresses central cardiorespiratory control mechanisms and sympathetic outflow. In addition, PB-induced accumulation of acetylcholine at peripheral cholinergic receptor sites resulted in bradycardia that is postulated to further reduce cardiac output, causing progressive circulatory shock. Pretreatment with atropine methyl nitrate protected against circulatory collapse by preventing PB-induced bradycardia.

1401 ACCELERATION OF ATHEROSCLEROTIC PLAQUE FORMATION IN Apo E$^{-/-}$ MICE BY EXPOSURE TO TOBACCO SMOKE.

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Epidemiological studies have implicated active and passive smoking with increased risk of cardiovascular diseases. The present study was performed to determine if exposure to sidestream cigarrette smoke (SSCS) influences the development of experimental atherosclerotic plaque formation in a rodent model. Female Apo E$^{-/-}$ mice (n=20), maintained on a high fat diet, were divided into two equal groups. One group was exposed to SSCS in a whole-body exposure chamber set at a particulate concentration of 22±5mg/m^3 for a total of 6 hrs each day, 5 days a week. The second group was exposed to filtered ambient air and served as control. Elevated levels of blood carboxyhemoglobin and pulmonary CYP 1A1 were used to ascertain effective exposure of animals to smoke. The animals were killed after 3 months of exposure and their serum and aorta were collected for analyses. Both groups exhibited high serum cholesterol and triglyceride concentrations but no differences between the control and smoke-exposed groups were noticed. Image analysis of entire intimal area of the aorta covered by grossly discernable lesions demonstrated a significant increase in the smoke-exposed group. In agreement with these data there were significant increases in the aortic content of free and esterified cholesterol following smoke exposure. These results clearly demonstrate an enhancing effect of tobacco smoke exposure on the development of lesions in an atherosclerosis susceptible mouse model. (Supported by KTRB 5-41110 and the Gill Heart Institute.)

1402 ALLYLAMINE AFFECTS GLUCOSE UPTAKE IN RAT VASCULAR SMOOTH MUSCLE CELLS.

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A streptozotocin diabetic rat model was used to investigate the effects of allylamine-induced atherosclerosis. Vascular smooth muscle cells (VSMC) from allylamine treated diabetic and non-diabetic rats were used to investigate deoxyglucose uptake as an insulin-sensitive response. VSMC from untreated animals exhibited a 1.8-fold increase over basal uptake. The observed effect appears to be mediated by specific glucose transporters as evidenced by inhibition of the effect in the presence of high glucose. Allylamine treatment inhibits both basal and insulin-stimulated uptake. In the absence of insulin approximately 33% inhibition was observed. This inhibition did not change significantly in the presence of insulin suggesting an alteration of insulin-sensitive response. However, cells remained sensitive to insulin effects following *in vitro* exposure of non-treated cells to 5μM allylamine during the course of the uptake. In VSMC from non-allylamine treated diabetic rats , basal and insulin-stimulated uptake were decreased by about 38% and 30%, respectively. Allylamine treatment does not decrease basal or insulin-stimulated uptake beyond the decrease attributed to the diabetic state. However, allylamine appears to exert an effect on glucose uptake in non-diabetic animals that is not insulin dependent. (Supported by NIH/NCRR RCMI Grant No. G12RR03045-11.)

1403 COLLAGEN AND ELASTIN DEPOSITION BY VASCULAR SMOOTH MUSCLE CELLS IN RESPONSE TO INHIBITION OF SEMICARBAZIDE-SENSITIVE AMINE OXIDASE.

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Aortic vascular smooth muscle cells (VSMC) have the highest tissue-specific activity of Semicarbazide-Sensitive Amine Oxidase (SSAO) - an amine oxidase who's physiological function is undefined. This cell type is also responsible for production of collagen and elastin within the mammalian aorta. Our laboratory has demonstrated that *in vivo* SSAO inhibition results in vasculotoxicity in developing rats exemplified by lesions of the medial elastin architecture. This study examines changes in collagen and elastin deposition by cultured rat VSMC exposed to natural and synthetic SSAO inhibitors. Collagen and elastin fractions were isolated from Rat VSMC cultures treated for 30 days with SSAO inhibitors. Semicarbazide ($1x10^{-6}$ M), benzylamine ($1x10^{-5}$ M), and (*E*)-2-phenyl-3-chloroallylamine (Marion Merrell Dow; $5x10^{-6}$ M) - which are relatively specific inhibitors of SSAO - substantially reduced the amount of mature elastin deposited by VSMC while only slightly affecting collagen deposition. $ -aminopropionitrile ($8x10^{-3}$ M) - an inhibitor of lysyl oxidase - predictably reduced insoluble col-

lagen deposition but also completely obviated mature elastin deposition. (*E*)-2-(3,4-dimethoxyphenyl)-3-fluoroallylamine (from $2.2x10^{-12}$ M to $2.2x10^{-9}$ M) - which exhibits affinity for both SSAO and lysyl oxidase - dramatically increased the amount of mature insoluble elastin deposited by VSMC. Altered elastin deposition caused by SSAO inhibition observed in this *in vitro* study strengthens the conclusion that *in vivo* aortic lesions caused by SSAO-inhibition are due to aberrations in elastin metabolism. (This work was funded in part by NIH HL-26189 to PJB and US EPA Fellowship U-915014-01-2 and NIEHS Toxicology Training Grant Fellowship T32ES07254 to SDL.)

1404 CYTOTOXICITY OF ERGINE TO BOVINE ENDOTHELIAL CELLS IN CULTURE.

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Fescue toxicosis is a poorly defined syndrome of herbivores that consume tall fescue (*Festuca arundinacea* Schreb.) that is infected with an endophytic-fungus (*Neotyphodium coenophialum*). Ergot and pyrrolizidine alkaloid toxins produced by the endophyte-infected plants have been incriminated causally in the tall fescue related morbidity and mortality. Little is known regarding which of the toxins in tall fescue cause(s) the lesions in this syndrome. We examined the cytotoxicity of ergine, an ergot alkaloid of tall fescue, to vascular endothelial cells *in vitro*. Monolayers of confluent bovine endothelial cells received a single administration of ergine (10^{-5}, 10^{-7}, or 10^{-9} M), control vehicle or no treatment, and the media or lysates were examined at 2, 24, 72, and 120 hours after treatment. Administration of ergine as a single dose, repeated doses every 48 hours or repeated doses of ergine at the time of cell feeding caused no significant change in release of lactate dehydrogenase into the media, number of adherent cells or protein content of the monolayers. In contrast, monocrotaline pyrrole, a positive control in our experiments, caused delayed and progressive cytotoxicity to the endothelial cell monolayers. From these data we conclude that the ergot alkaloid, ergine, has little effect on bovine vascular endothelial cells *in vitro*. (Supported by USDA grant 97-35204-4814.)

1405 DISPLACEMENT OF ^{3}H-KETANSERIN IN THE HUMAN UMBILICAL ARTERY BY MIANSERIN, TYRAMINE, AND SEROTONIN.

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We have demonstrated that cocaine produces toxicity in human umbilical arteries through inhibition of the presynaptic reuptake mechanism for norepinephrine and serotonin. Serotonin is a very potent constrictor of human umbilical arteries and norepinephrine and tyramine may produce vasoconstriction via serotonin receptors as well in this tissue. Tissue homogenates of umbilical arteries were used in competitive binding assays to determine displacement characteristics of unlabeled ketanserin, mianserin, serotonin, or tyramine (0.001 μ M - 100 μ M), and norepinephrine (10 μ M) using ^{3}H-ketanserin. The B_{max} of ^{3}H-ketanserin was 15 nmol/mg protein, and the K_D was 241 nM . ^{3}H-ketanserin was competitively displaced by mianserin, a serotonin$_2$ antagonist, with a K_D of 0.3 $\pm$ 0.1 μ M,. Serotonin competitively displaced ^{3}H-ketanserin at two sites with K_D values of 2.8 $\pm$ 1.9 nM and 2.7 $\pm$ 0.7 μ M. Tyramine was able to displace ^{3}H-ketanserin at two sites, with K_D values of 7.9 $\pm$ 7.8 nM and 36 $\pm$ 32 μ M, but norepinephrine was unable to displace ^{3}H-ketanserin, even at a concentration of 10 μ M. These results are paxadoxical to contractile studies where cocaine potentiation of norepinephrine was attenuated by ketanserin. The concentration-dependent displacement of ^{3}H-ketanserin by tyramine is a novel finding. The K_D for tyramine of 36 μ M is within the concentration range (10 - 100 μ M) which produced vasoconstriction. This provides evidence that tyramine is producing its contractile effects through a serotonin$_2$ receptor subtype. Based on the contractile responses to serotonin the functional site for serotonin has a K_D of 2.8 nM in human umbilical arteries.

1406 MENADIONE-INDUCED VASCULAR ENDOTHELIAL DYSFUNCTION AND ITS POSSIBLE SIGNIFICANCE.

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Although many studies have shown that treatment of menadione leads to cytotoxicity to endothelial cells, investigations of menadione's effects on blood vessels are limited. Our previous studies have shown that menadione can indirectly provoke alteration in vasomotor tone through platelet cytotoxicity. To determine if menadione directly affects vascular function, menadione itself was added to the aortic rings with or without intact endothelium. Treatment of menadione directly resulted in contraction of aortic rings with endothelium, but did not cause any effect on aortic rings without endothelium. Menadione irreversibly inhibited the acetylcholine- and histamine-induced relaxation of aortic rings with endothelium in a time and dose-dependent manner. Menadione treatment potentiated phenylephrine-induced vasoconstriction in aortic rings with endothelium. Consistent with these *in vitro* results, when menadione was administrated intravenously to rats, blood pressure increased significantly in a dose-dependent manner. Furthermore, menadione infusion suppressed the blood pressure reduction induced by acetylcholine. By demonstrating that menadione caused *in vitro* endothelial dysfunction (i.e., decreased relaxation and increased vasoconstriction in the organ bath experiments) and confirming that these results were consistent with *in vivo* observations, we have provided evidence suggesting that a quinone such as menadione can alter vasomotor tone through endothelial dysfunction. Such dysfunction could possibly contribute to vascular diseases.

1407 MECHANISM FOR MENADIONE-INDUCED ENDOTHELIAL DYSFUNCTION.

J H Chung, J Y Lee, M Y Lee and S M Chung. *College of Pharmacy, Seoul National University, Seoul, Korea.*

Our previous study showed that menadione causes endothelial dysfunction which results in decreased relaxation and increased contraction of blood vessels. In other tissues, menadione exerts cytotoxicity by two mechanisms of action: 1) oxidative stress via redox cycling and 2) arylation. This investigation examined the role of these two mechanisms in menadione-induced endothelial dysfunction. Menadione increased superoxide anion generation in aortic rings in a dose-dependent manner. Superoxide dismutase treatment inhibited both menadione-induced superoxide anion production and menadione-reduced vascular relaxation, suggesting that oxidative stress was involved in these two effects. Menadione also inhibited relaxation induced by the NO donor, sodium nitroprusside, in a dose-dependent manner. Menadione resulted in a dose-dependent reduction of cGMP levels accumulated by acetylcholine. This effect was also blocked by superoxide dismutase, suggesting that superoxide generated by menadione could play a role in the inhibition of the nitric oxide pathway. Relaxation to acetylcholine was also inhibited by benzoquinone, suggesting that protein arylation by benzoquinone may also contribute to impairment of relaxation. These results suggest that menadione can cause endothelial dysfunction in blood vessels by the inhibition of the nitric oxide pathway via superoxide generation and that arylation activity may also be another important mechanism. Collectively, these results indicate that quinones such as menadione which have the ability to induce oxidative stress and arylation reactions can also cause endothelial dysfunction and ultimately, possibly contribute to vascular diseases.

1408 DEVELOPMENTAL VASCULOTOXICITY ASSOCIATED WITH INHIBITION OF SEMICARBAZIDE-SENSITIVE AMINE OXIDASES.

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The endogenous substrate(s) and physiological function(s) of semicarbazide-sensitive amine oxidases (SSAO), enzymes exhibiting highest activity in vascular smooth muscle cells of the mammalian aortic wall, remain undetermined. This study examines the pathophysiological effects to the aortic wall resulting from specific *in vivo* SSAO inhibition. Weanling Sprague-Dawley rats were treated acutely or chronically with either semicarbazide hydrochloride or the chloro- and fluoroallylamine derivatives MDL-72274 or MDL-72145 (Marion Merrell Dow). Treatment with these compounds produced acute (6 and 24 hr) and chronic (21 day) lowering of SSAO activity in aorta and lung with little effect on the activity of the vital matrix form-

ing enzyme - Lysyl oxidase in aortas of chronically treated animals. Chronic SSAO inhibition produced lesions consisting of striking disorganization of elastin architecture within the aortic media accompanied by degenerative medial changes and metaplastic changes in vascular smooth muscle cells. No significant differences in the total weight of dry, defatted aortic elastin and collagen components were observed between chronically SSAO inhibited and control animals. However, the amount of mature elastin was lowered and mature collagen was raised in the aortas of animals treated chronically with semicarbazide. Descending thoracic aortic rings isolated from chronically SSAO inhibited animals had larger cross sectional diameters (i.e., exhibited dilation) when compared to corresponding rings from control animals. This study demonstrates that developmental toxicity - characterized by striking vascular lesions and dilated thoracic aortas - can result from specific *in vivo* SSAO inhibition, suggesting a role for SSAO in connective tissue matrix development and maintenance, and specifically in the development of normal elastin. (Supported in part by NIH HL-26189 (PJB), NIEHS T32ES07254 (SDL, AB), and US EPA U-915014-01 (SDL).)

1409 THEOPHYLLINE INDUCES SPLANCHNIC ARTERIAL DAMAGE IN RATS.

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The splanchnic vascular bed of the rat is susceptible to damage by vasodilating drugs, including phosphodiesterase (PDE) inhibitors. Theophylline, an extensively used PDE-inhibitor drug, has been well studied in animals with respect to its cardiotoxicity, but its vasotoxic potential to rats is not well characterized. Male Sprague-Dawley (S-D) rats (3/group) were given theophylline (100, 200 or 400 mg/kg) by oral gavage as a single dose or as 2-3 consecutive daily doses. Rats were killed 24 hr post-dose and splanchnic vascular beds evaluated histologically. Microscopic lesions (focal medial necrosis) were observed in splanchnic arteries of rats given single or repeated doses 3200 mg/kg. To measure pharmacological activity, male S-D rats (6/group) were given single doses of theophylline by oral gavage and challenged with bacterial lipopolysaccharide (LPS) administered intraperitoneally 90 min post-dose. Theophylline inhibited LPS-induced tumor necrosis factor alpha (TNFa) production (measured by sandwich ELISA in serum collected 90 min following LPS challenge) dose-dependently across the dose range evaluated (3.3-100 mg/kg) with a calculated ED50 of 31.9 mg/kg. The small dose multiple (~6x) between the vasotoxic dose and the pharmacological ED50 for theophylline illustrates remarkable sensitivity of the rat splanchnic vasculature to PDE inhibitor-mediated damage.

1410 AN ASSESSMENT OF THE ANTI-ANGIOGENIC POTENTIAL OF AE-941 IN THE CYNOMOLGUS MONKEY.

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The purpose of the study was to evaluate the anti-angiogenic potential of AE-941 in-vivo in the Cynomolgus monkey using a cutaneous wound healing model. Four healthy Cynomolgus monkeys were allocated to one AE-941 treated group (n=2) and one control group (n=2). The animals were dosed by gavage with AE-941 (50 mg/kg/day) or saline for a period of 7 or 14 days. On the day prior to administration of the first treatment, full thickness cutaneous incisions were made in the interscapular region and sutured in order to allow healing by first intention. At the end of the treatment period, the animals were euthanized and whole body formalin perfusion was performed. The wound sites were collected and paraffin sections were stained with H&E, Masson's Trichrome and Factor VIII-Related Antigen for histopathological examination and morphometric analysis (blood vessel density, vessel wall area/tissue area and blood vessel diameter). The microscopic findings at the incision sites were consistent with healing by first intention in all monkeys and the subacute inflammatory process appeared to be slightly decreased in animals treated with AE-941. Morphometry indicated a decrease in vascular proliferation (blood vessel density) and vessel wall area/tissue area in treated animals when compared to the controls. An increase in the proportion of blood vessels with a diameter of < 10 μm was noted after 14 days of treatment when compared to the control animal and to animals euthanized on Day 8. Using this cutaneous wound healing model in the non-human primate, AE-941 demonstrated histopathological and morphometric changes consistent with an anti-angiogenic effect in-vivo. This model was considered an appropriate evaluation method that could be used concurrently on animals used for preclinical safety studies without impacting upon toxicological endpoints. This would not only minimize the use of animals but also demonstrate pharmacological activity in the species selected to evaluate the safety.

1411 THE CHALLENGES OF USING COMMON MECHANISMS OF TOXICITY IN CHEMICAL REGULATION.

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The 1996 Food Quality Protection Act (FQPA) has mandated that cumulative risk assessments be performed on pesticides acting through a common mechanism of toxicity. This strategy has not previously been used in pesticide regulation by the US Environmental Protection Agency (EPA). FQPA requires that new questions be asked to determine which pesticides act through a common mechanism of toxicity, and subsequently what methodology is appropriate for the new cumulative risk assessments. The FQPA©mandated time frame for reregistration of pesticides has created substantial pressure for the rapid development of new principles and procedures. Since these new principles and methods are without precedent in the risk assessment process for pesticides, there is considerable controversy regarding identification of cases when such cumulative risk assessments should be performed, and, if required, how they should be conducted. The roundtable panelists will present some common principles which can be used in judging whether toxicants act by a common mechanism of toxicity, and how these general principles were used to consider the anticholinesterase (organophosphate and carbamate) insecticides as a case study. The roundtable will also indicate the limitations and difficulties which arise by utilizing a restricted definition of mechanism of toxicity. Additionally, the roundtable will suggest possible methods which could be used to perform cumulative risk assessments on toxicants judged to act through a common mechanism of toxicity, and will indicate how the EPA is currently utilizing the common mechanism approach in its implementation of FQPA. The roundtable is designed to introduce a number of the controversies and pending questions associated with the common mechanism of toxicity issue.

1412 A PARTNERSHIP APPROACH TO THE EVALUATION OF ALTERNATIVE MODELS FOR CARCINOGENICITY TESTING.

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The willingness of the agencies involved in the regulation of pharmaceuticals to accept data from newly proposed models of carcinogenicity testing (e.g. transgenic animals and the neonatal mouse model) has stimulated intense international interest in gaining experience and a greater understanding of the strengths and limitation of the specific models. The International Life Sciences Institute (ILSI) Health Environment Sciences Institute (HESI) is currently coordinating a collaborative research program to characterize the responsiveness of alternative models for carcinogenicity testing. Participating in this research effort are nearly 60 industrial, government and academic laboratories from the United States, Europe and Japan. The program involves the development of standardized protocols for variety of alternative assays and the use of prototype compounds selected on the basis of mechanistically meaningful carcinogenic activity or non-carcinogenicity in the rodent bioassay. In addition, collaborative research will be conducted in number of academic institutions to gain a greater understanding of the mechanistic basis for the responses of these new models, facilitating interpretation of the findings. Most of the studies are underway and results will be forthcoming throughout 1999. A workshop will be organized by ILSI in late 1999 to present the combined results of these studies. This roundtable will describe the scientific and regulatory basis for the collaborative research effort, and provide perspectives by some of the participating government, industry and academic scientists from the U.S., Europe and Japan on these models. Emphasis will be placed on the unique partnership that has been formed between these sectors to generate the necessary information to evaluate these models. The goal of the roundtable will be to stimulate discussion regarding the model the partnership provides for future cooperative activities aimed at evaluating new proposed approaches that may enhance toxicity assessments.

1413 ALIPHATIC ETHERS AS FUEL OXYGENATES: HEALTH EFFECTS AND REGULATORY ISSUES.

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The Clean Air Act Amendments of 1990 require the use of oxygenated fuel in certain regions of the USA to reduce carbon monoxide emissions. Currently, the aliphatic ether most widely used to oxygenate gasoline is methyl tert-butyl ether (MTBE). However, other compounds of similar structure such as ethyl tert-butyl ether (ETBE) and tert-amyl methyl ether (TAME) are being used on a more limited basis. Because MTBE is added to gasoline at up to ~15% w/v, an enormous amount is being produced. People are potentially exposed to these compounds at low levels while refueling and driving automobiles (inhalation) and drinking contaminated water (ingestion). Complaints from the public along with chronic adverse effects of MTBE in rodents precipitated the need to further investigate the potential health effects of these ethers in people. An overview of the general toxicity (MTBE, ETBE, and TAME) and cancer (MTBE) studies carried out in rodents will be covered to emphasize the key human risk assessment issues associated with these chemicals. Results from human exposure studies will be presented and comparative studies on metabolic fate, and acute toxicity of these ethers in rodents and people will be included. Studies describing the potential modes of action by which MTBE induces tumors in chronic rodent studies will be described. Current risk assessment issues will be discussed and how the available information on these ethers is being used to carry out a more realistic risk assessment of the potential human health effects from exposure to these compounds.

1414 NEUROTOXIC, DEVELOPMENTAL AND TUMORIGENIC EFFECTS OF METHYL *tert*-BUTYL ETHER.

J Benson. *Lovelace Respiratory Research Institute, Albuquerque, NM, USA.*

Methyl tert-butyl ether (MTBE) is the predominant oxygenate currently used in oxyfuels and reformulated gasoline (RFG) to reduce carbon monoxide emissions from automobiles. The numbers of individuals living in metropolitan areas using oxyfuels is in excess of 120 million. Short-term effects reported by some individuals exposed to MTBE in gasoline include headache, eye irritation, burning sensation in the nose and throat, cough, dizziness/disorientation, and nausea. These symptoms have not been reproducibly duplicated in either community studies or studies involving controlled human exposures. There are no data on the long-term health effects associated with human exposure to MTBE. Adverse health effects in laboratory animals exposed to MTBE are generally only observed at high exposure levels. Acute, reversible neurotoxic effects have been observed in rats inhaling 800, but not 8000 ppm MTBE. Developmental effects have been observed in offspring of rats and mice exposed to 1300 ppm and 300 ppm, respectively. The no adverse effect level for developmental effects in these species was 300-400 ppm. Repeated exposure by ingestion or inhalation to high levels of MTBE have produced: 1) lymphomas and leukemias in female Sprague-Dawley (SD) rats ingesting 250 and 1000 mg MTBE/kg/day; 2) testicular interstitial cell neoplasms in SD rats ingesting 1000 mg/kg/day and F344/N rats inhaling 3000 and 8000 ppm MTBE; 3) renal tumors in male F344 rats inhaling 3000 and 8000 ppm; and liver tumors only in female CD-1 mice inhaling 8000 ppm MTBE. Tumor responses in rodents have all occurred at concentrations exceeding expected human exposure by orders of magnitude. Because of this, it is important to understand the mode of action for these responses in rodents and determine their relevance to assessing human risk from MTBE exposure.

1415 TOXICITY AND TOXICOKINETICS OF ALIPHATIC ETHERS- HUMAN VS RODENT STUDIES.

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Aliphatic ethers, mainly methyl and ethyl tertiary butyl ethers (MTBE, ETBE) and methyl tertiary amyl ether (TAME) are widely used as gasoline oxygenates in order to improve combustion and thereby reduce harmful emissions. Exposure in the low ppm to ppb range occurs mainly during manufacturing, distribution, filling, gas station attendance, and driving, and occasionally via polluted drinking water. Animal studies show that nearly 100% of the radioactivity is excreted within 24 h after administration of 14C-labelled ether, suggesting low potential for tissue binding. In man, the three parent ethers have similar inhalation toxicokinetic profiles, with substantial respiratory uptake and relatively rapid multiexponential postexposure decay, conistent with rapid partitioning between tissues and slight accumulation in adipose tissue. The decay in blood and urine of the metabolites t-butyl alcohol (TBA) and t-amyl alcohol (TAA) is much slower, with half times of about 10 h and 6 h, respectively. These metabolites may be suitable for biological monitoring of gasoline exposure. No evidence of saturation was seen at exposure levels up to 75 ppm in man and up to 300 ppm in rodents. CNS function, mucosal irritation, nasal inflammation and lung function have been studied after short-term exposure of a small number of male volunteers. No effects of MTBE were seen after 2h exposure at 50 ppm in one study, whereas another reported heaviness in the head and mild irritation after 3h at 75 ppm. Slight mucosal irritation and slightly impaired pulmonary function were seen in men exposed to 50 ppm ETBE for 2h. Similar symptoms have been associated with MTBE in epidemiological studies, with presumably markedly lower exposure levels raising the issue of sensitive subpopulations.

1416 BIOTRANSFORMATION OF METHYL *tert*-BUTYL ETHER IN HUMANS AND RATS.

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Methyl tert-butyl ether (MTBE) is widely used as an additive to gasoline to increase oxygen content and reduce tail pipe emission of pollutants. Therefore, widespread human exposure may occur. To contribute to the characterization of potential adverse effects of MTBE, its biotransformation was compared in humans and rats after inhalation exposure. Human volunteers (3 males and 3 females) and rats (5 males and 5 females) were exposed to 4 and 40 ppm MTBE for 4 hours in dynamic exposure systems. Urine samples were collected for 72 h in 6 h intervals and blood samples were taken in regular intervals for 48 h in humans. In urine, the MTBE-metabolites tert-butanol, 2-methyl-1,2-butanol, 2-methyl-1,2-butane diol and 2-hydroxyisobutyric acid were quantified, MTBE and tert-butanol were determined in blood samples. After the end of the exposure period, blood concentrations of MTBE in rats (5.9 (1.8 (M) and humans (6.7 (1.6 (M) were similar after 40 ppm MTBE. MTBE was rapidly cleared from blood with a half life of 2.6 (0.94 in humans and 0.5(0.2 h in rats. The blood concentrations of tert-butanol were 21.8 (3.7 in humans and 36.7 (10.8 in rats after 40 ppm MTBE. Tert-butanol was also rapidly cleared from blood with half lives of 5.3 (2.1 h in humans and 0.4 (0.1 h in rats. In urine of both species, 2-hydroxyisobutyrate was recovered as major excretory product in urine. Both tert-butanol and 2-methyl-1,2-propane diol were minor metabolites. All metabolites of MTBE excreted with urine were also rapidly eliminated in both species. Elimination half lives for the different urine metabolites of MTBE were between 10 and 17 h in humans and 3.5 and 4.5h in rats. The obtained data indicate that MTBE-biotransformation and excretion is similar in rats and humans and MTBE and its metabolites are rapidly excreted in both species. Supported by the Health Effects Institute, agreement No. 96-3

1417 SPECIES-SPECIFIC TUMOR RESPONSES FOLLOWING EXPOSURE TO METHYL *tert*-BUTYL ETHER (MTBE): POTENTIAL MODES OF ACTION.

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MTBE is a gasoline additive used to decrease carbon monoxide emissions. In rats and humans, MTBE is metabolized to tert-butyl alcohol (TBA). MTBE and TBA are negative in genotoxicity assays and cause renal tumors in male rats following chronic exposure. Short-term inhalation exposure studies have shown that MTBE and TBA induce (2u-globulin nephropathy ((2u-N) and renal cell proliferation in male rats. These responses are associated with the development of renal tumors in male rats following chronic chemical exposure. The classification of both MTBE and TBA as chemicals that induce renal tumors mediated by (2u-N will have significant impact on the hazard identification of these chemicals. MTBE also caused liver tumors in chronically exposed female mice. MTBE increased liver weight and hepatic microsomal cytochrome P450 activity at 3 and 21 days and 16 wks without hepatotoxicity and transiently increased hepatocyte DNA synthesis at 3, but not 21 days or 16 wks. MTBE also produced endocrine modulations

suggestive of estrogen antagonism. These effects of MTBE were similar to those produced by unleaded gasoline (UG). However, unlike UG, MTBE lacked tumor promoting activity in DEN-initiated mice suggesting that MTBE produced liver tumors through a mechanism different from that of UG. The induction of Leydig cell tumors in male rats following exposure to nongenotoxic chemicals has been shown to be a consequence of the ability of a chemical to interfere with endocrine regulation. Information concerning the mode of action by which MTBE induces these tumors in rodents is necessary to evaluate if these responses are relevant in assessing human risk. (These studies were sponsored by the Oxygenated Fuels Association.)

1418 RISK ASSESSMENT OF ALIPHATIC ETHERS: CRITICAL ISSUES.

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The 1990 Clean Air Act Amendments require oxygenated fuels in certain areas of the U.S.A. with excessive levels of carbon monoxide or ozone. Methyl tert-butyl ether (MTBE) is the most widely used oxygenate and has also been used since the 1970s to enhance gasoline octane. The widespread use of oxygenates has led to increased interest in the fate and transport of, and the potential for human exposure to, these chemicals in air and water. Questions have also arisen about the possible risks to human health posed by oxygenated fuels or oxygenates alone. Although EPA has derived an inhalation reference concentration of 3 mg/m3 for noncancer effects of chronic MTBE exposure, the lack of an adequate data base has made it difficult to evaluate potential health risks related to other routes or durations of exposure to MTBE, particularly in drinking water. U. S. EPA's draft (1998)'Research Strategy for Oxygenates in Water' proposes that it may be possible to estimate oral toxicity risk using the inhalation toxicity data base on MTBE. Although pharmacokinetic modeling efforts would support this route-to-route extrapolation, to achieve credible results several issues have to be addressed related to species differences in the metabolism and toxicity of MTBE. Are the carcinogenic responses observed in rodent studies at relatively high concentrations relevant, qualitatively and/or quantitatively, to potential human carcinogenic responses? What are the modes of action underlying different carcinogenic effects in laboratory animals? Does the (2u-globulin protein account completely for the male rat kidney tumors? Do hormonal perturbations underlie male rat testicular, female mouse liver, and possibly other tumorigenic effects? If so, what are the implications for risk of other cancer and/or noncancer effects? What influence might organoleptic reactions have on exposure and effects? Answers to these and other questions will assist EPA in characterizing the potential health risks related to MTBE and other fuel oxygenates.

1419 THE ROLE OF DNA REPAIR IN MAINTENANCE OF GENOME STABILITY.

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The ability of cells to repair damage to their DNA induced by xenobiotics is a critical factor in determining whether these agents induce mutations which can lead to diseases such as cancer. Recently there have been major advances in our understanding of the molecular processes of DNA repair. This symposium will describe some of the recent progress in determining the mechanism of repair of damaged DNA and the relationship between lesion removal and mutations. Structural studies of proteins involved in base excision repair, the process required to remove oxidative base damage and certain types of radiation-induced lesions, coupled with functional studies now provide an understanding of the molecular basis of this repair process. This understanding has been advanced by in vitro studies using DNA-histone substrates (i.e., chromatin complexes) that demonstrate the mechanisms by which DNA-protein interactions result in damage recognition and repair. Simultaneously, advanced PCR techniques have been developed to provide information on the location of damage within the genome, allowing measurements of damage removal at specific DNA bases, to determine how persistence of such adducts is related to the location of mutations in tumors. Recent availability of transgenic animals with specific repair gene knockouts are allowing the identification of critical genes involved in repair of different types of DNA damage. Thus, information from both molecular and structural biology approaches, coupled with sensitive analytical techniques, are providing the basis for improved risk assessments for DNA damaging agents by facilitating low dose extrapolation and interspecies comparisons.

1420 DNA REPAIR PATHWAYS AND ENVIRONMENTAL GENOMICS.

S H Wilson. *Laboratory of Structural Biology, Division of Intramural Research, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA.* Sponsor: D L Springer.

There is an extreme variability in mammalian cellular susceptibility upon exposure to genotoxic agents, such as DNA alkylating agents. In some cases, a genetic defect in a DNA repair gene renders a cell hypersensitive to a genotoxic agent, e.g., XPA and UV light, whereas in others the cells become tolerant to the agent, e.g., MSH2 and MNNG (N-Methyl-N'-Nitro-N-Nitrosoguanidine). While a host of genes that play a role in susceptibility to such environmental exposures have been identified, the genetic variations in these genes occurring in the human population have only recently been systematically studied and analyzed for functional implications. In the specific case of many DNA repair genes, a variation in the gene, thus eliminating function of the gene product, carries no apparent cellular phenotype by itself, e.g., mouse cell lines null for DNA polymerase (and 3-methyladenine DNA glycosylase. However, when such cells are exposed to an environmental challenge, such as a DNA methylating agent treatment, a hypersensitive phenotype is readily apparent. Such cellular phenotypes may be additive when associated with multiple variations and, therefore, elimination of more than one DNA repair pathway from the cell renders the cell extremely hypersensitive. Applications of these concepts in the field of environmentally related diseases will be discussed.

1421 REPAIR RATES OF DNA LESIONS ALONG THE p53 GENE: CORRELATION WITH SKIN AND LUNG TUMOR MUTATIONAL SPECTRA.

G P Holmquist. *City of Hope, Dept. Biology, Duarte, CA, USA.* Sponsor: D L Springer.

Repair rates for chemically identical adducts can vary up to thirty fold from one nucleotide position to the next. This variation appears primarily due to local DNA sequence context affecting how the repair machinery recognizes and excises DNA damage. The pattern of repair rate variation, as with the sequence context dependent pattern of DNA damage, is distinct for different mutagens and both the damage pattern and repair pattern contribute to a mutagen's mutational spectrum, the frequency distribution of transitions and transversions induced along a gene by that mutagen. The hot spots for mutation are the more readily damaged and more slowly repaired of the nucleotide positions. We treat human fibroblasts with various mutagens and, by ligation-mediated PCR, measure a mutagen's patterns of damage and repair rates. For skin and lung tumors respectively in humans, the patterns of p53 mutations are correlated with damage and repair rate patterns of UVB-induced cyclobutane dimers and BPDE-induced guanine adducts. For Aflatoxin B1-associated liver tumors, such correlations are absent.

1422 ROLE OF DNA/HISTONE INTERACTIONS IN DNA REPAIR.

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Within a cell DNA exists as a complex with proteins and is referred to as chromatin; repair enzymes must deal with damaged DNA in this context. The most basic functional unit of chromatin structure, the nucleosome, is composed of 146 bp of DNA wrapped around an octamer of histone proteins. We have evaluated DNA damage and repair using an in vitro model of 5S DNA reassembled into nucleosomes. Our results show that there is a generalized suppression of BPDE damage toward the center of the nucleosome relative to naked DNA and that the magnitude of the suppression is equally distributed at all adducted bases. Neither the translational positioning nor the rotational setting are altered by BPDE damage. Surprisingly, however BPDE damage increased the affinity of damaged DNA for the histone octamer relative to undamaged DNA, suggesting that damaged nucleosomal DNA may be more poorly repaired than naked DNA. Currently we are using highly efficient Xenopus oocyte nuclear extracts to determine the rate of lesion removal at specific sites along the 5S sequence of naked and nucleosomal samples. Using these procedures we are evaluating a number of variables associated with DNA histone interactions of chromatin to determine how

they contribute to DNA repair processes, the site specificity of lesion removal and ultimately mutations. (Supported by US DOE Contract DE-AC-76RLO 1830.)

1423 KNOWLEDGE OF MOLECULAR DOSIMETRY AND ENDOGENOUS DNA ADDUCTS CAN IMPROVE RISK ASSESSMENT AND MANAGEMENT.

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The human genome is subjected to attack by a plethora of exogenous and endogenous electrophiles that can result in mutations if not repaired prior to DNA synthesis. Such mutations can result in initiation and progression of neoplasia. Recent developments in molecular biology and analytical chemistry are allowing new questions to be posed that critically evaluate the role of DNA adduct formation and repair. This talk will focus on the use of these highly specific and ultrasensitive tools to compare the amount of endogenous and exogenous adducts for such chemicals as ethylene and propylene oxide, vinyl chloride, butadiene, and oxidative stress inducers such as peroxisome proliferators and PCBs. Abasic sites occur in DNA as a result of incomplete DNA repair and depurination and also can result in mutations. By understanding dose-response relationships for this complex matrix of DNA damage, the scientific basis for risk assessment and management can be improved. (Supported in part by grants P42 ES5948, T32 ES7126 and F32 ES5779 from NIEHS and grants from the CMA and Health Effects Institute.)

1424 CHEMICAL MODIFIERS OF RESPONSE TO FOOD-BORNE MICROBIAL PATHOGENS.

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Do chemicals contribute to increased human and animal health risks from exposure to food-borne microbial pathogens and their toxins? Chemicals can alter biological processes that are critical determinants in the host response to microbial pathogens and their products. Conversely, microbial pathogens induce inflammatory responses that can modify susceptibility to xenobiotics. Outbreaks of disease due to contamination of food and water with well-known and newly emerged microbial agents have been on the rise in developed countries. Characterization of the toxins produced by such pathogens and their role in tissue injury and disease progression is a challenging area of research. For example, the Shiga toxin family, produced by Shigella and E. coli 0157:H7, includes proteins that modify the host immune response and play key roles in the development of gastrointestinal and renal disease. In addition, bacterial endotoxin arising from translocation from the intestine into the circulation can interact with food-borne chemicals, leading to injury to liver and other tissues. For example, endotoxin can interact with mycotoxins to promote expression of proinflammatory cytokines and activation of apoptotic processes. Several recently discovered food contaminants can alter the expression of microbial pathogen/toxin receptors and adhesion sites. Food contaminants may also alter translocation of the toxins or the pathogen itself and can increase intestinal permeability, resulting in altered response to food-borne microbial pathogens/toxins. Thus, multiple interactions need be considered when assessing risk of food-related disease.

1425 EMERGING FOOD AND WATER-BORNE MICROBIAL DISEASES.

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Food and water-borne illnesses in the US are common and result in an estimated 9000 deaths annually. The emergence of immunodeficient populations such as AIDS or transplant patients further increase the risk of such infections. Outbreaks of food or water-borne poisoning from pathogens such as E. coli 0157:H7, Shigella, Salmonella enteritidis, Campylobacter jejuni, Listeria monocytogenes, and Helicobacter pylori are of particular concern. While the basis for pathogenesis of these microbes is unclear, virulence can involve release of endotoxins and extracellular toxins as well as invasiveness mechanisms. Less is known about types of exposures and dose response relationships. Most food-borne pathogens are capable of inducing a response in the general population, however, the most susceptible populations for many pathogens are children, the elderly, and pregnant women. One understudied area is how chemicals and infectious microbes interact to heighten or reduce disease severity, though research is ongoing. In a classic example, hepatocellular carcinoma is more common in populations that consume large amounts of food contaminated with aflatoxin. Furthermore, individuals that test positive for the hepatitis B virus are a higher risk of developing hepatocellular carcinoma than uninfected individuals. Certainly, other pathogens and chemicals may show similar interactions. However, more information about the dose response, mechanism of action and types of exposures are needed to clarify these interactions.

1426 BIOCHEMICAL AND PHYSIOLOGICAL FACTORS THAT AFFECT PATHOGENESIS CAUSED BY SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI.*

V L Tesh. *Texas A&M University Health Science Center, College Station, TX, USA.* Sponsor: R T Riley.

Although infections with Shiga toxin-producing E. coli are associated with the development of bloody diarrhea, infections with these bacteria are now also recognized as the most common cause of pediatric acute renal failure in many developed countries. The in vitro cytotoxic activity of Shiga toxins has been known for decades, but the precise mechanisms by which the toxins target renal microvascular endothelial cells for damage in vivo remain unknown. Recently, it has become clear that Shiga toxins interact with different cell types in vitro in fundamentally different ways. We hypothesize that the development of the extensive vascular lesions seen in patients with acute renal failure requires the differential interaction of Shiga toxins with multiple cell types. Finally, patients infected with Shiga toxin-producing E. coli are frequently endotoxemic due to damage to colonic epithelium. This presentation will focus on the interaction of Shiga toxins with immune cells and the contributions of Shiga toxins, bacterial endotoxins and the host immune response in the development of life-threatening systemic complications.

1427 BACTERIAL ENDOTOXIN INTERACTIONS WITH FOOD-BORNE CHEMICALS IN THE LIVER.

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Systemic exposure to small amounts of bacterial endotoxin is commonplace through processes such as infection and translocation from the intestine into the circulation. Such translocation can be enhanced by dietary factors such as alcohol consumption. Recent animal studies revealed an interaction between endotoxin and hepatotoxic, food-borne mycotoxins such as aflatoxin B1 or phytotoxins such as the pyrrolizidine alkaloid, monocrotaline. That is, pronounced liver injury occurs when modest exposure to endotoxin is combined with a subthreshold dose of an hepatotoxic chemical. The monocrotaline-endotoxin synergy does not appear to be due to enhanced bioactivation of the hepatotoxin, and it is not reproduced in cultured hepatocytes. Rather, the enhanced liver injury is likely related to the inflammagenic activity of endotoxin, which includes stimulation of inflammatory cells, release of soluble inflammatory mediators and an activated coagulation cascade. Endotoxin activates Kupffer cells in liver sinusoids, and this activation is critical to the liver injury that endotoxin alone causes at large doses. Kupffer cell destruction protects against the interactive effects of endotoxin with certain hepatotoxicants (eg, allyl alcohol) but is without influence on others (eg, monocrotaline). This and other results suggest that the specific inflammatory factors that are critical to endotoxin-induced enhancement of liver injury may differ among hepatotoxicants. Much remains to be learned about the interactions of endotoxin and other microbial products with food-borne chemicals, but such interactions should be considered for their potential to influence food-related disease.

1428 AMPLIFICATION OF ENDOTOXIN-MEDIATED CYTOKINE EXPRESSION AND LYMPHOCYTE APOPTOSIS BY TRICHOTHECENES: A PARADIGM FOR MICROBE-TOXICANT INTERACTIONS.

J J Pestka. *Michigan State University, East Lansing, MI, USA.*

There is increasing evidence that human exposure to endotoxin (lipopolysaccharide, LPS) from gram negative bacteria such as E. coli and Salmonella may increase the susceptibility of individuals to tissue injury by a variety of chemical agents. Trichothecene mycotoxins are frequent food and indoor air

contaminants that include some of the most potent protein synthesis inhibitors known. Concurrent exposure of mice to small doses of LPS and the trichothecene vomitoxin (VT) results in rapid and markedly elevated mRNA expression and serum concentrations of proinflammatory cytokines as well as prominent lymphocyte apoptosis as compared to mice receiving LPS or VT alone. Augmentation of transcription factor activity is one potential mechanism for cytokine superinduction by trichothecenes. For example, VT impairs synthesis of the negative regulatory protein ((B-(thereby increasing NF-(B activity. Other evidence suggests that trichothecenes increase cytokine mRNA stability thus favoring increased translation. Lymphocyte apoptosis following LPS and trichothecene co-exposure is likely to be mediated by elevated TNF-(expression and TNF-(mediated sequelae such as corticosterone and prostaglandin E2. In addition, trichothecenes can synergize with TNF-(or other apogenic signals to directly induce lymphocyte apoptosis thus further magnifying this response. Study of the interactions between LPS and trichothecenes will provide insight into the potential for increased sensitivity to chemical-mediated immune dysfunction among individuals exposed to gram negative bacteria or their inflammatory products.

1429 XENOBIOTIC-INDUCED ALTERATIONS IN GLYCOSPHINGOLIPID RECEPTORS, MICROBIAL TOXIN BINDING, ADHESION, AND TRANSLOCATION: POTENTIAL MODIFIERS OF DISEASE RESPONSE.

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Several common food-borne xenobiotics have the potential to alter the expression of microbial pathogen/toxin receptors and adhesion sites in digestive epithelia and other target tissues. In addition, microbial toxin translocation and intestinal permeability can be modified by xenobiotics altering an organism's response to common food-borne pathogens. Fumonisins (FBs) are fungal toxins found in corn and corn products. Consumption of FB-contaminated corn can result in direct exposure of the digestive epithelia to FB concentrations which inhibit glycosphingolipid (GSL) biosynthesis by human intestinal and other epithelial cells. Thus, FBs and other food-borne inhibitors of GSL biosynthesis modify the expression of receptors and adhesions sites for microbial pathogens/toxins. For example, expression of the cholera toxin, Shiga-like-, and vero-toxin (VT) GSL receptors are decreased in epithelial cells exposed in vitro to FB1. Cholera toxin receptor expression is also altered in the tissue of rodents fed FBs. While expression of the VT receptor is decreased in vitro, VT translocation is increased by FB; suggesting FB alters regulatory processes that are independent of GSL expression. FBs and other fungal toxins, such as deoxynivalenol, have been shown to alter transepithelial permeability, in vitro. These and other studies indicate that food-borne toxin-induced alterations in pathogen/toxin adhesion, internalization, and translocation could significantly modify disease expression in response to common microbial pathogens such as Salmonella and E. coli.

1430 ENVIRONMENTAL JUSTICE: SOCIOECONOMIC INEQUITIES AND POPULATIONS AT RISK.

R Snyder. *Environmental and Occupational Health Sciences Institute and Rutgers University, Piscataway, NJ, USA.*

It has been recognized by minority groups across the nation that many of their communities contain potentially polluting industries which utilize chemicals or treat chemical wastes, develop natural resource, engage in developing or testing weapons, etc. The communities, sensing potential adverse impacts on their health, have appealed to the federal government for help and one response has been the Environmental Justice: Partnerships for Communication program established by NIEHS. The grant program supports studies aimed at determining whether the claims of impacts on health of the people in these communities can be substantiated via environmental health research. There are currently 12 research grants funded (approx. $3 million) by the NIEHS to perform community generated research, usually with the help of a university based research group, aimed at evaluating the impact of local industry on public health. The active participation of stakeholders in the development and performance of research provides new opportunities for interactions and mutual understanding between academics and neighborhood occupants. We will discuss research strategies, environmental contributions to asthma, health effects of PCB's, chemical exposure to migrant farm workers, and role of the community in developing research proposals. (Supported by ES07723.)

1431 THE COMPLEX INTERACTION OF POVERTY, POLLUTION, AND HEALTH STATUS.

K Olden. *National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA.*

Several health surveys reveal that major disparities exist between the health status of the overall population of the U.S. and that of its socioeconomically disadvantaged groups. A recent county-by-county study of the U.S. population demonstrated that life expectancy was decreased by as much as 15 years in those communities experiencing the most severe poverty. While behavioral and lifestyle factors, nutrition, and access to health care services are important contributors to the increased morbidity and mortality among socioeconomically disadvantaged populations, environmental and occupational exposures, over which these individuals have little control, are likely to play a prominent role. Viewed from this perspective, the high morbidity and mortality among the poor might derive in part from the nation's social, economic, public health, and environmental policies. Indisputably a possible interaction of environmental pollution, poverty, and disease burden has gained greater prominence as a legitimate public health issue. Lead poisoning serves as a well-documented example of an association of poverty, pollution, and illness. What is now needed is data that more clearly establish a link between disproportionate exposure to environmental carcinogens and toxicants and a resulting disproportionate morbidity and mortality in the poor. The great need at this time is to conduct exposure assessment studies that define actual exposure levels, to investigate genetic and racial differences in susceptibility to these exposures, and to identify biomarkers of disease risk. Use of these three elements in future studies would bring a refinement in the data set that would be optimal in determining the boundaries of the environmental justice issue. The National Institute of Environmental Health Sciences has instituted several new projects to help achieve this data set. These include the Exposure Assessment Project, the Environmental Genome Project, and the Environmental Justice Grants.

1432 ENVIRONMENTAL JUSTICE AND HEALTH: OVERVIEW OF RESEARCH QUESTIONS, CONCEPTS AND METHODS.

S Wing. *Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill, NC, USA.* Sponsor: R Snyder.

The environmental justice movement is challenging biomedical researchers to consider exposure assessment and environmental epidemiology within the context of community-based struggles against the inequitable distribution of hazardous exposures, and social inequalities in health. Research questions are being re-framed to reflect concerns about multiple exposures, multiple outcomes, susceptible sub-populations, and the socioeconomic consequences of disproportionate environmental burdens. Concepts of race and class, central to environmental justice research, are being re-cast as complex biosocial phenomena. Problems with exposure assessment and low statistical power in epidemiological studies have created a demand for geographical analysis using routine data sources, while community concerns have led to a demand for more sensitive assays and biomarkers, as well as for consideration of neuro-endocrine and central nervous system-mediated effects of chemical exposures. Poor access to medical care introduces ethical problems in research design but is also seen as an injustice that follows from socioeconomic and environmental disadvantage. Quantitative studies of the distribution of environmental pollution in North Carolina are used to illustrate the way that epidemiological research is responding to environmental justice concerns. Over 1,300 manufacturing facilities that report to the Environmental Protection Agency's Toxic Release Inventory, and approximately 2,500 confined hog feeding operations registered with the NC Division of Water Quality, are considered in relation to racial, economic and susceptibility characteristics of local communities. Methods of presentation of results reflect the importance of community needs for research findings that can be effectively communicated to a wide audience.

1433 EPIDEMIOLOGICAL & EXPOSURE ASSESSMENT STUDIES OF ASTHMA & LUNG DISEASE IN CHILDREN IN BORDER TOWNS.

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A series of epidemiological and exposure studies of lung diseases in school children & parents in relation to environmental contamination have been conducted in Arizona-Sonora border towns stemming from discussions with community-university-agency prevention partners; this is one of several joint efforts determined though open community communications, based on community health concerns. They have been designed & operated biculturally in full collaboration. Air pollution in Nogales, Son. exceeds US & Mexican standards for PM10, and are high in US border towns. Ozone & CO are also high in all the border towns. However, asthma prevalence rates (7-8%) in the 3 towns studied to date do not differ, but other chronic respiratory symptoms are higher in Nogales, Son., and in homes with tobacco smoking. Differences are also related to socio-economic-environmental inequities. Also, daily symptoms and peak flow relate to daily air pollutant & temperature levels. Community & school fora are being planned to discuss results and plan school & community-based prevention/intervention programs. Studies continue in other border towns. (This work supported by ADHS & ALA AZ grants.)

1434 INTEGRATING RESEARCH INTO THE COMMUNITY-HEALTH EFFECTS OF PCBs IN A NATIVE AMERICAN POPULATION.

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The Mohawk Nation at Akwesasne lies in the shadow of three aluminum foundries which have contaminated local waters with PCBs and disrupted traditional life styles which depended on fishing as an occupation and food source. For the past ten years a university-community partnership has existed which attempts to determine the degree of PCB contamination in the community, what if any health effects have resulted from PCB exposure, educate community members on sources of exposure and work with the community on strategies and methods of cleanup. A major part of this partnership involves educating the scientists on the culture and values of this distinct community. Initial efforts were focused on determination of breast milk PCB levels, correlated with reported consumption of local fish. While a direct relation was observed prior to 1990, most women of reproductive age stopped eating local fish in response to fish advisories, and present levels are not above background. Considerable concern remains about cognitive, developmental and reproductive effects in children born during the time of PCB exposure, and disease in adults who were exposed for long periods of their life. Research attempting to evaluate these effects is ongoing. Other concerns relate to which diseases occur more commonly in this community than other populations, and this question is being pursued by analysis of clinic records of the Indian Health Service. In addition, through an Environmental Justice grant from NIEHS, a major program of community environmental health education, outreach and training has been implemented which has focused on making scientific information available to the community in a culturally relevant and understandable fashion.

1435 CHEMICAL EXPOSURE AMONG SEASONAL AND MIGRANT FARMWORKERS: COMMUNITY-BASED EPIDEMIOLOGY.

S A Quandt and T A Arcury. *Wake Forest University School of Medicine, Winston-Salem, NC, USA.* Sponsor: R Snyder.

Seasonal and migrant farm workers are at risk for exposure to agricultural chemicals that have short- and long-term health effects. Measuring this exposure, communicating the risk to farm workers and to farmers , and reducing exposure risk require social and cultural knowledge as well as toxicological and epidemiological data. This presentation uses data from ongoing research in North Carolina, which is designed to develop and test effective interventions to reduce farm worker exposure to workplace chemicals. We demonstrate the need to integrate methods from social science, epidemiology, and toxicology in a community participation framework to effectively address exposure issues for this population. In this project, community participation includes partnership with a community-based organization, an advisory committee of farm workers and farmers, and community forums. Formative research data show that the application of toxicological techniques to measure exposure is complicated by the local tobacco farming system with its numerous dispersed plots with individualized chemical application schedules. Data from in-depth interviews and focus groups with community members reveal belief systems that need to be incorporated into efforts to communicate risk. Many farmworkers do not believe they can be exposed unless they physically sense the wet chemicals. They consider exposure effects to be short-term, and know little of possible long-term consequences. Farmers do not believe that farmworkers are exposed to chemicals because the farmworkers do not mix or apply the chemicals. Using a community participation framework that involves both farm workers and farmers has provided access to data necessary for shaping an effective strategy for communicating risk information and potentially, reducing risk. (Funded by NIH grant ES08739.)

1436 DEVELOPMENT OF SUCCESSFUL COMMUNITY-BASED RESEARCH PROJECTS FROM A COMMUNITY PERSPECTIVE.

P M Shepard. *West Harlem Environmental Action, Inc., New York, NY, USA.* Sponsor: R Snyder.

The Environmental Justice Movement has illuminated the fact that people of color are disproportionately and excessively exposed to chemical and physical hazards in the environment. While there are egregious localized instances of environmental injustice, in aggregate, urban populations of color exposed to a multitude of hazards represent a sizable proportion of those who are affected by environmentally unjust policies and practices. To address the problem of environmental injustice, an effective public health approach must include a broad range of disciplines. The role of scientists, researchers, clinicians and community-based organizations is crucial to addressing health, environmental exposures, public communication, and priorities for research. Successful community-university partnerships are key to successful community-based research and interventions that are culturally sensitive and appropriate; ethical; address community concerns and priorities; provide educational components that can sustain the intervention; and provide for appropriate dissemination of findings to research participants and the community before submission to the media. This presentation will discuss these issues.

1437 REGULATION OF SUBCELLULAR LOCALIZATION OF THE ARYL HYDROCARBON RECEPTOR (AHR).

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The aryl hydrocarbon receptor (AhR) mediates the toxicity of dioxins and related compounds. In the absence of ligand, AhR is found in the cytosol. Binding of ligand activates AhR to relocalize to the nucleus, dimerize with its DNA-binding partner ARNT (aryl hydrocarbon receptor nuclear translocator), and bind dioxin-responsive enhancers (DREs) to stimulate or repress transcription of target genes. Although it has been well established that inactive AhR is cytosolic, little is known of how the localization of AhR is regulated. AhR contains both a nuclear localization signal (NLS) and a nuclear export signal (NES). In order to study the regulation of AhR localization, we transfected cos-1 cells with an expression vector containing myc-tagged full-length murine AhR. Transfected cells were treated with leptomycin B, a specific inhibitor of the nuclear export receptor CRM-1. In the absence of exogenous ligand, leptomycin B treatment caused a shift in AhR localization from cytosolic to nuclear. This result supports the hypothesis that AhR shuttles between the nucleus and cytosol independent of the presence of exogenous ligand, and that CRM-1 is required to maintain the cytosolic localization of unliganded AhR.

1438 OMEPRAZOLE AND RELATED BENZIMIDAZOLES ARE Ah RECEPTOR LIGANDS.

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The Ah receptor (AhR) is a ligand-dependent transcription factor that mediates many of the biological and toxicological actions of 2,3,7,8-tetra-

chlorodibenzo-p-dioxin (TCDD) and related chemicals. Although several substituted benzimidazoles have been shown to be relatively potent inducers of CYP1A in human and rat hepatocytes, their inability to competitively bind to the AhR suggested that they mediated their effects via an AhR ligand-independent manner. Utilizing the CALUX (Chemically Activated LUciferase eXpression) cell bioassay (employing cell lines stably transfected with a TCDD/AhR-responsive firefly luciferase reporter gene) and an inducible AhR-dependent DNA binding (gel shift) assay, we have examine the ability of several benzimidazoles, including: omeprazole, (OMP) thiabendazole (TBD), albendazole (ALB) and others to activate the AhR signaling system. Although all three chemicals induced luciferase reporter gene activity in stably transfected rat hepatoma and guinea pig adenocarcinoma cells, only OMP induced in human hepatoma and all were inactive in mouse hepatoma cells. Gel shift analysis revealed that nuclear extracts from OMP-, TBD- or ALB-treated guinea pig adenocarcinoma cells contained activated AhR complexes. The ability of OMP, TBD and ALB to competitively inhibit the binding of [3H]TCDD to the guinea pig cytosolic AhR, clearly indicates that these chemicals are indeed AhR ligands. The somewhat greater inducing potency of these chemicals in intact cells, as compared to their potency *in vitro*, suggests that these chemicals may be converted within the cell to more potent activators. (Supported by the NIEHS (ES07685) and Superfund (ES04699).)

1439 ACQUIRED RESISTANCE TO TCDD-MEDIATED SUPPRESSION OF ESTROGEN-DEPENDENT MCF-7 TUMOR GROWTH.

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We previously reported that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) transiently suppressed 17β-estradiol (E_2)-dependent growth of MCF-7 xenografts in immunosuppressed BDF_1 mice. To further elucidate this antiestrogenic action of TCDD we now report that the initial two week suppression of MCF-7 tumor growth by TCDD (5 μg/kg body weight) is coincident with expression, in the tumor, of the mRNA encoding the E_2 4-hydroxylase CYP1B1 (10 fold over untreated controls). This had declined 40-50% by the third week, at which time the tumor was growing. Levels of estrogen receptor and aryl hydrocarbon receptor mRNAs were essentially unchanged (80-100% and 110% of control respectively) and CYP1A1 mRNA remained low (30-100 fold less than CYP1B1). Three results suggest that host-mediated events underlie the loss of MCF-7 sensitivity to TCDD-mediated growth inhibition. (1) MCF-7 tumors that have become unresponsive to the growth inhibitory effects of TCDD are still E_2 dependent and TCDD sensitive when transplanted into naive mice. (2) MCF-7 tumor growth was not inhibited by TCDD when transplanted into mice that had been pre-treated with TCDD for two weeks. (3) Chemoresistance of MCF-7 tumors to TCDD was almost immediate when this tumor was transplanted into the highly TCDD-sensitive C57 strain, whereas in the less TCDD-sensitive DBA strain there was a two-week lag (similar to BDF_1) for induction of chemoresistance. These results are consistent with an inducible AhR-mediated mechanism of acquired resistance to TCDD, possibly involving hepatic sequestration of the xenobiotic. The results of these studies have temporal implications for TEF calculations as applied to risk assessment for chronic TCDD exposure. (ES03561)

1440 DIFFERENCES IN LOCALIZATION OF TCDD IN CENTRILOBULAR AND PERIPORTAL HEPATOCYTES GIVE INSIGHT INTO THE MECHANISM OF GENE EXPRESSION.

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Dose-response relationships for TCDD suggest a differential sensitivity of liver cell types to the induction of CYP1A1 and CYP1A2 and that the induction of CYP1A2 causes a sequestration of TCDD. Therefore, this study examined the dose-dependent effects of TCDD in unfractionated, centrilobular and periportal hepatocytes after acute exposure (3 days) of female Sprague-Dawley rats to a single oral dose of 0.01-10.0 μg [^{3}H]TCDD/kg. Whole hepatocytes were isolated by a collagenase perfusion technique; whereas centrilobular and periportal enriched-hepatocytes were isolated by a dual pulse digitonin-collagenase perfusion system. Examination of the isolated liver cells for alaninamino-transferase activity (ALT), a marker for periportal hepatocytes, showed a 3-fold elevation in ALT activity in periportal cells as compared to centrilobular cells, which was unaffected by dose of TCDD. A dose-dependent increase in localization of TCDD was accompanied by a dose-dependent increase in EROD and MROD activities in all liver cell populations. The centrilobular hepatocytes exhibited a 2.5 to 4.0-fold higher concentration of TCDD as compared to the periportal hepatocytes at doses up to 0.3 μg TCDD/kg. Centrilobular hepatocytes also exhibited an elevated MROD activity as compared to the periportal hepatocytes at doses up to 0.3 μg TCDD/kg. This is the first study to show that a dose-dependent difference in distribution of TCDD exists between centrilobular and periportal cells which may be due to regional differences in CYP1A2 induction resulting in a sequestration of TCDD in centrilobular hepatocytes at doses up to 0.3 μg TCDD/kg. (Abstract does not necessarily represent USEPA policy).

1441 TCDD AND DES INHIBIT FETAL THYMOCYTE DEVELOPMENT IN VITRO BY DISTINCTLY DIFFERENT MECHANISMS AS SHOWN BY STUDIES OF BCL-2 TRANSGENIC MICE.

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Both 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and diethylstilbestrol (DES) induce thymic atrophy. Although similar effects have been observed on thymic stem cells the precise molecular mechanisms by which either agent induces thymic atrophy are still undetermined. In a previous study, we found that TCDD and DES inhibited C57BL/6 murine fetal thymocyte organ cultures (FTOC) at different stages of development. Now using a bcl-2 transgenic (TG) mouse model, we have further investigated the mechanisms of TCDD and DES on fetal thymocyte proliferation, differentiation, maturation and apoptosis. Thymocyte development in FTOCs was inhibited by either TCDD (10 nM) or DES (20 μM) in both bcl-2 TG+ mice and TG- littermates. However, the reduction of cell number induced by DES in bcl-2 TG+ mice was significantly less than the level of inhibition in TG- mice. On the other hand, there was no difference in the level of reduction from TCDD-exposed TG+ or TG- FTOC. While TCDD induced production of more mature CD8 cells in either strain, DES mainly yielded cells in the $CD4^-CD8^-$ (DN) stage in TG- mice. The anti-apoptotic bcl-2 transgene overcame some of the DES blocking of DN thymocyte development, allowing more cells to differentiate into CD4 single positive cells. Analysis of cell cycle showed that TCDD inhibited cell proliferation in the $G_{0/1}$ stage, whereas DES blocked cell cycle at the G_2/M phase. While TCDD did not induce apoptosis in FTOC, DES induced apoptosis in the TG- FTOC. These apoptotic cells were mainly in the DN subpopulation. This apoptosis could be prevented by the overexpression of bcl-2 in the TG+ mice. Taken together, our results demonstrate distinct mechanisms for thymic atrophy induced by TCDD and DES in vitro. (Supported by NIEHS grants ES07216, ES04862 and ES01247.)

1442 CELL PROLIFERATION IN THE LIVERS OF FEMALE RATS CHRONICALLY EXPOSED TO BOTH 2,3,7,8-TETRA CHLORODIBENZO-*P*-DIOXIN (TCDD) AND 17β-ESTRADIOL.

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An understanding of the mechanism of hepatocarcinogenesis induced by TCDD will serve to reduce uncertainties in the risk assessment and setting of guidelines for human exposure to dioxin-like compounds. In chronic tumor promotion studies with dioxin, the dose-dependent increase in cell proliferation rates and formation of altered hepatic foci has been shown to be ovarian hormone-dependent. The aim of the present study was to test the hypothesis that estrogen is involved in the induction of cell proliferation by TCDD. DEN-initiated ovariectomized Sprague-Dawley female rats, treated with 100 ng TCDD/kg/day, were co-treated in the presence or absence of continuous 17β-estradiol administration for 20 or 30 weeks. Dose-dependent increases in serum 17β-estradiol levels and uterine weight were observed in estrogen-treated animals. Hepatic bromodeoxyuridine labeling indices in the liver were significantly lower in ovariectomized animals treated with TCDD after 30 weeks compared with intact TCDD-treated animals. Cell proliferation was significantly reduced, relative to control animals, in the livers of

animals exposed to either TCDD, 17β-estradiol, or TCDD and 17β-estradiol for 20 weeks and was significantly reduced by 17β-estradiol treatment, but not TCDD, after 30 weeks. There was no effect of supplemental estrogen treatment on cell proliferation in TCDD-treated ovariectomized rats. These data indicate that continuous treatment with 17β-estradiol alone cannot compensate for the effect of ovarian hormones on the induction of cell proliferation in TCDD-treated rats.

1443 CLARIFICATION OF DIFFERENTIAL TOXICITY OF TCDD TOWARD C-SRC KNOCKOUT AND WILD-TYPE MICE.

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Previously we have reported the results of our preliminary study on the toxic effects of TCDD *in vivo* in male c-src -/+ B6,129-src-tm1Sor mice. Two major deficiencies of the above study were: (a) the low number of individuals available at that time. (b) in some of the comparison tests, C57BL/6J mice were used as wild-type control mice. Since then we increased our laboratory breeding program and thereby the availability of -/+, -/-, and true littermate wild-type +/+ individuals as well as a genetically stable strain of B6,129 mice, B6,129-Fos-tm1Pa (c-src and c-fos +/+) mice. The results of *in vivo* toxicity tests, involving 8 to 15 mice per test group, showed that there are considerable variations expressed in toxicity within each group of c-src deficient mice, indicating that B6,129-Src-tm1Sor mice still harbor varying genetic backgrounds. Nevertheless, when a large enough number of individuals were tested, two toxic effects were found to be less expressed in src-deficient mice. They were: excess fatty deposits and mottled appearance of liver which were commonly observed in TCDD-treated wild type mice, but not in c-src deficient mice. The former trend was also confirmed by both liver lipid analysis and histological examinations of affected livers. On the other hand, src deficient mice showed consistently more severe cases of hepatomegaly as compared to wild type B6,129 mice. C57BL/6J mice cotreated with geldanamycin or quercetin, two known protein kinase inhibitors also showed the same differential responses of liver as toward TCDD as c-src knockout mice, the reduction of TCDD's toxicity in terms of the weights of pancreas and adipose tissues were less pronounced (statistically significant difference from those treated only with TCDD). Also, TCDD's effect to reduce bodyweight gain and thymus were less expressed in mice cotreated with these inhibitors. These tendencies however, were not statistically significant. (Supported by NIEHS ESO5233, ESO03575, and ESO05707.)

1444 THE EFFECTS OF TCDD ON THE ACTIVATION OF OVA-SPECIFIC DO11.10 TRANSGENIC T CELLS IN ADOPTIVELY-TRANSFERRED MICE.

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The activation of a naive CD4+ T lymphocyte is dependent on several key signals, primarily mediated by ligation of the antigen-specific T cell receptor and accessory molecules. Disruption of these signals may result in an inability to respond to a specific antigenic challenge. Previously, it has been shown that exposure to the environmental contaminant 2,3,7,8-tetrachlorodibenzo-*p* -dioxin (TCDD) suppresses the generation of T-dependent immunity, both humoral and cell-mediated. The mechanism of TCDD-induced immune suppression remains to be defined. We hypothesize that exposure to TCDD suppresses the activation of naive CD4+ T cells, and prevents their expansion and differentiation into effector T helper cells capable of driving T-dependent immune responses. To test this hypothesis, we adoptively-transferred DO11.10 transgenic T cells into syngeneic recipients allowing us to track the *in vivo* activation of naive, OVA-specific CD4+ T lymphocytes following exposure to antigen. Exposure of adoptively-transferred mice to TCDD (15 &g/kg) resulted in a 60-80% suppression of OVA-specific IgG1 and IgG2a antibodies without affecting production of anti-OVA IgM. TCDD did not alter the *in vivo* expansion or activation of OVA-specific CD4+ T cells as determined by flow cytometry, however, spleen cells from TCDD-treated mice produced reduced levels of T cell-derived cytokines following *in vitro* restimulation, suggesting defective differentiation of OVA-specific T cells. Production of IL-12, a cytokine produced by antigen-presenting cells (APC) which drives T cell differentiation, was also significantly suppressed in adoptively-transferred mice exposed to TCDD indicating defective activation of APC. Administration of recombinant IL-12 to TCDD-exposed mice elevated anti-OVA IgG2a titers to levels normally found in vehicle-treated OVA-immunized mice, yet had only partial or no effect on raising anti-OVA IgM and IgG1 titers, respectively. These data suggest that TCDD suppression of T cell-dependent humoral immunity is a consequence of dysfunctional APC-mediated differentiation of CD4+ T helper cells. Furthermore, these results highlight the potential of the DO11.10 adoptive transfer system to assess possible immunotoxic effects of other xenobiotics in an antigen-specific transgenic mouse model. (Supported by NIEHS grant ES003966.)

1445 VARIATION IN AH RECEPTOR EXPRESSION AND CYTOCHROME P450 INDUCIBILITY IN A DIOxiN-EXPOSED POPULATION.

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Knowledge regarding both human individual variation in response to dioxin and susceptibility factors for dioxin-related adverse health effects should reduce uncertainty in human risk assessment for exposure to dioxin-like compounds. Utilizing a human peripheral blood mononuclear cell model, we evaluated dioxin exposure-response relationships in an exposed human population to identify factors that determine individual variation in biological response to dioxin. The present study examined aryl hydrocarbon receptor (AHR), aryl hydrocarbon receptor nuclear translocator (ARNT), cytochrome P4501A1 and 1B1 mRNA levels by quantitative RT-PCR, in resting and cultured peripheral blood lymphocytes from 104 individuals occupationally exposed to a wide range of TCDD and other dioxin-like compounds (plasma dioxin toxic equivalents [TEQ] up to 915 ppt lipid). In lymphocyte cultures from these individuals (n=85), there was a 20-fold, 22-fold and 14-fold range of *in vitro* TCDD-inducible CYP1A1 enzyme activity, CYP1A1 mRNA, and CYP1B1 mRNA levels, respectively. AHR and ARNT mRNA expression varied 27-fold and 20-fold, respectively, and AHR expression correlated weakly with some measures of individual CYP1 inducibility. There was no clear relationship between plasma dioxin TEQ and AHR, ARNT, or TCDD-inducible CYP1A1 or CYP1B1 expression. These results indicate there are individuals with a wide range of responsiveness to dioxin, related to AHR expression as well as other factors, and this may represent an altered sensitivity to the biological effects of dioxins.

1446 HUMAN DIOXIN EXPOSURE ASSESSMENT BASED ON BLOOD ANALYSES: EXPOSURE DIFFERENCES ATTRIBUTABLE TO GENDER, AGE, DIET, AND COUNTRY.

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Measurement of human blood dioxin concentration is currently used to estimate body burden and exposure in workers and the general public. We have found that exposure varies with age, gender, and country of residence, and also may vary with diet. Both congener patterns and exposure levels differ among countries. In pooled blood samples taken between 1988 and 1991, blood lipid TCDD toxic equivalent (TEQ) levels of dioxins and dibenzofurans were 42 pg/g in Germany and 41 pg/g in the USA. From relatively less industrialized central China and Hanoi, Vietnam, TEQ levels in pooled samples were 5 and 10 pg/g, respectively. Increasing levels of dioxins, dibenzofurans, and coplanar PCBs with increasing age were found in blood of 134 individuals in Germany in 1994. A similar age difference had been found earlier in central China for dioxins and dibenzofurans. TEQ levels for dioxins, dibenzofurans, and coplanar PCBs were 20% higher for women than for men in pooled blood samples collected in the USA in 1997. Mean blood lipid TEQ levels also were higher for individual German women than men tested in 1996. Almost all the human body burden of dioxins is from food ingestion, especially foods of animal origin. In blood taken in 1997 from one man and one woman who for approximately 3 decades had been vegans, strict vegetarians consuming no food of animal origin, total TEQ levels of dioxins, dibenzofurans, and coplanar PCBs were less than 1/3 that of the general population. Measurement of dioxins and dibenzofurans captures only a portion of the TEQ body burden. In individual analyses of blood lipids for dioxins, dibenzofurans and coplanar, mono-ortho, and di-ortho PCBs in fifty Michigan men in 1992, the PCBs accounted for most of mean total TEQ.

1447 EARLY RESULTS FROM A DIOXIN GENOME PROJECT.

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In an attempt to better define the cellular and molecular mechanisms of toxicity for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), we have initiated a comprehensive survey of gene-expression alterations using an expressed sequence tag (EST) approach. This approach utilizes the partial sequencing and comparison of randomly selected clones from cDNA libraries derived from TCDD-treated and control groups. To generate the ESTs in our survey, cDNA libraries were constructed from the livers of male C57BL/6J mice treated i.p. with either 30 nmol/kg TCDD solubilized in a dioxane vehicle or the vehicle alone. Following treatment, animals were sacrificed after 48 hrs and the mRNA isolated from the liver of each mouse. Synthesis of cDNA was performed on mRNA samples pooled from the livers of three animals and the resulting transcripts were cloned unidirectionally into a plasmid vector. From each library, clones were randomly selected and sequenced from the 3' direction. ESTs were then assembled into clusters of overlapping sequences based on stringent assembly parameters and the identity of each cluster was established via BLAST searches against the GenBank database. Based on preliminary analysis of approximately 2,000 total clones, increases were observed in CYP1A2 ($p < 0.001$), aldolase B ($p = 0.1$), and CYP1A1 ($p = 0.1$) while a significant decrease was detected in mouse urinary protein (MUP) ($p < 0.001$). Other genes, such as albumin, maintained expression levels despite treatment ($p > 0.5$). In addition, preliminary results also show an increase in the number of unique transcripts in TCDD-treated animals as compared with controls which highlights the complex change in gene expression following TCDD treatment. Of these transcripts, nearly a dozen represent TCDD-inducible genes that have not yet been reported as upregulated by dioxin. The results will become increasingly informative as the number of clones sequenced continues to grow.

1448 DIOXIN INTAKE FROM FOOD IN THE UNITED STATES GENERAL POPULATION.

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Almost all the burden of dioxins in humans is from food ingestion, especially from meat, fish, and dairy products. We recently conducted a nationwide food sampling to determine levels of dioxin-like chemicals in the US food supply and to calculate daily intake. Concentrations of dioxins and dibenzofurans, and also coplanar, mono ortho, and di-ortho PCBs were measured. Twelve separate analyses were conducted on pooled batches of foods from 110 samples purchased in 1995 in supermarkets in Atlanta GA, Binghamton NY, Chicago IL, Louisville KY, and San Diego CA. The food category with highest TCDD toxic equivalent (TEQ) pg/g, whole weight, was freshwater fish (1.7 pg/g); and the lowest was a simulated vegan (vegetarian) diet (0.08 pg/g). Based on our analyses and using USDA survey data on food consumption, we estimated mean daily intake of TEQ pg/kg BW for age 1-11 to be 5.44 pg/kg BW for males and 5.36 pg/kg BW for females; age 12-19, 3.07 for males and 2.35 for females; age 20-79, 2.24 for males and 2.00 for females, and age 80+, 1.71 for males and 2.04 for females. Mean daily intake of TEQ for US breast-fed infants - based on analyses of human milk collected in 1995 - was 37.6 pg/kg BW. "Acceptable daily intake" or "risk specific dose" in the US, in Europe, and recommended by WHO are 0.01 (US EPA dioxin draft document) or 1-10 pg/kg BW/day (WHO). For adults (age 20-79) PCDDs, PCDFs, and PCBs each contributed about 1/3 of dietary TEQ intake, when non-detects were calculated at 1/2 the level of detection. When calculated with non-detects = 0, mean daily intake for adults, age 20-79, was 0.80 pg/kg BW, with 60% of TEQ contributed by PCBs, 30% by PCDDs, and 10% by PCDFs.

1449 DECREASED INTRACELLULAR ZINC LEVELS ALTER MITOCHONDRIAL MEMBRANE FUNCTION AND INCREASE CASPASE-3 ACTIVITY IN HL-60 CELLS.

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Low levels of extracellular zinc (Zn) have previously been associated with the induction of apoptosis. The objective of this study was to assess the cellular responses to low intracellular Zn concentration in human premyelocytic leukemia cells (HL-60). Cells were grown in media (DMEM + 7.5% fetal calf serum) containing 0.5, 25, or 50 μ M Zn, then removed at indicated time points over 48 h for analysis by flow cytometry or fluorescence microscopy. Apoptotic cells were identified by their inability to retain rhodamine 123 (Rh123), a measure of the breakdown of mitochondrial membrane potential and an early indicator of apoptosis. Cells grown in 0.5 μ M Zn medium over 48 h exhibited a continuous decrease in intracellular Zn concentration as measured by the Zn-specific fluorophore, zinquin ($P<.01$). Zn levels were maintained in cells grown in 25 or 50 μ M Zn media. There was a decrease in Rh123 retention by the cells after 18 h in 0.5 μ M Zn media. However, the addition of 10 μ M Zn at or before 12 h of culture in 0.5 μ M Zn medium restored Rh123 retention to control levels. Caspase-3 activity, a recognized target for Zn inhibition, was four-fold higher in the cells cultured for 48 or 72 h in 0.5 μ M Zn compared to 24 h of culture in 0.5 μ M Zn or to cells cultured in 50 μ M Zn. In addition, cell cycle analysis of BrdU incorporation demonstrated a loss of cells in the S phase and an increase in the sub-G_0/G_1 peak when cells were cultured in 0.5 μ M Zn medium. These data indicated that decreasing intracellular Zn levels preceded alterations in mitochondrial membrane potential and the activation of the caspase cascade leading to apoptosis.

1450 APOPTOSIS IN CELLS LACKING MITOCHONDRIAL DNA.

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Mitochondria play a central role in regulating apoptosis. Release of mitochondrial cytochrome *c* is an early and common signaling event after a variety of apoptotic stimuli. The loss of cytochrome *c* triggers superoxide production from mitochondria and contributes to the oxidation of the cellular thiol-disulfide redox state. In this study, we tested wthether depleting mitochondrial DNA would affect these signaling mechanisms during apoptosis. We found that cells lacking mitochondrial DNA (ρ0) could still undergo apoptosis similarly to control ρ+ cells in response to staurosporine, and the cytochrome *c* dependent caspase activation mechanism was well preserved. Release of cytochrome *c* was accompanied by the release of adenylate kinase, another mitochondrial intermembrane protein. Measurement of cellular GSH and GSSG contents showed that the thiol-disulfide redox status change, which was observed as a dramatic oxidation in ρ+ cells, was inhibited in ρ0 cells. Thus, the bio-energetic function of mitochondria can be separated from mitochondrial signaling of apoptosis and mitochondria are the principal source of the oxidative signal.

1451 STUDY OF APOPTOSIS IN THYMOCYTES OBTAINED FROM γ-GLUTAMYL TRANSPEPTIDASE (GGT) DEFICIENT KNOCKOUT MICE.

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Previous work from our laboratory has shown that a decrease in glutathione (GSH) can lead to cell damage and even cell death. We hypothesize that cellular GSH homeostasis depends on mitochondrial GSH. We have shown that in GGT-deficient mice cytosolic GSH as well as mitochondrial GSH is significantly decreased. In this study we investigated how this impairment in GSH homeostasis may relate to apoptosis. It is widely accepted that apoptosis is accompanied by a depletion of GSH. It has also been established that thymocytes treated with dexamethasone (DEX) undergo a step-wise dysregulation of mitochondrial function involving the mitochondrial permeability transition (MPT) leading to apoptosis. It's been known that MPT is influenced by thiol status. However, the question of how cytosolic and mitochondrial GSH relates to apoptosis remains complex and not very well understood. Mice were treated in vivo for 8 hours with DEX. Thymocytes

were isolated and subjected to flow cytometry to analyze dysregulation of mitochondrial function as it relates to apoptotic events. We determined mitochondrial membrane potential (DiOC6, JC-1), cardiolipin oxidation (NAO), changes in lipid packaging (MC540), cytosolic and mitochondrial calcium (calcium green-am, Rhod 2-am), glutathione (MCB), as well as early and late apoptotic cells (annexin V, 7-aaD) in thymic subpopulations. Our results show that GGT-deficient mice were significantly more susceptible to DEX treatment. We conclude that the observed decrease in mitochondrial GSH increased their susceptibility to MPT and apoptosis. Studies are currently underway to investigate if the GSH precursors, N-acetylcysteine and 2-oxo-4-thiazolidine-carboxylic acid, can reverse the observed susceptibility to apoptosis in the GGT-deficient knockout mice.

1452 MITOCHONDRIAL DEPOLARIZATION IS AN EARLY EVENT IN PHOTODYNAMIC THERAPY-INDUCED APOPTOSIS IN CHO CELLS.

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Photodynamic therapy (PDT) is currently used as a promising cancer treatment against a variety of tumors. The mechanisms underlying PDT induced cell death remain unclear. In this study, we first examined the subcellular localization of the phthalocyanine photosensitizer Pc-4 in Chinese hamster ovary (CHO) cells. Cells were incubated with 200 nM Pc-4 for 2 h. Subsequently, red fluorescence of Pc-4 was imaged using confocal microscopy. Co-staining cells with MitoTracker Green demonstrated that Pc-4 was localized to mitochondria. To examine further the role of mitochondria in Pc-4 induced apoptosis, Pc-4-loaded CHO cells were co-loaded with MitoFluor Green, a mitochondrial memrane potential sensitive fluorophore. Radiation of Pc-4 with a red light (>600 nm; 1.53 kJ/m^2) caused mitochondrial depolarization after 10-30 min. Subsequently, caspase-3 became activated followed by apoptotic cell death. Cyclosporin A (1-10 μ M), a specific inhibitor of the mitochondrial permeability transition, prevented onset of apoptosis in a dose-dependent manner; 20 μ M cyclosporin A decreased apoptosis from 50% to 20% at 5 h. In conclusion, these results suggest that mitochondrial dysfunction induced by onset of the mitochondrial permeability transition is a critical event in the PDT-induced apoptotic death.

1453 MITOCHONDRIAL DEPOLARIZATION PRECEDES BAX TRANSLOCATION TO MITOCHONDRIA.

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Alteration of mitochondrial function plays a critical role in apoptosis. Previously, we showed that staurosporine induced mitochondrial depolarization, which is accompanied by cytochrome *c* release in pheochromocytoma (PC6) cells. Here, we studied changes of mitochondrial membrane potential in relation to insertion of pro-apoptotic protein Bax to mitochondria using confocal microscopy. We fused green fluorescent protein (GFP) to the amino terminus of Bax and transfected the GFP-Bax into PC6 cells. Transfected cells displayed a diffuse pattern of fluorescence indicating that Bax was initially localized to the cytosol. Cells were also loaded with TMRM, a mitochondrial membrane potential indicating fluorophore. To induce apoptosis, cells were treated with staurosporine (5 μ M). Mitochondrial membrane potential and Bax redistribution were monitored simultaneously in single living cells. Individual cells behaved asynchronously and lost their mitochondrial membrane potential and begun the process of Bax translocation at variable times. However, mitochondria depolarized before the translocation of Bax from the cytosol to mitochondria. Subsequently, cells began to die apoptosis. In conclusion, our results show that Bax insertion to the mitochondrial membrane occurs considerably later than mitochondrial depolarization and cytochrome *c* release in staurosporine-induced apoptosis.

1454 INDUCTION OF APOPTOSIS BY CYCLOSPORINE A IN RAT HEPATOCYTES.

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The role of cyclosporine A (CsA) during apoptosis has not been clarified yet and there is a lot of controversial information in the current literature. CsA acts as an inhibitor of apoptosis in some cellular systems, induction of programmed cell death has been observed in others. In rat hepatocytes and isolated mitochondrial fractions of the liver, CsA was very often used as a specific inhibitor of mitochondrial Ca^{2+} release and as a blocker of the mitochondrial permeability transition (MPT), both processes involved in the inhibition of apoptosis. Neither inhibition nor induction of apoptosis by CsA has been described in rat hepatocyte primary cultures. It was the purpose of the present study to characterize the effects of CsA on apoptosis at the morphological and biochemical level and to distinguish apoptosis from necrosis. Rat hepatocytes cultured for 4 or 20 h with CsA at concentrations of 0, 10, 25 and 50 μM were investigated for chromatin condensation, DNA fragmentation (TUNEL) and Annexin V reaction. Four hours after CsA treatment apoptosis increased dose-dependently without any enzyme leakage through the cytoplasmic membrane. In parallel the activity of the cysteine proteases caspase-3 (CPP 32) and caspase-6 (Mch 2) but not caspase-1 (ICE) were increased. The specific caspase inhibitors Ac-DEVD-CHO and Ac-VEID-CHO inhibited caspase activation and attenuated CsA-induced apoptosis. After 20 hours of CsA incubation apoptosis was further increased and accompanied by cytochrome c release into the cytosol. Necrosis appeared with typical leakage of cytosolic enzymes. Ultrastructural evaluations by TEM confirmed necrosis after 20 hour but not after 4 hours. The decreased mitochondrial membrane potential $\Delta \Psi_i$ and the increased cytosolic concentrations of $[Ca^{2+}]_i$, which were the result of extracellular uptake between 1-4 hours of incubation, might be regarded as early events triggering the cascade of apoptosis which results in proteolysis and DNA fragmentation. Our findings suggest that in rat hepatocyte cultures CsA specifically induces apoptosis at early time points, overlapping with necrosis after longer treatment.

1455 LEUKOTOXIN DIOL IS A POTENT AND SPECIFIC INDUCER OF THE MITOCHONDRIAL PERMEABILITY TRANSITION.

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Leukotoxin (9,10-epoxyoctadec-12-enoate) is biosynthesized from linoleic acid by inflammatory cells upon activation and is converted by a biologically ubiquitous enzyme, epoxide hydrolase, to its toxic diol metabolite, leukotoxin diol. Leukotoxin has been proposed to be one of the causative agents involved in Acute Respiratory Distress Syndrome (ARDS), however, the mechanism of leukotoxin-induced cell death has not been characterized. We show for the first time, by spectrophotometric measurement of mitochondrial swelling, that leukotoxin diol, but not its epoxide precursor, is a potent and specific inducer of the mitochondrial permeability transition (MPT) and that cyclosporin A completely inhibits leukotoxin diol-induced MPT.

1456 SUBCHRONIC DOXORUBICIN ADMINISTRATION INCREASES MITOCHONDRIAL-MEDIATED CELL INJURY BY CALCIUM IONOPHORES.

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Recent research implicates interference with mitochondrial bioenergetics to be a critical factor responsible for the cardiomyopathy that limits the clinical efficacy of doxorubicin (DXR) antineoplastic therapy. We found that DXR interferes with mitochondrial calcium homeostasis and that cardiomyocytes isolated from rats treated subchronically with DXR are highly sensitive to calcium-induced cell killing. Since mitochondria play an important role in calcium signaling pathways, we questioned whether cardiomyocytes from DXR treated rats would express an exaggerated sensitivity to the cytotoxic response to agents that increase cytosolic calcium concentrations. Adult male Sprague-Dawley rats received 6 weekly (s.c.) injections of either 2 mg/kg doxorubicin or saline and were killed 7 days following the last injection. Cardiomyocytes were isolated and exposed in vitro to increasing concentrations of A23187. Cell killing was measured by trypan blue dye exclusion. Cytosolic calcium concentration was estimated fluorometrically with FURA-2AM and mitochondrial membrane potential was measured with TMRM. A23187 caused a dose-dependent increase in cytosolic calcium, depolarization of mitochondrial membranes and cell killing. There was no discernable difference in cytosolic calcium response to A23187 between cardiomyocytes from DXR and saline-treated rats. However, cells from DXR-treated rats were more susceptible to A23187-induced mitochondrial depolarization and cell killing. Cyclosporin A was not cytoprotective. The results

implicate a critical role for disruption of mitochondrial calcium homeostasis in the manifestation of doxorubicin cardiotoxicity. (Supported by NIH grant HL-58016.)

1457 ACETAMINOPHEN, AND ITS NONHEPATOTOXIC REGIOISOMER, INDUCE CELL DEATH BY ANOMALOUS APOPTOSIS *IN VIVO* AND *IN VITRO*.

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The biochemical events accompanying cell death by acetaminophen (*N*-acetyl-*p*-aminophenol, APAP), and its relatively nonhepatotoxic regioisomer *N*-acetyl-*m*-aminophenol (AMAP), are mostly unknown and have been examined using both *in vivo* and *in vitro* model systems. Hepatotoxic doses of APAP (600 mg/kg, i.p., $B6C3F_1$ mice) induced mitochondrial cytochrome *c* relocation and DNA fragmentation events normally associated with apoptosis. Although nucleosomal ladder formation was not evident for either compound, genomic DNA fragmentation (*ca.* 50 kb), a robust indicator of apoptotic nuclear changes, was detected using CHEF gel analysis. Surprisingly, effector caspase activation (caspase 3-mediated PARP, caspase 6-mediated lamin A or DEVDamc cleavage) was not observed with APAP treatment. AMAP treatment (600 mg/kg, i.p.), however, resulted in lamin A and atypical PARP cleavage despite no evidence of cytochrome *c* leakage. APAP bioactivation and resultant cell death in a differentiated, TGFα -transgenic, hepatocyte cell line (TAMH) was observed at concentrations, and over time frames, relevant to clinical overdose situations. Chromatin margination and 50 kb fragment formation *in vitro* confirmed the alterations to nuclear morphology found *in vivo*. Comparable AMAP-induced cytotoxicities were not observed in this cell line as expected from the *in vivo* hepatotoxicity of these compounds. The relative cytotoxic potency of APAP and AMAP was reflected in dose-related collapse of mitochondrial membrane potential which preceded loss of cellular reducing equivalents, as assessed by multiparameter flow cytometry. This contrasted with the sequence of events observed during apoptotic cell death induced by actinomycin D/TNF. In conclusion, APAP and AMAP-mediated cell death *in vivo* and *in vitro* does not resemble classical apoptosis but instead represents an atypical, possibly truncated, version of this form of cell death. (Supported by grants #CA74131 to NF, #GM25418 to SDN & #GM51916 to SB.)

1458 ROTENONE-INDUCED APOPTOSIS IN LIVER CELLS: INVOLVEMENT OF THE MITOCHONDRIAL MEMBRANE PERMEABILITY TRANSITION (MPT).

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Mitochondrial dysfunction, in particular the mitochondrial membrane permeability transition (MPT), has been implicated in the cascade of events involved in the execution of apoptosis. Fluorescent microscopy of Hoechst 33258-stained WB-F344 cells was utilized to examine the effect of rotenone, Atractyloside (Atr), and Cyclosporin A (CsA) on apoptosis. A time- and concentration-dependent increase in apoptosis was observed following treatment with Atr and rotenone (7.7 and 11.7 fold over control). CsA, an inhibitor of the MPT, was utilized to examine the role of the MPT in the induction of apoptosis by rotenone and Atr. When cotreated with 5.0 μM rotenone, 7.5 and 10.0 μM CsA inhibited rotenone induced apoptosis. A similar effect was observed following CsA cotreatment with Atr. To inhibit rotenone induced apoptosis, CsA exposure was necessary within 20 minutes of rotenone exposure. Examination of mitochondrial function by staining with the mitochondrial membrane potential ($\Delta \psi_m$)-selective fluorochrome, MitoTracker Red, demonstrated that rotenone (5.0 μM)- and Atr (5.0 μM)-induced a reduction of the $\Delta \Psi_m$ within 15 minutes and throughout the duration of the study (6 hours) to approximately 65% and 50% of control. However, cotreatment with concentrations of CsA that inhibited the rotenone- and Atr-induced apoptosis also prevented the rotenone- and Atr-induced reduction of the $\Delta \Psi_m$. The apoptogenic effect of rotenone and Atr thus appears to occur rapidly (within 20 minutes) and is irreversible once mitochondrial damage occurs. The inhibition of the rotenone and Atr induced apoptosis and mitochondrial dysfunction by CsA suggests that the MPT may be involved in the induction of apoptosis by rotenone.

1459 OXIDATIVE STRESS IS A MEDIATER OF DOPAMINE AND CYANIDE INDUCED NEURONAL APOPTOSIS.

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Excessive exposure to dopamine (DA) produces degeneration of dopaminergic neurons. Since impaired mitochondrial metabolism might enhance DA toxicity, KCN was used to produce hypoxia, and alter mitochondrial function. Differentiated PC12 cells were exposed to DA, KCN, or a combination of the two. LDH assays indicated that both DA and KCN induced a dose and time dependent cell death and that their combination produced an additive cytotoxicity. Apoptotic death was both dose and time dependent for DA and KCN. DA/KCN produced an increase in apoptosis, indicating impaired mitochondrial function enhances DA cytotoxicity. In mechanistic studies, cells were pre-treated with a series of compounds and incubated with DA (300μ M) and/or KCN (100μ M) for 24 hours. Nomifensine, a DA re-uptake inhibitor, rescued nearly 50 % of the cells from DA and DA/KCN induced apoptosis. Pre-treatment with SOD (37 U/ml), catalase (500 U/ml), N-acetylcysteine (100μ M), or glutathione (100μ M) blocked both DA and KCN apoptosis indicating involvement of oxidative stress. Buthione sulfoximine, an inhibitor of glutathione synthesis, increased the apoptotic response. DA, KCN and DA/KCN produced a significant increase in intracellular ROS generation. The NOS inhibitor, L-NAME and uric acid, a peroxynitrate scavenger, blocked apoptosis and ROS production, supporting the involvement of NO. z-VAD, a caspase inhibitor, protected cells from apoptosis indicating activation of a cell death pathway. It is concluded that DA and KCN neurotoxicity involves ROS and NO mediated oxidative stress as an initiator of an apoptotic cascade. (Supported by NIH grant ES04140.)

1460 EFFECTS OF ATMOSPHERIC PARTICULATE MATTER ON BASAL AND BACTERIA-INDUCED DEGRANULATION OF HUMAN PERIPHERAL BLOOD LEUKOCYTES.

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The effects of three standard particulate materials (PM) (i.e., silica, diesel and urban) on the release of primary granules, secondary granules, and histamine from peripheral blood leukocytes were determined using whole blood assays. In these assays, fixed Staphylococcus aureus bacteria, a known inducer of degranulation and histamine secretion, were used as a positive control. Bacteria were found to induce the release of primary granules, as determined by the release of p-Nitrophenyl-N-acetyl-β -D-glucosaminidase and myeloperoxidase activity, secondary granules as determined by the release of lactoferrin, and histamine. Pretreatment of cells with either silica or diesel PM did not induce the release of any of the markers analyzed or dramatically alter the ability of bacteria to liberate these agents from the cells. Although urban PM did not affect the release of primary granules, this material caused release of lactoferrin from secondary granules. Urban PM was also found to be a potent inducer of histamine release. Fractionation studies revealed that soluble (aqueous) fractions and to a lesser extent, insoluble fractions of urban PM contained histamine releasing activity. The results indicate that simple assays of leukocyte degranulation and histamine release can be used to differentiate various types of PM. Furthermore, they demonstrate that both soluble and insoluble components of ambient PM can mediate leukocyte degranulation. The release of these bioactive components may be an important contributing factor to the adverse health effects associated with PM exposure.

1461 VANILLOID (CAPSAICIN) AND ACID SENSITIVE IRRITANT RECEPTORS UNDERLIE MOUSE STRAIN-SENSITIVITY TO PARTICULATE MATTER (PM).

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The causes of strain-species differences in response to chemically-induced inflammation remain unresolved. BALB/c and C57blk (B6) mice, exposed intratracheally to residual oil fly ash (ROFA, 3 mg/kg), were examined 24 hr later for signs of airway inflammation. BALB/c showed significantly higher numbers of neutrophiles and increased airway hyperresponsiveness to iv metacholine, whereas B6 mice showed no change in either endpoint. Dorsal

root ganglion (DRG) sensory neurons, which enervate the upper and lower airways, were cultured from BALB/c and B6 fetal mice. These cultures were exposed (4 hr) to ROFA (25-50 μg/ml), coal fly ash (25,50 mg/ml), capsaicin (1-3 μM) or acid (pH 5.0 and 6.5 for 15 min, then culture medium for 4 hr). In all instances, DRG neurons from BALB/c mice released significantly higher levels of the proinflammatory cytokine, IL-6 into their nutrient media compared to B6 mice. Finally, single cell recordings of intracellular calcium were taken from cultured DRG, loaded with the fluorescent calcium indicator dye Fluo-3 AM, in response to pH 6.5 and capsaicin (0.5 μM). These data indicated that significantly higher numbers of BALB/c neurons responded with increases in intracellular calcium compared to B6 neurons, but that qualitatively the responses (i.e., peak amplitudes) were equivalent between strains. Taken together these data suggest that irritant receptors (i.e., capsaicin, acid sensitive) located on sensory nerve fiber terminals subserve PM-induced airway inflammation and are quantitatively different in responsive and nonresponsive mouse strains. (This abstract does not reflect EPA policy.)

1462 PARTICULATE MATTER AND CHARGED SYNTHETIC POLYMER MICROSPHERE ANALOGUES ACTIVATE BRONCHIAL EPITHELIAL CELLS AND SENSORY NEURONS THROUGH CAPSAICIN AND ACID RECEPTORS.

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Residual oil fly ash (ROFA), an industrial particulate pollutant, causes airway inflammation and hyperresponsiveness in rodents. Recent data showed that exposure of BEAS-2B bronchial epithelial cells to ROFA elicited inflammatory effects (e.g. increases in intracellular calcium, cytokine transcript and release), which were inhibited by capsazepine (CPZ), an antagonist of capsaicin receptors, and by amiloride, an antagonist of acid receptors (Veronesi et al., Toxicol. Appl. Pharmacol., *in press*). In this study, we examined whether physico-chemical characteristics of particles contributed to the inflammatory changes recorded in culture. Electrophoresis showed that 'field' ROFA particles carry a negative surface potential, as indicated by their zeta potential of -25 - -35 mV. Synthetic polymers microspheres (SPM) were synthesized, that resemble ROFA particles with 2-6 μm diameter and a zeta potential of -29 mV. The effects of ROFA and SPM were examined on BEAS-2B cells and sensory dorsal root ganglion (DRG) neurons. ROFA and SPM caused an immediate increase in intracellular calcium which was inhibited by CPZ and amiloride, respectively. In addition, exposure of BEAS-2B cells and DRG neurons to ROFA or SPM caused the release of IL-6, which was completely blocked by CPZ and partly blocked by amiloride. In both cell types capsaicin and pH 6.5 increased intracellular calcium and caused the release of IL-6 in a receptor-mediated fashion. These data suggest that the acidic environment associated with the negative zeta potential of the particles can activate capsaicin and acid receptors. We propose that activation of these irritant receptors triggers the subsequent release of inflammatory cytokines and neuropeptides which initiate and sustain neurogenic inflammation in the airways. (This abstract does not reflect EPA policy.)

1463 PHYSICOCHEMICAL COMPONENTS OF PARTICULATE MATTER CONTRIBUTE DIFFERENTIALLY TO INFLAMMATORY RESPONSES IN SENSORY NEURONS.

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Airborne particulate matter (PM) consists of complex aggregates of elemental and organic carbons, metals, sulfates and microbial contaminants. Its heterogeneous composition has complicated identifying the mechanism(s) which underlie the symptoms of airway inflammation. We examined the physicochemical characteristics of various urban and industrial PM in relation to their inflammatory effects in cultured sensory dorsal root ganglion neurons (DRG). The components of PM were separated by centrifugation and filtration, and characterized in terms of particle size, surface charge and pH in buffered solutions. The results show that most PM remain acidic in solution, even after multiple washings. The different PM contained particles of variable sizes, including ultrafine particles. The 'field' PM particles, their washed particle core and the ultrafine particles, obtained after 22 μm filtration, contained a variable surface charge as indicated by their zeta potentials. Exposure of DRG to PM caused a differential release of the proinflammatory cytokine IL-6 depending on the source and on the fraction of PM tested. Finally, pretreatment of DRG with antagonists of capsaicin receptors (i.e., capsazepine) or acid-sensitive receptors (i.e., amiloride or benzamil) inhibited the PM-induced IL-6 release in a differential manner. Collectively, these data support our hypothesis, that PM initiate inflammation by activation of capsaicin and acid-sensitive irritant receptors located on various airway target cells. In addition, the data indicate that soluble, acidic and charged particle components of PM contribute differentially to the inflammatory effects. (This abstract does not reflect EPA policy.)

1464 NUCLEAR FACTOR–KAPPA BETA (NF-KB) ACTIVITY IN RAT ALVEOLAR MACROPHAGES EXPOSED TO RESPIRABLE ORGANIC PARTICLES AND ANTIOXIDANTS.

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Activation of the transcription factor NF-kB by respirable organic particles (ROP) facilitates oxidative damage in lung through increased expression of pro-inflammatory genes. In previous studies, we found that NF-kB in alveolar macrophages was activated maximally at 2 hours following <I>in vitro</I> treatments with ROP. Because of the recent interest in identifying oxygen radical pathways of NF-kB activation, we have investigated inhibitory effects by specific antioxidant agents. NR8383 rat alveolar macrophage cells were treated with ROP, with and without antioxidants, and analyzed for NF-kB activation employing the electrophoretic mobility shift assay (EMSA). Densitometry analysis of EMSA gels revealed the following reductions in NF-KB activation (reported as % of positive control). 200 units/ml superoxide dismutase reduced activation by 51%, whereas catalase had no inhibitory effect, strongly suggesting a primary role for the superoxide anion radical, but not hydrogen peroxide, in the activation of NF-kB in alveolar macrophages. Furthermore, only a 27% reduction in NF-kB activation was observed following cell treatments with 1 nM melatonin, an efficient scavenger of hydrogen peroxide. A series of vitamin E species, alpha-tocopherol succinate, vitamin E acetate, and Trolox, a vitamin E derivative, at 100 uM reduced NF-kB activation 63%, 33%, and 38%, respectively, clearly indicating that vitamin E analogs differ in their potencies of NF-kB inhibition. One uM pyrrolidinedithiocarbamate and 20 mM N-acetyl cysteine, which have effects on diverse oxygen free radical pathways, reduced NF-kB activation 54% and 66%, respectively, providing further support for a general role of reactive oxygen species in NF-kB activation. Ongoing studies are exploring the role of lipopolysaccharide receptors and calcium regulatory pathways on NF-kB activation in lung cells following exposure to particulate matter. (Support from NIOSH and USACEHR.)

1465 FREE RADICAL GENERATION BY WOOD SMOKE PARTICLES AND IN VITRO DNA DAMAGE.

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As a component of our investigations into the pathophysiology of smoke inhalation, the present study characterizes the free radicals generated by combustion of Western bark (fir and pine), using the technique of electron spin resonance (ESR) . Smoke was generated by heating the wood chips in a crucible furnace at a constant temperature of 400°C and an air flow of 6 L/min. Smoke was filtered through Whatman 1 filters or bubbled through saline over a 1 min period beginning at 0, 5, 10, 15 and 20 min after generation of the smoke. ESR analysis of the particles trapped by the filters revealed the generation of carbon-centered free radicals similar to those generated by coal or diesel particles. These radicals were observed at all time points and may be associated with the surface or the core of the particles. Addition of H_2O_2 generated hydroxyl radicals (•OH). There was no direct correlation between the generation of •OH and the level of carbon-centered free radicals. In aqueous media, wood smoke particles also generated •OH. Incubation of wood smoke particles with DNA, in vitro, caused DNA strand breaks as measured by gel electrophoresis. The H_2O_2 scavenger, catalase, the •OH scavenger, formate, and the iron chelator, deferoxamine, inhibited the DNA strand breaks, while H_2O_2 enhanced them. These data suggest that free radical reactions mediated by wood smoke particles may play an important role in the cellular injury associated with smoke inhalation.

1466 LUNG CELL INJURY INDUCED BY DIESEL EXHAUST PARTICLES (DEP) IN VIVO AND IN VITRO: EFFECTS OF OZONE (O_3) AS A CO-POLLUTANT.

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DEP are a frequent, and sometimes major, constituent of ambient air particulate matter. DEP may contribute to the increased morbidity (e.g., increased pulmonary infections) and mortality associated with PM. These particles coexist in the ambient air with O_3 which may ozonize DEP to alter DEP chemical composition and subsequent toxicity. We examined the effects of DEP, and ozonized DEP, on pulmonary toxicity in vitro and in vivo. To generate ozonized particles, DEP were exposed to 0.1 ppm O_3 for 48 hr. For in vitro studies, cultures of human airway epithelial cells were initially exposed for 2 or 24 hr to HBSS (as control), unexposed DEP, ozonized DEP, or air-exposed DEP at $\leq$ 500 μg/ml. Viability among the treatment groups, assessed by LDH release, was not different from control at 2 and 24 hr. At 24 hr, an increase in prostaglandin E_2, an important immunoregulatory lipid, after melittin stimulation was observed for ozonized DEP-exposed cells, but not for other exposures. To examine the in vivo effects of unexposed DEP and ozonized DEP, 60 day old Sprague-Dawley rats were intratracheally instilled with 500 μg of particles and lungs lavaged 24 hr later. Rats instilled with ozonized DEP, but not DEP, had a significant increase in lavage protein (85% increase compared to control animals; $p<0.05$). Lavage neutrophils in both DEP and ozonized DEP treated rats increased similarly compared to saline-instilled controls. The data from these in vivo and in vitro studies suggest that ozonized DEP is more potent than unexposed DEP in inducing lung toxicity and injury. This change in the biological potency of DEP induced by O_3 exposure is likely due to ozonation of organic components. O_3 and DEP, and possibly other organic-rich particulate matter, may act together in the induction of increased human morbidity and mortality. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

1467 MOBILIZATION OF IRON FROM SIZE-FRACTIONED COAL FLY ASH IN HUMAN AIRWAY EPITHELIAL (A549) CELLS.

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Coal fly ash (CFA), a contributor to particulate air pollution, contains as much as 15% iron by weight. Some of the pathological effects after inhalation may be due to reactive oxygen species produced by the redox cycling of iron. The objective of this study was to compare sized fractions of CFA and to determine whether iron could be mobilized in A549 cells and if this correlated with mobilization of iron by citrate *in vitro*. Fly ash samples produced by burning 3 types of coal were collected as fine (<2.5 μm), coarse (2.5-10 μm) or >10 μm size fractions. Iron was mobilized by 1 mM citrate from all 3 size fractions of all 3 coal types to levels as high as 56.7 nmol of Fe/mg of CFA after 24 h. With all 3 coal types, more iron was mobilized by citrate from the <2.5 μm fraction than from the fractions >2.5 μm. The mobilized iron was in the Fe(III) form and the amount mobilized had no relationship to the total iron present in the ash. A549 cells were treated with CFA and the amount of the iron storage protein, ferritin, was determined after 24 h as in indicator of intracellular iron mobilization. Ferritin levels were increased by as much as 11.9-fold with 20 μg coal fly ash/cm^2 cells. Higher ferritin levels were observed from treatment with the <2.5 μm than the >2.5 μm fly ash with 2 of the 3 types of coal studied. The amount of iron mobilized by citrate correlated with the amount of ferritin induced in A549 cells treated with CFA. The results of this study suggest that mobilization of iron in A549 cells was related to the size of the particles and the speciation of iron. These results support concerns by the EPA that particulate air pollution <2.5 μm is of greater importance for health concerns (Supported by a grant from HEI.)

1468 TOXICITY OF COBALT-CONTAINING DUST FOR RAT TYPE II PNEUMOCYTES AND ALVEOLAR MACROPHAGES.

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Occupational exposure to cobalt-containing dust has been associated with the development of interstitial lung disease. Cobalt (Co) is used in many industrial applications, e.g. as a binder for tungsten carbide (WC) in hard metal (CoWC: 6%Co, 94%WC). We investigated the in vitro toxicity of different forms of Co for type II pneumocytes (AT-II) and alveolar macrophages (AM). Primary cultures of rat AT-II and AM were incubated for 24 hours in 24-well plates with Co metal, Co^{2+} ion or CoWC. In some experiments inserts were used to separate particles from cells and EDTA was used to chelate Co ions. As an index for cell toxicity, we determined mitochondrial activity using the MTT test. Co particles (5-25-100μg/well) caused a dose-dependent toxicity in AT-II (77-59-25% viability of control) and AM (93-72-58% viability of control). There was no change in toxicity when the particles were placed in an insert. The presence of EDTA decreased the toxicity of Co particles for AT-II, but not for AM. The presence of WC (i.e. CoWC) increased minimally the toxicity of Co for AT-II and definitely for AM, but only when the particles were in direct contact with the cells. EDTA was able to decrease the toxicity of CoWC for AT-II and AM. Medium in which 100μg Co particles had been placed in an insert for 24h (0-24h) was toxic for the AT-II and resulted in 15% viability compared to control cells. However, when the same (but now oxidized) particles were placed in fresh medium (24-48h), they caused much less toxicity (viability 71% of control). $CoCl_2$ (10^{-4}-$4x10^{-4}$-10^{-3}M) caused a dose-dependent toxicity in AT-II (79-69-42% viability of control) and AM (90-67-35% viability of control). Our results suggest that the toxicity of Co for AT-II is mainly due to the production of Co ions during the dissolution of the particles. For AM, the toxicity of Co is due in part to the Co ions and in part to the contact with the cells, especially in the presence of WC. These results suggest that Co causes toxicity via different mechanisms in AM and AT-II.

1469 TOXICITY TESTING OF AIRBORNE PARTICULATE EXTRACTS USING THE NEUTRAL RED BIOASSAYS WITH KERATINOCYTES AND BRONCHIOTREACHEAL CELLS.

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Airborne particulate matter has been reported as one of principal causes of respiratory illnesses, acute bronchitis and asthmatic attacks in the world. Toxicity testing of particulate matter has been based mainly on traditional mutagenic assays determined by the widely used Salmonella/microsome bioassay. Normal human epidermal keratynocytes (NHEK) have been recently used in our laboratory to compare toxicities of adsorbed compounds to particulate matter. To establish stronger correlations between toxic effects and respiratory tract diseases, the Neutral Red Bioassay using normal human bronchial epithelial cells (NHBEC) were employed. Total suspended particles, less than 100μ m (TSP) and 10μ m (PM10) were processed using Soxhlet extraction methodology with solvents of different polarities (hexane, dichloromethane, acetone). A seasonal screening using both type of cells were performed in order to establish comparisons between cell types. Extracts of standard reference material (SRM 1649, urban dust) were tested using both types of cells as a reference point for comparison. The mutagenic activities of dichloromethane and polar extracts (acetone) from SRM 1649 have been reported to be relatively similar (20-23 revertants/μg) and was found to be 3-4 times higher than that of airborne particulates collected from a New Jersey site. The data proves evidence that moderately polar and polar fractions from SRM 1649 contain mutagenic organic material. Cytotoxic levels based on the neutral red uptake of bronchial cells exhibited a similar NR_{50} range for hexane extracts when compared to NHEK. The moderately polar and polar extracts from the same samples showed a wide difference among themselves for both type of cells. NR_{50} values ranged from 1 to 6 μg/mL (NHEK) and 6 to 43 μg/mL (NHBEC), for the SRM extracts. None of the control extracts from a reference site exhibited any detectable level of toxicity during the winter. Acetone extracts showed a broad difference in toxic effects when compared with NHEK and NHBEC. In summary, bronchial epithelial cells showed more sensitivity to extracts collected throughout the year at both sites.

1470 FURTHER CHARACTERIZATION OF THE IMMUNE RESPONSE TO ENZYME ALLERGENS IN THE BDF1 MOUSE USED IN THE MOUSE INTRANASAL TEST: LACK OF INFLUENCE OF SOURCE OF ANIMALS.

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The mouse intranasal (IN) test was developed as a method to assess the immunogenic potential of detergent enzymes. The BDF1 mouse was chosen based upon its robust IgG1 antibody response to certain enzymes and its

propensity to generate IgE antibody to protein antigens. Numerous detergent enzymes have been tested in this strain and the potency estimates match those obtained in the guinea pig intratracheal test which correlate with skin test res-ponses in occupationally exposed humans. Since the BDF1 is a hybrid of 2 inbred strains, the potential role the source of animals may have on the response to enzymes was tested. BDF1 mice obtained from 2 suppliers in the United States (US): Charles Rivers (CRL) and Taconic Farms (TF), 2 breeding sites from the same supplier in the US (CRL-MI, CRL-NC) and a third supplier (Harlan) in the United Kingdom (UK) received IN doses of enzyme in detergent matrix or saline vehicle on days 1, 3 and 10. Sera were collected on day 15 and IgG1 antibody titer to enzyme assessed by ELISA. The response to Alcalase in saline was similar in animals obtained from the 2 US suppliers: TF ED_{50} =2.24μ g, CRL ED_{50} =2.35-2.61μ g. The response to Alcalase in detergent was similar among the CRL US sourced animals: CRL-MI ED_{50} =0.25-0.39μ g, CRL-NC ED_{50} =0.25-0.34μ g. The response to the amylase Termamyl was also similar among the US CRL sourced animals: CRL-MI ED_{50} =0.042-0.091μ g, CRL-NC ED_{50} =0.056-0.087μ g. The response to Alcalase and Savinase was very similar between the US and UK sourced animals. The ED_{50} to Alcalase in UK animals was 0.37 μ g. The ED_{50}s to Savinase in US animals =0.25-0.38μ g and in UK animals = 0.32μ g. These data show that the IgG1 antibody response of BDF1 mice to different enzymes is consistent regardless of the source of animals.

1471 SENSITIZATION POTENTIAL OF A PIPERIDINYL CHLOROTRIAZINE DERIVATIVE IN A MURINE LOCAL LYMPH NODE ASSAY AND IG-E TEST.

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A spectacular outbreak of occupational asthma in a polymer processing plant was found to be due to a novel UV-stabilizer, a piperidinyl chlorotriazine derivative (PCT; CAS # 52185-43-0). In this study we further investigated the sensitization potential of PCT using a local lymph node assay (LLNA), which has been proposed as a valid method to identify chemical sensitizers (Basketter *et al.*, Fd Chem Toxic, 32, 1994, 543-547). PCT [10 and 20 % (w/v) in dimethylformamide (DMF)] was tested in female BALB/c mice [n=3-7; 3 experiments]. During 3 consecutive days, 25 μl of PCT or vehicle were applied on the dorsum of both ears. On day 5, the mice were injected intravenously with 20 μCi [^{3}H]-thymidine. After 5 h, the auricular draining lymph nodes were excised and radioactivity was measured as an index of lymphocyte proliferation (Stimulation Index=SI). Our previously reported tests using low concentrations [0.05, 0.5, 2.0 % (w/v) in DMF] had been negative. However, after application of 10 % and 20 % solutions we found a significant increase in lymph node weight (1.3- and 1.5-fold, resp.) and cell proliferation (SI=2.7 and 3.2, resp.). We also used the mouse IgE test, which has been proposed to identify *respiratory* sensitizers (Dearman *et al.*, J Appl Toxicol, 12, 1992, 317-323). Female BALB/c mice [n=4-5] received 50 μl of PCT [10 or 20 % (w/v) in DMF] or vehicle on each shaved flank under occluded patch or 25 μl of the same solutions on the dorsum of both ears during 3 consecutive days. On day 8, 25 μl of the same solution diluted 1:1 with vehicle were applied to the dorsum of both ears. On day 15, serum IgE was determined using a sandwich ELISA. A significant increase in IgE was found for the 20 % group in both experiments (3- and 1.7-fold, resp.). We conclude that PCT is a respiratory sensitizer in mice when tested at high concentrations. This is in contrast with the very low concentrations of PCT that appear to have induced severe occupational asthma in humans.

1472 DETECTION OF SKIN SENSITIZERS IN THE LLNA: AN ANALYSIS BY CHEMICAL CLASS.

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The murine local lymph node assay (LLNA) has been developed as a method for the prospective identification of chemicals with the potential to cause contact sensitization. As part of an extensive evaluation and validation program, the performance of the local lymph node assay with over 200 chemicals has been compared with results obtained using relevant guinea pig test methods and/or with available human data. In the investigations described here, we have compared LLNA with guinea pig test data according to the chemical class of test materials. An examination of 14 different chemical classes revealed a very high level of agreement. Differences between the LLNA and guinea pig test methods were seen only with the lactones and esters. Such differences are believed to have been exaggerated (in the case of esters) by the very small numbers of compounds tested, or due possibly (in the case of lactones) to species differences in response. For all other chemicals classes, the LLNA and guinea pig test methods yielded comparable results. Collectively these data provide additional evidence for the general utility of the LLNA as a routine method for the identification of skin sensitizing chemicals. (Supported by NIEHS under contract N01-ES-85424.)

1473 OXAZOLONE-INDUCED ALLERGIC SKIN INFLAMMATORY RESPONSE IS PREDOMINATED BY MIXED PATTERNS OF CYTOKINE PRODUCTION AND NEUTROPHIL INFILTRATION.

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Allergic contact dermatitis (ACD) is traditionally classified as Type IV hypersensitivity. Previous studies employing local lymph node assay and *in vitro* organ and cell culture system documented an important role of Th1 cytokine production and CD4+ T cell in chemical-induced allergic contact dermatitis. However, a comprehensive *in situ* study of skin inflammatory response in terms of cytokine production and composition of infiltrating cells and its relation to the development of ACD largely remains absent. In this study, B6,129 mice were either sensitized with 0.5% oxazolone or control vehicle on abdominal skin and challenged five days later on ear. The dermal inflammatory response was evaluated by immunohistochemistry at 24, 48 or 72 hours post challenge. At 24 and 48 hours post challenge, the dermal cellular infiltrate consisted of CD4+ T cells, CD8+ T cells, mast cells, macrophages and neutrophils for animals sensitized with either vehicle or oxazolone. Interestingly, neutrophil were the predominant cell type. However, at 72 hours post challenge, while neutrophil are still the dominant infiltrating cells in the dermis, there is a significant increase in numbers of CD4+ T cells and CD8+ T cells in both groups. Among these inflammatory cells, large numbers of cells producing Th1 (IL-2, IFN-gamma), Th2 (IL-4, IL-10 and IL-13), and proinflammatory (IL-1beta and TNF-alpha) cytokines were consistently observed. Small numbers of infiltrating cells produced IL-12 or RANTES. Results of this study suggest that 1) both Th1 and Th2 cytokines, along with proinflammatory cytokines, are present in the inflammatory skin. 2) neutrophils are the dominant inflammatory cells. 3) chemical-induced allergic contact dermatitis may not be a traditional Type IV hypersensitivity response in terms of requirement for prior sensitization, pattern of cytokine production and type of inflammatory cells.

1474 LANGERHANS CELL MIGRATION INDUCED IN MICE BY SODIUM LAURYL SULFATE REQUIRES INTERLEUKIN 1α.

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Epicutaneous exposure of mice to skin sensitising chemicals induces the migration of Langerhans cells (LC) from the epidermis and their subsequent accumulation as immunostimulatory dendritic cells (DC) in lymph nodes draining the site of application. We have reported previously that this response is dependent upon receipt by LC of two epidermal cytokine signals; one provided by tumor necrosis factor α (TNF-α) and the other by interleukin 1β (IL-1β). Exposure of mice to the skin irritant sodium lauryl sulfate is also associated with the stimulation of LC migration, an event shown previously to be dependent upon TNF-α . Since SLS is thought not to induce in mice local IL-1β expression, we have investigated the contribution of IL-1α to SLS-induced LC migration. Systemic pretreatment of mice with a neutralising anti-IL-1β antibody was without effect on the accumulation of DC in draining lymph nodes measured 18 hours following topical exposure of mice to 10% SLS, under conditions where this antibody inhibited (by 75%) the response to the contact allergen oxazolone (Ox). Pretreatment of mice with a neutralising anti-IL-1α antibody, however, prevented completely both the reduction in epidermal MHC class II+ LC and the accumulation of DC in draining nodes and measured 4 hours and 18 hours, respectively, following exposure to SLS, but failed to inhibit Ox-induced responses. These data suggest that there may exist a differential requirement for IL-1α and IL-1β during irritant- and allergen- induced cutaneous responses with IL-1α substituting for IL-1β in the regulation of irritant-induced LC migration.

1475 EVALUATION OF DENDRITIC CELLS FOR IN VITRO DETECTION OF SENSITIZING CHEMICALS.

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After application of haptens to the skin, Langerhans cells (LC) that are immature dendritic cells (DC) of the skin, secrete some cytokines and migrate to secondary lymphoid organs leading to activation of naive T cells. To assess which cytokines are produced by LC after an application of haptens, we have isolated $CD34^+$ hematopoietic progenitor cells from human cord blood through positive selection using anti-CD34 mAb and goat anti-mouse IgG coated microbeads. Isolation of $CD34^+$ cells was achieved using Minimacs separation column. After purification, $CD34^+$ cells were cultured with GM-CSF and h-TNF-α for 12 days in order to obtain Langerhans cells /dendritic cells. At day 12, surface marker analysis was performed using cytofluorometry. These LC/DC were positive for $CD1a^+$ (83%), CD83 (23%), B7-1 (40%) and ICAM-1 (80%). These cells showed a good uptake of antigens as measured using FITC-dextran and cytofluorometry. To assess the expression of cytokine mRNA after hapten stimulation, we added to LC/DC dinitrochlorobenzene (DNCB), a chemical agent known to induce cutaneous reactions. Results obtained using quantitative RT-PCR with a standard competitor DNA, showed that DNCB induced a three time fold increase in IL-1β mRNA expression as compared to controls. Maximal expression was obtained 15 min after the application of the hapten. These experiments showed that haptens can induce expression of IL-1β in cultured human dendritic cells. We are currently analyzing the expression of IL-12, MIP-1α and MIP-1β mRNAs in LC/DC after DNCB stimulation.

1476 EXAMINATION OF HUMAN PERIPHERAL BLOOD DERIVED DENDRITIC CELLS FOR USE AS ANTIGEN-PRESENTING CELLS IN AN *IN VITRO* LYMPHOCYTE PROLIFERATION ASSAY.

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Dendritic cells (DC) are professional antigen presenting cells which are widely distributed in the body and are key for initiating T cell mediated immune responses. Methods for culturing human DCs from peripheral blood precursors have been developed by several laboratories. We were interested in examining DCs produced by these methods and evaluating their ability to present low molecular weight chemical haptens to sensitized T cells in an *in vitro* lymphocyte proliferation assay. Immature DCs (iDCs), were generated by culturing human peripheral blood mononuclear cells for 7 days in the presence of the cytokines Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) and Interleukin-4 (IL-4). When examined by flow cytometry, these cells were found to be MHC class II $HLA\text{-}DR^+$, $CD80^+$, $CD11b^+$, $CD45RO^+$ and $CD36^-$. iDCs were treated with the test allergen, dinitrobenzenesulfonic acid (DNBS), then cultured with 10^5 autologous T cells at several responder (T-cells) to stimulator (DCs) ratios (R:S). The cultures were incubated for four days, pulsed with ^{3}H-thymidine and harvested 18-20 hours later. T-cells cultured with DNBS haptenized iDCs at a 10:1 R:S demonstrated the greatest proliferation based on ^{3}H-thymidine incorporation compared with control cultures. Mature DCs (mDCs) were generated from iDCs by culturing them in the presence of GM-CSF, IL-4, TNF-α, IL-1β, IL-6 and PGE_2 for 2 additional days. These cells, in addition to being $HLA\text{-}DR^+$, $CD80^+$, were also found to be $CD83^+$. When mDCs haptenized with DNBS were used as antigen presenting cells, sensitized T cells demonstrated the greatest proliferation at an R:S of 50:1. Increased T cell proliferation above controls was also observed at an R:S ratio of 100:1. These initial results suggest that mDCs are better antigen presenting cells for chemical haptens.

1477 mRNA EXPRESSION BY CULTURED HUMAN BLOOD-DERIVED DENDRITIC CELLS: ASSOCIATION WITH SKIN SENSITIZATION POTENTIAL.

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Langerhans cells (LC) are a specialized form of dendritic cell found within the epidermis which are important for the development of contact sensitization and other cutaneous immune responses. The study of LC has been constrained by the fact that they represent only a minority population. However, methods have been developed recently that allow the expansion in culture of LC-like cells from precursors within a number of human tissues. Human peripheral blood mononuclear cells depleted of T and B lymphocytes by positive selection were cultured in the presence of interleukin-4 and granulocyte/macrophage-colony stimulating factor. After five days of culture, approximately 60% of cells displayed a LC-like phenotype, with characteristic dendritic morphology and cell surface expression of CD83, MHC class II and CD1a (determined by flow cytometry). The majority of the remaining cells was CD14+ monocytes/macrophages, while CD3+ T cells and CD19+ B cells represented minor populations. Cytokine expression was analyzed by semi-quantitative RT-PCR. In common with skin-derived LC, blood-derived DC expressed constitutively mRNA for interleukins (IL) -1β, IL-6 and IL-18. Following exposure of blood-derived DC to the chemical sensitizer 2,4-dinitrofluorobenzene (DNFB; 10.7pM) in 0.01% dimethyl sulfoxide (DMSO), IL-1β mRNA expression was up-regulated in cells derived from 2 out of 5 donors. Under the same conditions, 0.01% DMSO alone was without effect. The variation in IL-1β up-regulation may reflect subtle differences in culture conditions, particularly minor variations in cytokine concentration. These data suggest that with further optimisation, up-regulation of IL-1β mRNA expression by blood-derived DC might prove a useful marker of skin sensitizing activity.

1478 EVALUATION OF HUMAN IRRITANTS AND WEAK TO MODERATE SENSITIZERS USING A MODIFIED LLNA AND AN IRRITANCY/PHENOTYPING ASSAY.

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One reported weakness of the murine Local Lymph Node Assay (LLNA) is its inability to differentiate weak sensitizers and strong irritants. In these studies, we evaluated a panel of irritants and weak to moderate sensitizers in a modified LLNA and an irritancy/phenotypic analysis assay. Compounds initially tested included p-xylenol, thiomerosal, formaldehyde, benzocaine, menthol, and phenol. The LLNA protocol was modified to include pre and post ear measurements as an indication of irritation. The irritancy/phenotypic analysis method also used ear swelling as an indication of irritancy but evaluated sensitization by an elevation of the %B220+ and/or %IgE+B220+ (IgE-mediated) draining lymph node cell populations. In both assays, female BALB/c mice were topically exposed on the ear pinnae for 4 consecutive days. Ear measurements were taken prior to exposure on day 1 and 24 hr after final exposure on day 5. For the LLNA, [^{3}H]-thymidine incorporation was measured on day 5 and for phenotypic analysis cell populations were examined on day 10. Consistent with the human literature, thiomerosal and formaldehyde were identified as irritants and sensitizers in both the modified LLNA and the irritancy/phenotypic assay. Additionally, the IgE+B220+ lymph node cell population was elevated in animals exposed to formaldehyde in agreement with reports of human contact urticaria following exposure to this compound. Phenol, a strong human irritant with a low sensitization index, was positive for irritation and sensitization in these assays. Benzocaine gave a false negative response for sensitization in both assays when tested at a 50% concentration in acetone. Although reported to be human irritants, menthol (50%), and p-xylenol (10%) were negative for both irritation and sensitization at the highest concentrations tested. (These studies were supported in part by the NIOSH/NIEHS interagency agreement # Y02ES10189.)

1479 USE OF THE MURINE LOCAL LYMPH NODE ASSAY TO ASSESS THE RELATIVE SKIN SENSITIZATION POTENCY OF TWO PROTEIN COUPLING REAGENTS.

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The murine local lymph node assay (LLNA) was used to evaluate the contact allergenic potency of two protein coupling reagents, N,N-dicyclohexylcarbodiimide (DCC) and 1-ethyl-3-(3'-dimethylaminopropyl)-carbodiimide (EDC). Using a standard LLNA protocol, groups of five female CBA/J mice each were treated with concentrations ranging from 0.01, 0.03, 0.1, 0.3 and 1.0% DCC in isopropyl acetate and 0.1, 0.3, 1.0, 3.0 and 10% EDC in acetone/distilled water (1:1). Range-finding irritation studies employing clinical observations and ear thickness measurements were used to guide dose selection. Irritation was also monitored in the main studies. Draining auricular lymph nodes were evaluated in individual animals and a concurrent positive control (DNCB, 0.1%) was included. Separate groups of five animals each

were also included for vehicle and naive controls. Both DCC and EDC produced a clear dose-related increase in lymphocyte proliferation in draining auricular lymph nodes compared to vehicle controls and stimulation indices of ≥ 3 were obtained for all but the lowest concentrations tested for each compound. Therefore, the threshold for skin sensitization was between 0.01 and 0.03% for DCC and between 0.1 and 0.3% for EDC. DCC was equipotent to DNCB in this assay. The results of the study show that both DCC and EDC have the potential to induce contact hypersensitivity and the potency of DCC appears to be 10 times higher than EDC at equivalent concentrations. The guinea pig maximization test for these two compounds yielded parallel results.

1480 COMPARISON OF MURINE MODELS FOR THE IDENTIFICATION OF POTENTIAL CHEMICAL SENSITIZERS.

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Efforts have been underway to develop a phenotypic analysis assay for the identification and differentiation of chemicals with the potential to induce irritation and IgE mediated and T cell mediated sensitization. The purpose of these studies was to compare results obtained with this assay to those obtained in the Mouse Ear Swelling Test (MEST) and the Local Lymph Node Assay (LLNA). The Irritancy/Phenotypic analysis assay utilizes the endpoints of ear swelling for irritation and the expression of B220 and IgE on the lymph node cells draining the site of chemical exposure as an indicator of sensitization. By measuring the increase in ear swelling, the MEST evaluates the elicitation response of previously sensitized mice following chemical challenge. A mouse ear swelling irritancy assay is used to determine the minimal irritating concentration of each chemical to be used as the challenge concentration in the MEST. The LLNA utilizes [^{3}H]-thymidine incorporation into draining lymph node cells as a measure of lymphocyte proliferation during the sensitization phase. Known human sensitizers, potassium dichromate (PDC) and 2,4-dinitrocholobenzene (DNCB), and the irritant methyl salicylate (MSC) were used for the dose response comparison studies. Female BALB/c mice were exposed topically to the chemicals following the protocol for each assay (four consecutive daily exposures for both the irritancy/phenotypic analysis and LLNA and three days of induction followed by a single challenge eight days post initial exposure for the MEST). DNCB was identified as a sensitizer in all three assays at concentrations as low as 0.25%. MSC tested negative at all concentrations in the MEST, LLNA, and phenotypic analysis assay. PDC tested positive as a sensitizer in the LLNA at concentrations as low as 0.25%, while it tested positive only at the 0.5% concentration at the 48 hr measurement of the MEST. Results obtained in these studies from the Irritancy/Phenotypic analysis assay were comparable to those for the MEST and LLNA. (These studies were supported in part by the NIOSH/NIEHS interagency agreement #Y02ES10189.)

1481 EFFECT OF VEHICLE ON POTENCY ASSESSMENT IN THE LOCAL LYMPH NODE ASSAY.

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The murine local lymph node assay (LLNA) has been validated for the identification of skin sensitization hazards. Contact allergens are identified as a function of proliferative responses induced in draining lymph nodes and this provides the opportunity also of assessing relative potency by reference to dose response analyses. In the current investigations, the known skin sensitizer 1,4-dihydroquinone has been tested in 7 vehicle systems in each of two independent laboratories. The results from the two laboratories were almost identical. The dose response data from the pairs of LLNAs were interpolated using a statistical model to derive the estimated concentration of 1,4-hydroquinone necessary to cause a 3-fold stimulation of proliferation, the EC3 value. The vehicles and mean EC3 values obtained were as follows: methylethylketone 0.07%; acetone 0.08%; acetone/olive oil (80/20 $^{v}/_{v}$) 0.15%; dimethylformamide 0.22%; dimethylsulfoxide 0.4%; propylene glycol and acetone/saline (50/50 $^{v}/_{v}$) vehicles both yielded negative results. Thus the results demonstrate that the apparent potency of a contact allergen can depend critically on the vehicle in which it is presented. These data reveal that the vehicle in which a chemical is encountered in the skin can have a significant impact upon skin sensitization potency. The implication is that accurate assessment of risk to humans will require an appreciation of the likely condition of exposure.

1482 SEROPREVALENCE OF NATURAL RUBBER LATEX-SPECIFIC IgE ANTIBODY IN NON-HEALTHCARE WORKERS.

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Serological assays for latex (LAT)-specific IgE antibody are extensively used in the diagnosis of LAT allergy. We present the results of testing sera with two FDA 510K cleared, LAT IgE kits (CAP System and AlaSTAT Microplate [ALA]). Total IgE levels were also measured. The sera were obtained from 381 workers employed in numerous, non-healthcare industries over the last ten years and stored frozen . Twenty-six sera gave positive (pos) results using the ALA assay (6.82%), while 24 yielded pos results (6.30%), using the CAP assay (pos cutoff= ≥ 0.35 $kIU_{(A)}/L$). The assays agreed on the pos or negative (neg) status of 349 samples (91.6%). Discordant results (ALA pos, CAP neg) occurred in 17 sera (4.46%), while ALA neg, CAP pos results occurred in 15 sera (3.94%). Both assays agreed on the pos status of 9 sera (2.36%). The mean total IgE of all sera (N=374) was 144.19 ± 284.00 kU/L (± SD). There were no significant differences (P=NS) between the mean ALA and CAP assays' measurements of LAT antibodies for all 381 participants, yielding 0.28 ± 0.19 and 0.34 ± 0.59 $kIU_{(A)}/L$, respectively. CAP and ALA results were significantly correlated, (0.271, P<0.01). When sera with only pos LAT IgE antibodies were evaluated, CAP yielded significantly higher results than ALA (1.71 ± 1.93 (N=24) vs. 0.75 ± 0.55 $kIU_{(A)}/L$ (N=26), (P<0.05). Pearson correlation analyses of all sera indicated significant, albeit low, associations between total IgE levels and LAT specific IgE levels (ALA=0.117, P<0.05; CAP=0.411, P<0.01). These data indicate that both the CAP and ALA LAT specific antibody kits essentially agree on the seroprevalence of LAT IgE in non-healthcare workers (6.30% vs. 6.82%), respectively, and also agree with the seroprevalence (ALA, 6.4%) reported in 1000 blood donors (Ownby, 1996). The CAP assay gave significantly higher mean levels of LAT specific antibody in pos individuals than the ALA assay. However, both assays identify different individuals as being "pos", most probably since these assays detect different subsets of antibodies, presumably to different LAT allergen epitopes present in the kits.

1483 MURINE IMMUNE RESPONSES TO NATURAL RUBBER LATEX PROTEINS.

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It has been estimated that up to 17% of the 5.5 million U.S. health care workers (HCW) demonstrate IgE antibodies to natural rubber latex (NRL) products; an even higher prevalence of latex allergic spina bifida patients have been reported (as high as 70%). Furthermore, different allergen specific IgE profiles have been associated with HCW as compared to spina bifida patients. Although the exposure routes which elicit allergic responses are identifiable, the route(s) by which NRL sensitization occurs remains unclear. Our objective is to develop murine models representative of different routes of exposure to better understand latex sensitization and for testing intervention methodologies. Female BALB/c mice were treated for ~2 months subcutaneously (sc) or topically (abraded or intact skin sites) with latex proteins ranging from 1.5μg - 200μg per exposure. Following sc exposure, total IgE levels were elevated in a dose response fashion beginning at day 14 and peaked near 12,000ng/ml following 8 weekly injections of 200μg. Likewise, IgE levels were increased 9-fold after two weeks of daily applications to abraded skin sites using 150μg of latex protein diluted in acetone. Fifty μg applications to abraded skin sites also resulted in greater than a 4 fold elevation in total IgE by day 16 whereas intact skin exposure produced no increases until day 37. By day 53, total IgE concentrations following 50μg topical exposures were comparable between "abraded" and "intact" mice. Antigen specificity was demonstrated when *in vitro* stimulation of splenocytes (sc exposed mice) with 20μg/ml latex proteins caused a 500% increase in ^{3}H-thymidine uptake. Immunoblots identified IgE specific for all of the major latex allergens (Hev b1-7) following either sc or topical latex exposure. Functional, latex specific IgE was demonstrated in a murine Active Cutaneous Anaphylaxis assay within 5 minutes of intradermal latex protein challenge (~10μg) in the ear. These results indicate that BALB/c mice should serve as an acceptable model to study hypersensitivity to NRL. (This project is supported in part by NIOSH/NIEHS Interagency Agreement #Y02ES10189.)

1484 TYPE I HYPERSENSITIVITY TO LATEX AMONG GLOVE MANUFACTURING WORKERS IN MALAYSIA.

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Previous studies indicate individuals in certain groups may have an increased risk for developing a latex allergy. This includes healthcare workers, rubber workers, atopic individuals, spina bifida patients and others who have undergone multiple surgical procedures. The study objective was to determine the extent of latex allergy in a Malaysian glove manufacturing facility where potential exposure to latex may be greater. Employee interviews and skin prick tests (SPT) were conducted. At least 16 allergens were used in the SPT including dust mites, grasses and latex. Sera was collected for measurement of latex-specific IgE antibodies. Airborne allergen concentrations, total proteins and antigenic proteins from the gloves in the production facility were determined. Results from 323 predominantly Banglideshi workers indicate a 2% incidence of latex sensitivity as determined by SPT. The mean age was 29.7 years and mean duration of exposure to rubber gloves was 5 years. The study has been expanded to include all nationalities employed at the manufacturing plant. Of those employees testing positive to latex, 80% were also positive to at least one other allergen, most typically dust mites, cockroach or watermelon. Airborne allergen concentrations ranged from 8–30,000 ng/m^3 which correlated with antigenic protein and total protein content of gloves sampled from different sites within the facility. The development of latex hypersensitivity does not appear to be increased in this population. These findings are comparable or lower than those reported in earlier studies conducted with glove factory workers.

1485 THE ROLE OF ENDOTOXIN IN THE DEVELOPMENT OF LATEX ALLERGY.

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Several investigators have hypothesized that endotoxin may act as an adjuvant, enhancing the development of an IgE response to natural rubber latex proteins following respiratory exposure. These studies were conducted to evaluate the role of endotoxin in the development of latex sensitization following two other relevant routes of exposure, topical and subcutaneous (sc). Groups of female BALB/c mice were exposed to 0, 5, 10 or 25μg of lipopolysaccharide (LPS) alone or in conjunction with 25μg of non-ammoniated latex (NAL). For topical studies mice were dosed 5 days a week to the shaven, derm-abraded, dorsal thorax. For sc studies animals were exposed once weekly by injection between the scapulae. Total IgE levels were monitored weekly and at the termination of the study; splenocyte proliferation assays were preformed and cytokine levels were quantified on *in vitro* splenocyte cultures. Following topical administration, animals simultaneously exposed to NAL and LPS (all doses) showed approximate 50% decreases in total IgE production as compared to animals exposed to NAL alone. Animals exposed sc to NAL and LPS demonstrated more dramatic decreases in IgE levels with values reaching less than 10% of the levels in mice exposed to NAL alone. A similar decrease in *in vitro* splenocyte proliferation was seen. A minimal effect on proliferation was seen following *in vitro* stimulation with NAL in splenocytes from animals topically sensitized to NAL in the presence of LPS. However, in animals sc sensitized to NAL in the presence of LPS an approximate 50% reduction in proliferation occurred. An increase in IL-2 levels were seen in splenocyte culture supernatants from these animals, which may hinder the class switch to IgE. Data from these studies suggest that the route of exposure to endotoxin may play a role in modulation of the IgE response to latex allergens. (These studies were supported in part by NIOSH/NIEHS interagency agreement Y02ES10189.)

1486 REDUCED PROINFLAMMATORY CYTOKINES IN FEED-RESTRICTED RATS EXPOSED TO HOUSE DUST MITE ANTIGEN.

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Feed restriction (FR) has been shown to reduce allergic responses to house dust mite (HDM) antigen in rats, which include less pulmonary inflammation, lower antigen-specific IgE in serum, and reduced antigen-specific lymphcyte proliferation in pulmonary lymph nodes. To understand the underlying mechanisms, both Th1/Th2 and proinflammatory cytokine expression was assessed in female Brown Norway rats fed either *ad libitum* (AL) or at feed intakes equal to 75% that of AL intake. Through an intratracheal instillation of HDM, rats were sensitized after 3 weeks of feed restriction then challenged 2 weeks later. Rats were euthanized and pulmonary lymph nodes collected 7 and 14 days after sensitization as well as 2 and 7 days after challenge. Although expression patterns of Th1/Th2 and proinflammatory cytokines were demonstrated in AL and FR rats exposured to HDM, no FR-related change was observed in IL-1β , IL-2, and IL-4 mRNA expression in pulmonary lymph nodes. However, IFN-γ expression level in FR rats was similar to that in AL rats prior to challenge and 2 days post challenge, it was higher in FR rats than that in AL rats 7 days after HDM challenge. While the expression of IL-6 and TNF-α was lower in FR rats than in AL rats 7 days after HDM sensitization, it elevated to the same levels as AL rats after HDM challenge. These data revealed Th1/Th2 and proinflammatory cytokine profiles in rats exposed to HDM antigen and indicated that dietary restriction reduced proinflammatory cytokine expression after sensitization. (This abstract does not necessarily reflect the views and policies of US EPA.)

1487 COMPARISON OF ALLERGIC RESPONSES TO HOUSE DUST MITE (HDM) AMONG LOCALLY AND SYSTEMICALLY SENSITIZED SUCKLING, WEANLING, AND ADULT BROWN NORWAY RATS.

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The model of HDM allergy in adult Brown Norway (BN) rats has been a useful tool for exploring the mechanisms of allergic disease and the impact of environmental pollutants on the development and expression of disease. Because the incidence of childhood asthma is increasing, it is necessary to adapt the HDM model to younger animals. In this study, allergic responses to Derf1, a purified HDM antigen, were compared among systemically and locally immunized 8-week-old adult, 3-week-old weanling, and 10-day-old suckling BN rats. Systemically immunized animals received subcutaneous injection of saline or 10μ g Derf1. Locally immunized animals received saline or a total of 10μ g Derf1 in 2 intratracheal (IT) instillations 48 hours apart. Twelve days after sensitization, rats were IT challenged with 10μ g Derf1. At 2,4, and 9 days post challenge, total IgE, antigen specific proliferation of lymph node and spleen cells, and pulmonary inflammation were measured. These data show that while local and systemic administration of antigen, without the use of adjuvant, caused allergic sensitization in adult and weanling animals, serum IgE levels were substantially higher in systemically immunized animals. Maximal pulmonary inflammation occurred at 2 days in adult rats, but was delayed until 4 days in weanlings. This immunization protocol was not sufficient to induce allergic sensitization in suckling rats. Future studies will investigate alternative immunization protocols, including multiple Derf1 instillations. (Funded by the NCSU/EPA Cooperative Training Program in Environmental Science Research, Training Agreement CT826512010 with North Carolina State University. This abstract does not reflect EPA policy.)

1488 A FLOW CYTOMETRIC METHOD TO SCREEN FOR BERYLLIUM SENSITIZATION.

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Human exposure to beryllium can result in chronic beryllium disease (CBD), an immune system-mediated disease resulting in loss of lung function, formation of granulomas, and fibrotic scarring. Medical monitoring relies on an assessment of beryllium sensitization based on the lymphocyte proliferation test (LPT), which currently measures ^{3}H-thymidine incorporation into DNA of beryllium-stimulated and -unstimulated peripheral blood lymphocytes (PBL). We present a flow cytometric assay that uses the quenching effect of bromodeoxyuridine on Hoechst 33258 DNA fluorescence to study the proliferative activity of PBL. This method allows the enumeration of cells in each G_1, S, and G_2/M phases of up to three successive cell cycles. PBLs were isolated from known CBD patients and normal controls and tested in culture for their response to either phytohemagglutinin P (PHA), tetanus, or beryllí-

um. PHA and tetanus caused similar increases in proliferation of cells from both CBD patients and normal controls. Increased cell proliferation to beryllium was only seen with blood cells from CBD patients but not normal controls. Beryllium elicited a positive proliferative response in cultured PBL that ranged from 4-6% at day 4 and 3-5% at day 6 of culture, respectively. Exogenous factors such as serum type and cytokines were tested with the goal of improving the sensitivity and specificity of the assay. Maximal proliferative response to beryllium was seen with 10% fetal bovine serum as compared to human AB serum or Nu serum. Interleukin 2 enhanced the survival of cultured cells but did not increase the total number of proliferating cells in response to beryllium. The proposed assay may prove useful in studying the etiology of CBD and for refining clinical assays currently used to identify beryllium-affected workers. (Supported by Department of Energy Cooperative Agreement #DE-FCO1-95EW55084).

1489 AN EVALUATION OF GUINEA PIG HYPERSENSITIVITY TESTS FOR ABILITY TO DETECT SYSTEMIC HYPERSENSITIVITY POTENTIAL OF DRUGS.

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Guinea pig assays developed primarily to detect contact sensitizers have also been used to screen compounds for potential to produce systemic hypersensitivity. We examined the FDA Adverse Event Reporting System (AERS) and New Drug Application (NDA) reviews and found that 151 new molecular entities (NMEs) approved for marketing between 1993 - 1998 could be evaluated. Approximately 10% of adverse drug reports (ADRs) associated with these drugs appeared to be systemic hypersensitivity reactions. An analysis of NDA reviews demonstrated that guinea pig hypersensitivity assays had been conducted on 32% of the evaluable NMEs. Positive results in guinea pig assays had been reported for 18% of these drugs. Comparison of findings in guinea pig assays and ADRs indicates an approximate 20% probability that a positive result in any guinea pig assay would be predictive of clinical systemic hypersensitivity. Analysis of NMEs approved from 1978 - 1993 suggests that, in general, guinea pig assays were not predictive of systemic hypersensitivity reactions. It is unclear if potential sensitizers had been eliminated prior to marketing based on positive findings in guinea pig assays, but analysis of available data tends to suggest that this is not the case.

1490 TH1/TH2 PHENOTYPE OF HUMAN β-LACTAM SPECIFIC LYMPHOCYTES IN IMMEDIATE OR DELAYED HYPERSENSITIVITY REACTIONS.

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β-lactam antibiotics elicit CD4+ and CD8+ T cell-mediated immune responses that play a central role in allergic reactions. However, the involvement of a Th1- or a Th2-like differentiation remains unclear. We investigated the expression of IL-4 and IFN-γ mRNA by quantitative RT-PCR in patients-derived peripheral blood lymphocytes following specific *in vitro* stimulation. Samples were collected from a total of 12 patients with clinical manifestations of β-lactam immediate hypersensitivity (urticaria, angioedema, shock), 8 patients with delayed reactions (maculopapular rash, contact dermatitis), and 11 control subjects chronically treated with β-lactams and with no reported allergic reaction. PBMC were stimulated with either free antibiotics or antibiotic-HSA conjugates. The immediate type reactions were characterized by induction of IFN-γ mRNA only (6 out of 12), or induction of both IL-4 and IFN-γ (5 out of 12). Lymphocytes from one patient did not respond. IFN-γ mRNA was induced mainly following stimulation with conjugated drugs while IL-4 mRNA expression was mainly triggered by free drugs. Delayed-type reactions were characterized by induction of IFN-γ mRNA and a total lack of IL-4 response. Lymphocytes from control subjects never produced IL-4 and a more than twofold induction of IFN-γ mRNA was observed in only one case. These data show that delayed-type reactions are characterized by Th1-like responses and that immediate reactions are not linked to typical Th2-like responses.

1491 INTERLEUKIN-4 POTENTIATES IL-2-INDUCED HUMAN PENICILLIN-SPECIFIC CD4+ T CELLS PROLIFERATION THROUGH REGULATION OF CYCLIN-DEPENDENT KINASE INHIBITORS.

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β-lactam antibiotics are haptenic compounds known to induce hypersensitivity reactions. Clinically, penicillin-induced allergies include urticaria, angioedema, shock, and bullous skin eruptions. These toxic effects involve activation of CD4+ helper T lymphocytes and/or CD8+ T lymphocytes, as well as production of specific antibodies. We previously showed that interleukin-4 (IL-4) plays a dominant role in controlling Th1- or Th2-like primary immune response to the hapten penicillin G (PNG) in the mouse. The present work focuses on the effects of IL-4 on differentiation of human penicillin-specific T lymphocytes derived from allergic patients. Such lymphocytes proliferate in response to penicillin in the presence of irradiated autologous antigen-presenting cells (APCs: EBV-transformed B lymphocytes). This proliferation is MHC-restricted since no proliferation is induced with allogeneic APCs. Long-term culture of this cell population in the presence of IL-4 and IL-2 induces selection of a pure CD4+ population. The cell-cycle analysis of CD4+ penicillin-specific T lymphocytes showed that IL-4 increases IL-2-induced G1/S cell cycle transition. Cell cycle is in part regulated through an interplay between cyclins, serine/threonine cyclin-dependent protein kinases, and cyclin-dependent kinase inhibitors (CDKIs). We show that IL-4 decreases expression of CDKIs in human penicillin-specific T lymphocytes. These data underline the key role of IL-4 in regulation of drug-specific T cell responses.

1492 HUMORAL IMMUNE RESPONSE TO A SEVOFLURANE DEGRADATION PRODUCT IN THE GUINEA PIG FOLLOWING INHALATION EXPOSURE.

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Compound A (2-fluoromethoxy-1,1,3,3,3-pentafluoro-1-propene) is produced by reaction of the inhalation anesthetic, sevoflurane, with CO_2 absorbents. Compound A has been reported to directly react with protein. Since adduction of proteins can transform them into antigenic material, Compound A was assessed for its ability to produce a humoral immune response. Male outbred Hartley guinea pigs (500-600 g, N=7) were exposed via inhalation for 4 hr to a subtoxic level (100 ppm) of Compound A, 3 times, at 42 day intervals. Blood samples obtained at 2, 14, 28 and 40 days after each exposure were measured for ALT, creatinine, and urea nitrogen and for the presence of antibodies to trifluoroacetylated guinea pig albumin (TFA-GSA). All indicators of liver and kidney injury remained within normal range throughout the course of the study. A humoral immune response to TFA-GSA was observed following each exposure with a titer appearing by day 14 after exposure, peaking near day 28, and resolving to normal levels by day 40. The titer levels were approximately equivalent after each exposure and about one-third that previously seen in guinea pigs after multiple exposures to halothane. Compound A would appear to have the ability to form antigenic adducts during inhalation exposure. These findings are similar to those observed for halogenated inhalation anesthetics that have been linked to cases of immune-medicated idiosyncratic hepatitis and indicate that Compound A exposure may pose the same hazard. (Supported by Baxter Pharmaceutical Products Inc.)

1493 EVALUATION OF BENZO(A)PYRENE IN THE NZBWF1 MOUSE MODEL OF AUTOIMMUNITY.

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Potent immunosuppressive compounds such as cyclophosphamide and TCDD have been shown to enhance the immune response when administered at low doses. Benzo(a)pyrene (BaP) has been shown to act in a similar manner, enhancing the antibody forming cell response to sRBC at low dose levels. The objective of this study was to determine if exposure to BaP would exacerbate autoimmunity in genetically predisposed NZBWF1 mice. NZBWF1 mice spontaneously develop an autoimmune disease similar to systemic lupus erythematosus seen in humans. NZBWF1 mice (5 weeks of age at beginning of treatment) were dermally exposed to BaP (0.2, 2, 20 mg/kg) daily for thirty days. Vehicle mice were treated with an acetone: olive

oil mixture (9:1). No significant change in body weights or kidney and thymus weights was observed; however, animals treated with 20 mg/kg BaP had statistically significant increased liver (20%) and spleen (93%) weights. Using ELISA methodology, several autoimmune parameters were evaluated by detecting the presence of antibody to self and non-self antigens. When compared to vehicle mice, no difference in levels of serum IgG total antibodies or antibody titers to DNP-HSA, dsDNA, or laminin were observed in animals treated with BaP. While lower dose levels are currently under investigation, initial results indicate that dermal exposure to BaP for thirty days does not exacerbate autoimmunity in NZBWF1 mice. (Supported in part by NIEHS Contract ES55387 and Immunotoxicology Training Grant ES07087.)

1494 ACCELERATION OF AUTOIMMUNITY BY THREE CHLORINATED PESTICIDES WITH ESTROGENIC EFFECTS.

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Systemic lupus erythematosus is an autoimmune disorder in which women are affected more frequently than men. Data from both human and animal studies support the findings that estrogens can have an adverse impact on the course of the autoimmune process. Considerable interest has focused on the possible environmental factors that may contribute to the development of autoimmune disorders in general, and lupus in particular. Recently, a wide array of common environmental contaminants has been shown to possess estrogenic effects. Specifically, environmental toxicants including DDT, chlordecone, and methoxychlor have been shown to have estrogenic effects in both in vitro and in vivo systems. However, the effects of these compounds on immune-mediated processes such as lupus have not been well studied. We examined the effects of these three agents on the course of an autoimmune disorder in mice using the (NZB x NZW)F1 (BWF1) mouse model of lupus. Each compound was tested at a dosing rate verified to produce estrogenic effects (uterine hypertrophy) in vivo. All three compounds were found to accelerate the course of lupus in ovariectomized BWF1 mice. Specifically, mice treated with DDT, chlordecone or methoxychlor developed an accelerated production of anti-dsDNA antibodies, an accelerated onset of renal disease, and an increased early mortality. The results suggest that chlorinated pesticides with estrogenic effects may pose a distinct risk to individuals with lupus and related autoimmune diseases. (Supported by ES 08551.)

1495 MULTIPLE IMMUNE FUNCTIONS IN RATS FED ECHINACEA.

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Echinacea purpurea (ECH) is found as the sole ingredient or in combination with other herbs in herbal products advertised as protective against colds and flu. Herbalist have traditionally recommended the use of the echinacea plant to stimulate immune system. Research using both human cells and rodent models suggest that this recommendation may be well founded. In our current study, two doses (50 and 250 mg/kg) of a liquid ECH preparation were fed to 6 wk old male rats. At the conclusion of the six week ECH feeding period the following immune functions were evaluated: 1) B cell-mediate antibody production; 2) T cell-mediated delayed-type hypersensitivity; 3) non-specific cell-mediated natural killer cell cytotoxicity. We found no significant difference between vehicle- and ECH-fed rats in the immune functions assayed. To address the possibility that tolerance to the immunostimulatory actions of ECH may occur over a 6 week feeding period, we examined antibody production in female and male rats fed ECH (250 mg/kg for 2 weeks) starting on the day of the first antigen injection. The rats were sacrificed and blood collected one week following the second antigen injection. We found no difference in antibody production between ECH- (6.2 ± 1.4) and vehicle- (6.8 ± 1.7) fed male rats, however, the female rats fed ECH exhibited a significant reduction (4.0 ± 1.2, $p < 0.01$) in antibody production compared to their vehicle control (9.0 ± 1.0). Our findings of no effect on immune function following 6 weeks of ECH feeding at doses that exceeded recommended human doses, no effect on antibody production in male rats, and a significant reduction in antibody production in female rats fed the high dose (250 mg/kg) of ECH for 2 weeks, fails to confirm the efficacy of commercially prepared echinacea as an immunostimulatory agent in our rat model.

1496 ORAL EXPOSURE TO BUTOXYACETIC ACID (BAA) DOES NOT MIMIC THE EFFECTS OF ITS PARENT COMPOUND, BUTOXYETHANOL (BE), ON SYSTEMIC IMMUNE RESPONSES.

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Immunomodulatory effects of glycol ethers have been studied in several rodent models. Previous studies in our lab indicated that 10-day oral dosing with 2-butoxyethanol (BE) enhances systemic immune responses in female BALB/c mice. The objective of this study was to determine whether oral dosing with BE's primary metabolite, butoxyacetic acid (BAA), would produce the same enhancement. Mice were given either 0, 50, 150 or 400 mg/kg/day BAA for 10 days by oral gavage and sacrificed 72 hours later for assessment of systemic immune parameters. We found no significant effects on lymphocyte blastogenesis in response to either B- or T-cell mitogens (lipopolysaccharide and concanavalin A, respectively). There were no significant effects on blastogenesis in the mixed lymphocyte response. We also observed no differences in natural killer cell activity or in cytotoxic T cell activity among any of the BAA doses when treatment groups were compared to the control group. Based on these findings, it appears that the immunoenhancement observed with oral BE administration is not due to the action of BAA.

1497 EFFECT OF CHEMICAL MODIFICATIONS ON A B CELL STIMULATORY OLIGONUCLEOTIDE.

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Phosphorothioate oligodeoxynucleotides have been shown to induce a dose-dependent immune stimulation in rodents. The immunostimulatory effects are independent of the antisense target and unrelated to pharmacological activity. Stimulation has been observed with most phosphorothioates, but the levels of stimulation are highly variable due, in part, to sequence differences. Although palindromes (i.e. the CpG motif, unmethylated CpG dinucleotide flanked by two 5' purines and two 3' pyrimidines) play a critical role in the stimulatory effects of oligos, other sequence-related elements may also be involved. To better understand the influence of various structural and chemical modifications on immunostimulation, ISIS 12449 (5'ACC-GATAACGTTGCCGGT GACG 3'), a highly stimulatory phosphorothioate oligodeoxynuceotide, has been modified and its effects studied in a standard *in vitro* B cell stimulation assay using splenocytes from CD-1 mice. In general, stimulatory oligomers lose activity as their length is decreased. The stimulatory effects of ISIS 12449, and other oligomers, were also attenuated, respectively, by modifying the 2' position within the oligonucleotide with methoxy ethyl and the addition of 5-methyl cytosine. Combination of the modifications further decreased stimulation, but did not completely abrogate the effects. Alterations in the base positioning in relation to the CpG motif indicated that both base identity and position within the molecule also influenced biological effects. These data suggest that multiple factors contribute to the immunostimulatory effects of antisense oligonucleotides. Although, certain sequences may contain components that are highly stimulatory, immunostimulation may be ablated through chemical modifications.

1498 FUMONISIN ALTERS MACROPHAGE ACTIVATION AS MEASURED BY LUMINOL-DEPENDENT CHEMILUMINESCENCE.

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Fumonisin B1 (FB1) is a sphingosine analog mycotoxin purified from *Fusarium* species which contaminate corn and other crops worldwide. FB1 inhibits complex sphingolipid metabolism and causes a variety of animal diseases including hepatotoxicity, nephrotoxicity, equine leukoencephalomalacia and porcine pulmonary edema. In order to elucidate a possible cellular mechanism of fumonisin toxicity, an *in vitro* model was established using the murine macrophage RAW 264.7 cell line. Luminol-dependent chemiluminescence (LDCL), an *in vitro* technique to measure the oxidative burst in macrophages after phagocytosis, was used to characterize our previous observation that pretreatment with 10μM FB1 for 48 hours increased zymosan-stimulated LDCL of RAW 264.7 cells. Zymosan-stimulated LDCL enhancement by FB1 was dose-dependent, duration-dependent and reversible upon removal of FB1. In contrast to enhancement of zymosan-

stimulated LDCL, FB1 pretreated RAW 264.7 cells displayed a greatly reduced phorbol myristate acetate (PMA)-stimulated LDCL. This decrease was also FB1 dose- and duration-dependent. Differential RAW cell responses based on activating stimuli suggest that FB1 may alter cell signaling events by influencing secondary messengers or membrane integrity, perhaps via changes in cellular sphingolipid pools. To evaluate model validity, adherence-purified primary macrophages from mouse spleen cell suspensions and peritoneal washes were treated *in vitro* with 10 μM FB1. An increase in zymosan-stimulated LDCL was observed in these cells indicating the possibility that changes in macrophage function may occur during *in vivo* exposure to FB1. However, when female B6C3F1 mice were treated by gavage for 0, 3, 6, 9 or 12 days with 12 or 20 mg/kg/day FB1, no treatment-dependent alterations in zymosan-stimulated LDCL were observed.

1499 PARADOXICAL IMMUNOMODULATORY EFFECTS IN α-CD3/α-CD28-STIMULATED PRIMARY SPLENIC AND THYMIC T-LYMPHOCYTES FOLLOWING ACUTE TGF-β1 EXPOSURE *IN VITRO*.

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Transforming growth factor-β1 (TGF-β1) is a 25 kDa hematopoietic cytokine which modulates a diverse array of biological activities including immune cell function. It has only recently been demonstrated that TGF-β1 elicits immunostimulatory effects, in addition to, the more thoroughly characterized immunosuppressive effects. Interleukin-2 (IL-2)/IL-2 receptor signaling, G1 to S-phase cell cycle transition, and lymphoid apoptosis have been proposed as putative mechanistic targets of TGF-β1-mediated immunomodulation in T-lymphocytes. The objective of the current studies was to characterize the concentration-dependent immunomodulatory effects of TGF-β1 on T-cell receptor-dependent immune cell function. Resting or α-CD3/α-CD28-stimulated primary B6C3F1 mouse splenic or thymic T-lymphocytes were incubated with TGF-β1 (0-10 ng/ml). Steady-state IL-2 mRNA expression, IL-2 protein secretion, and cell proliferation were quantified using qualitative RT-PCR, ELISA, and 3H-thymidine incorporation, respectively. IL-2 protein secretion was either enhanced or inhibited following low (0.0001 to 0.1 ng/ml) or high (10 ng/ml) concentration TGF-β1 exposure, respectively. Additionally, TGF-β1 impaired cell proliferation in a concentration-dependent manner (0.001 - 10 ng/ml). The stimulatory effect was dependent on the addition of TGF-β1 prior to, or at the time of, T-cell activation. In conclusion, we have identified several factors, including the concentration of TGF-β1, the mode of T-cell activation, and the temporal relationship between T-cell activation and TGF-β1 exposure which, at least in part, modulate whether TGF-β1 elicits an immune stimulatory or an immune suppressive response on T-lymphocytes following acute exposure. These data support the hypothesis that the *immunomodulatory* effects of TGF-β1 are regulated by multiple divergent molecular pathways. (This research was supported by NIEHS Grant P01 P42ES04911-08C.)

1500 EVALUATION OF TOXIC SHOCK SYNDROME-ASSOCIATED TOXIN CONTAMINATED TAMPONS IN A RABBIT MODEL.

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Toxic shock syndrome (TSS) has gained concern because of its association with tampon use. The aim of this study was to develop an in vivo test system for detection of endotoxin (lipopolysaccharide; LPS) or toxic shock syndrome toxin-one (TSST-1) contaminated tampons. This study was designed to measure the febrile response in rabbits following intravenous injection of extracts of LPS or TSST-1 from contaminated tampons. LPS or TSST-1 were extracted from tampons contaminated with different concentrations(0, 250, 500, 1000 EU/ml) of each toxin. Rabbits were intravenously injected with these extracts, and body temperature was measured for 180 minutes following injection. The results of this study demonstrated that the i.v. administration of extracts of LPS or TSST-1 induced a febrile response in a dose-dependent pattern. Thirty minutes after injection, the temperature was increased up to 0.8-1.0°C in rabbits injected with 20 or 40 EU/kg LPS. The data also indicated the minimum pyrogenic threshold for rabbits was 5 EU/kg of LPS, which induced a minor temperature change (<0.2°C). At 180 minutes after injection of extracts from tampons contaminated with 50-200 EU of LPS, body temperature was increased 0.3-0.6°C. These data suggest that the pyrogen test in rabbits can be applied for the biological evaluation of toxic shock syndrome associated-toxin contaminated tampons or medical devices.

1501 SUBCHRONIC ORAL TOXICITY OF THIODIGLYCOL IN RATS.

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Thiodiglycol, a hydrolysis product of sulfur mustard, has been shown to be present in the ground and surface water at several military installations. In order to establish toxicity standards for thiodiglycol to facilitate environmental remediation, a subchronic oral study was conducted in male and female Sprague-Dawley rats. Preliminary studies indicated that thiodiglycol had a relatively low toxicity by the oral route. Neat thiodiglycol was administered via gavage, five days per week, over a period of 90 days. Three groups of rats, consisting of 10 males and 10 females each, received test material at the rate of either 50, 500, or 5000 mg/kg/day. A fourth group served as a sham control. In addition to weekly measurement of individual body weight and food consumption, urine, blood and tissue samples were collected at or near study termination. Thiodiglycol was not lethal to rats nor did it produce visible signs of toxicity at dosage levels as high as 5000 mg/kg/day. The measurable signs of toxicity included decreased body weight gain, lower absolute body weight, increased urine output, lower urine pH, higher urine specific gravity, appearance of granular casts in the urine and increased kidney weights. These signs were found in both sexes at the 5000 mg/kg/day dosage level. Additionally, liver, testes and brain to body weight ratios were significantly higher than controls at 5000 mg/kg/day. In females at that dosage, adrenal to body weight ratios were higher than corresponding controls. The only effects noted at 500 mg/kg/day, and only for females, were lower urine pH and higher urine specific gravity. No compound-related histopathological changes were found. The NOAEL for subchronic oral exposure to thiodiglycol, as determined from this study, is 500 mg/kg/day.

1502 PRECLINICAL TOXICITY STUDIES OF THE PHOSPHO-DIESTERASE IV INHIBITOR CI-1018 IN RATS.

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These studies were conducted to evaluate in rats the toxicity of the type IV phosphodiesterase inhibitor CI-1018 in support of clinical trials. Daily CI-1018 administration at 100, 300, and 1000 mg/kg for 2 weeks, caused dose-related gastrointestinal (GI) lesions and vasculitis in females at 300 mg/kg and in males at 1000 mg/kg. Vasculitis and GI inflammation were associated with significant morbidity, also greater in females, at 1000 mg/kg. Gender differences in sensitivity to CI-1018 correlated with approximately 2-fold greater plasma concentrations in females. In addition, epididymal edema and seminiferous tubular dilatation occurred at very low incidence at ≥ 100 mg/kg. Based upon these data, 13-week studies of CI-1018 in rats were conducted at doses of 30, 100, and 300 mg/kg. Despite systemic exposures comparable to those achieved in the previous 2-week study, as indicated by similar Cmax and AUC values, CI-1018 was well tolerated for 13 weeks at these doses and was without significant pathological change. Remarkably, gastrointestinal inflammation and vasculitis and epididymal effects were absent after 13 weeks of treatment suggesting possible accomodation to CI-1018. Additional studies demonstrated that doses up to 2000 mg/kg in males and 1000 mg/kg in females, with correspondingly greater systemic exposures, could be achieved by giving CI-1018 in the diet and escalating doses weekly over the first 4 weeks of treatment. In addition to previously noted target tissues, inflammation of the tarsal joint was identified at low incidence at these higher subchronic exposures to CI-1018.

1503 PRECLINICAL TOXICOLOGY PROFILE OF CI-1012 IN RATS.

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CI-1012 is a novel agent which induces zinc ejection from zinc fingers contained in the nucleocapsid p7 (NCp7) protein, a retroviral structural protein with multiple roles in early and late phases of the HIV-1 infection cycle. The objective of these studies was to characterize the toxicity of CI-1012 in rats in support of human clinical trials. CI-1012 given to rats by gavage at 100, 250, 500, and 1500 mg/kg for 2 weeks was associated with significant clinical signs of toxicity, body weight loss and deaths at 1500 mg/kg. Reductions

in red blood cell counts occurred at 1500 mg/kg and suppression of bone marrow hematopoiesis was evident at ≥ 250 mg/kg. Compensatory extramedullary hematopoiesis in spleen was observed at ≥ 100 mg/kg. Renal tubular degeneration occurred at 500 and 1500 mg/kg. Histiocytosis in spleen was observed at 1500 mg/kg and lymphadenopathy, dose-dependent incidence and severity, was present at all doses. Liver weight was increased without corresponding histopathological change. Histopathologic evidence of gastric irritation was present at ≥ 250 mg/kg. In chronic studies, CI-1012 was given to rats at 50, 100, and 500 mg/kg for up to 26 weeks. Gastric irritation occurred at ≥ 50 mg/kg and increases in liver and thyroid weight, both without histopathologic correlates were observed at 500 mg/kg and at ≥ 100 mg/kg, respectively. The data suggest that effects seen in 2-week studies at 500 mg/kg and below were relatively mild and non-progressive when CI-1012 is given chronically at these doses.

1504 SETTING INVENTORY LIMITS OF CHEMICALS FOR EMERGENCY PLANNING, HAZARD ASSESSMENT, OR SAFETY ANALYSIS.

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Two different methods are in use for establishing the safety of facilities, processes, or operations involving hazardous chemicals. One sets inventory limits using Occupational Safety and Health Administration (OSHA) threshold quantity (TQ), Environmental Protection Agency (EPA) Clean Air Act Amendment threshold quantity (CAA-TQ), threshold planning quantity (TPQ) or reportable quantity (RQ), values published in the Federal Register. The second method uses toxicological consequence estimates at different receptor points (e.g., facility boundary, 100 m, site boundary) of concentration limits established for this purpose. These include such parameters as EPA acute exposure guidance level (AEGL), emergency response planning guideline (ERPG), and immediately dangerous to life or health (IDLH) values. These two methods were compared by estimating the potential downwind concentrations of all chemicals on the OSHA Process Safety Management regulation TQ list. EPA CAA-TQ, TPQ, and RQ, values were extracted for these chemicals. Only 50 of the 134 unique chemicals with TQs also have CAA-TQs, 60 have TPQs, and 72 have RQs. In general, CAA-TQs > TQs > TPQs > RQs, although there are exceptions. Only 7 of 60 TQs are less than TPQ values for that chemical. Conservative release fractions at 25 ° C and dispersion conditions were used to calculate potential airborne concentrations at 100 m downwind of the assumed release of TQ quantities of each chemical. These calculations were repeated for all chemicals on the TQ list that also had CAA-TQs, TPQs or RQs. These concentrations were compared with ERPG values wherever possible. Every TPQ to ERPG ratio was greater than unity. For RQs, only 3 of 23 RQ to ERPG-1, 5 of 30 RQ to ERPG-2, and 7 of 30 RQ to ERPG-3, ratios were less than unity. Most of the other RQ to ERPG ratios were one to three orders of magnitude > 1. These results demonstrate the undesirability of using regulatory quantities established for different purposes to include these chemicals in hazard assessments, or as inventory limits. It is recommended that concentration-limits established specifically for short-term acute exposures, such as AEGLs or ERPGs, be used in safety-related applications.

1505 TWO-WEEK CONTINUOUS INTRAVENOUS INFUSION OF PROBENECID IN DOGS.

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Probenecid is a renal tubular transport-blocking agent used concurrently with other drugs to inhibit their renal secretion to decrease potential toxicity. In a range finding study, continuous IV infusion of 25 or 50 mg/kg/hr probenecid in male beagle dogs resulted in marked clinical signs (prostration, mydriasis, convulsions, tachypnea, miosis, emesis, spasms, hypoactivity) with plasma concentrations ≥ 408 μ g/mL within 21 hrs. In a subsequent 2-week study, 3 dogs/sex/group were infused with probenecid at 0, 2, 5, or 10 mg/kg/hr. Dogs were observed daily and changes in food consumption, body weight, hematology, serum biochemistry, and thyroid hormone levels were evaluated. Pathological examination was conducted. Plasma probenecid levels were measured on Days 1 and 14. Females had reduced food consumption at all doses. There was a non dose-dependent decrease (30% - 65%) in thyroxine (T_4) concentration from pretest to Day 14 in all probenecid groups. Triiodothyronine (T_3) was not affected. Mild thyroid follicular epithelial hypertrophy was noted in all probenecid groups with increased incidence at 10 mg/kg/hr with no changes in thyroid weight. Liver weights increased 16% and 24% in males at 5 and 10 mg/kg/hr, and 14%, 25%, and 40% in females at 2, 5, and 10 mg/kg/hr, respectively. Plasma probenecid levels generally increased with dose on Days 1 and 14, with means of 253 and 243 μ g/mL in males and females, respectively, at 10 mg/kg/hr on Day 1. However, Day 14 levels were approximately 35% lower than Day 1 levels. These results, and those from concurrent studies conducted in rats, suggest that the thyroid changes associated with continuous IV infusion of probenecid may be related to hepatic induction and increased elimination of T_4 with possible disturbances of the hypothalamic-pituitary-thyroid axis.

1506 SUB-ACUTE EFFECTS OF CONTINUOUS INTRAVENOUS INFUSION OF PROBENECID IN RATS FOR 2 WEEKS.

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Probenecid is a renal tubular transport blocking agent used as an adjunct with other drugs to inhibit their renal secretion. Its effects have not been evaluated following continuous intravenous infusion. In the present study, groups of 10 Wistar rats/sex were continuously infused for 2 weeks at 5, 10, and 20 mg/kg/hr through vascular access ports. Clinical examination, including changes in food consumption and body weight, were monitored throughout treatment, and clinical laboratory analysis, and pathological examination were conducted at termination. Plasma concentrations of probenecid were measured on Days 7 and 14. No changes were noted in animals given 5 mg/kg/hr. At 10 mg/kg/hr, changes were limited to decreases in circulating levels of thyroxin (T_4) (50%-65% less than concurrent controls). At 20 mg/kg/hr, a higher incidence of clinical signs was noted, consisting of chromodacryorrhea, hypoactivity, reduced or absent feces, and decreased food consumption and body weight gain. One female and 1 male were euthanatized in moribund condition on Days 4 and 11, respectively. Pathological changes noted at 20 mg/kg/hr included increased absolute and relative liver weights (17%-21%), and mild thyroid follicular cell hypertrophy in males. T_4 plasma concentrations decreased 70% in males and 49% in females, while triiodothyronine (T_3) decreased 18% in males. Plasma Probenecid concentrations increased more than dose-proportionally between 5 and 20 mg/kg/hr, and between Day 7 and Day 14. Concentrations at 5 mg/kg/hr were below the limit of quantification (10 μ g/mL), while at 10 mg/kg/hr they were within the intended therapeutic range of 30-150 μ g/mL. Higher concentrations were achieved at 20 mg/kg/hr (279-658 μ g/mL). These results, and those from concurrent studies conducted in dogs, suggest that thyroid changes induced by probenecid could be associated with disturbances of the hypothalamic-pituitary-thyroid axis consequent to increased hepatic clearance of T_4.

1507 TERFENADINE EVOKED PROLONGATION OF THE QTc INTERVAL IN ISOLATED PORCINE PURKINJE FIBRES - AN EXPERIMENTAL MODEL FOR THE ASSESSMENT OF ARRHYTHMOGENIC POTENTIAL IN MAN.

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Since the discovery that terfenadine, the non-sedative H_1-antihistamine produced a prolongation of the QTc interval, which can lead to the development of Torsades de Pointes in man , there has been a need for the development of an experimental model capable of predicting the arrhythmogenic effect of new non-cardiovascular medicinal products in man. The aim of this study was to investigate the electrophysiological effects of terfenadine using conventional microelectrode techniques in porcine cardiac Purkinje fibres. Each preparation was stimulated at a supramaximal voltage with a cycle length of 1000 ms and 1 ms duration. Increasing concentrations of terfenadine (10^{-6} M to 10^{-5} M) were administered to each preparation and, following a 30 minute incubation period, the maximum velocity of action potential upstroke (Vmax) and action potential duration (APD) were recorded. The results obtained showed that terfenadine (3 x 10^{-6} and 10^{-5} M) dose dependently increased action potential duration ($APD_{90,60,30}$). This reached significance at the highest dose of terfenadine ($p < 0.05$, n=10). Terfenadine had no significant effect on Vmax. In conclusion, these results support the usefulness of porcine cardiac Purkinje fibres in predicting the potential for QTc interval prolongation in man of new non-cardiovascular medicinal products.

1508 TOXICITY AND CARCINOGENICITY OF TACRINE IN B6C3F1 MICE.

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Tacrine (1,2,3,4-tetrahydro-9-acridineamine hydrochloride; Cognex®) is a centrally active reversible acetylcholinesterase inhibitor approved for the treatment of Alzheimer's disease. The chronic toxicity and carcinogenicity of tacrine was characterized in B6C3F1 mice as part of the preclinical safety assessment. Tacrine was administered in diet at 100, 200, 300 or 400 mg/kg for 13-weeks and 50, 100, or 200 mg/kg for 104 weeks to groups of up to 60 male and 60 female mice. Controls received normal diet. Dosing at 400 mg/kg tacrine was terminated after 1 week due to severity of the clinical signs and at 300 mg/kg body weight loss was dose-limiting for the carcinogenicity study. In the 13-week study, body weight gain suppression occurred at all doses. Microscopic evaluation revealed minimal hepatocellular hypertrophy at all doses and single cell necrosis of centrilobular hepatocytes in males at 200 mg/kg and both sexes at 300 mg/kg. In the carcinogenicity study, tacrine reduced body weight gain at 100 and 200 mg/kg and increased mortality at 200 mg/kg. Survival did, however, exceed 50% in all groups at 104 weeks. Liver was a target organ both for tacrine-induced neoplastic and non-neoplastic changes. There was an increased incidence of foci of hepatocellular alteration and nuclear atypia at all doses. Incidence of single cell necrosis was also increased males at 50 and 200 mg/kg. Tumor analysis showed an increased incidence of hepatocellular adenoma and carcinoma at 100 and 200 mg/kg at which plasma concentrations exceeded 100 ng/mL or 3 times human therapeutic levels. Carcinoma was diagnosed in 5, 7, 14, and 20 males and 3, 2, 16, and 41 females in controls and at 50, 100 and 200 mg/kg, respectively. Adenoma occurred in 8, 5, 19 and 25 males and 4, 9, 18, and 45 females in controls and at 50, 100, and 200 mg/kg, respectively. The mechanism of tumor induction is unknown but may be related to the minimal hepatocellular cytotoxicity and/or genotoxicity in the highly susceptible B6C3F1 strain of mice.

1509 PANCREATIC ACINAR CELL TUMORS IN WISTAR RATS TREATED WITH TACRINE, A COGNITION ACTIVATOR FOR 104 WEEKS.

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Tacrine (1,2,3,4-tetrahydro-9-acridinamine, hydrochloride; Cognex®), a cognition activator approved for the treatment of Alzheimer disease, was evaluated in a 2-year carcinogenicity study in Wistar rats. Three groups of 60 Wistar albino rats per sex received tacrine in the diet at daily doses of 5, 10, and 15 mg/kg for 104 weeks. An additional group of 60 rats per sex received no drug and served as controls. Survival was adequate in all groups for evaluation of tumorigenicity. Body weight gain during the study was suppressed by 15% and 19% in males, and by 18% and 29% in females at 10 and 15 mg/kg, respectively, relative to controls. In general, morbidity and mortality were attributed to a spectrum of pathologic alterations that were unrelated to compound administration and were considered common age-related changes. Absolute pancreatic weights were increased in both sexes at the high dose. A statistically significant increased incidence of exocrine pancreatic (acinar cell) tumors was identified in treated males and females. These tumors in treated rats were observed within an unusually high background incidence of pancreatic exocrine tumors in concurrent controls. The incidence of pancreatic acinar cell adenoma was 31, 33, 35, and 43 for males and 1, 0, 7, and 6 for females in controls and at 5, 10, and 15 mg/kg, respectively. The incidence of pancreatic acinar cell carcinoma was 10, 9, 12, and 18 for males and 0, 2, 2, and 4 for females in controls and at 5, 10, and 15 mg/kg, respectively. There were no treatment-related increases in other tumour types. Biologically, malignant pancreatic exocrine tumors in the rat were considered of low grade because they were not invasive, rarely metastasized, were of late onset, and did not compromise survival. Further, tacrine-induced pancreatic exocrine neoplasia in the rat bears little histologic or biologic resemblance to pancreatic cancer in man which is primarily of ductal rather than acinar cell origin.

1510 SINGLE AND REPEAT DOSE DISPOSITION OF AG2034 IN LOW FOLATE MICE.

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The toxicity of GARFT inhibitors is often cumulative, and significantly enhanced under low folate conditions in mice. These effects may occur as a result of cellular accumulation of the inhibitors following polyglutamation. Studies were therefore initiated to determine the accumulation of the GARFT inhibitor, AG2034, in female C3H mice. Animals fed either a standard diet or a folate deficient diet for two weeks prior to a single intravenous administration of ^{14}C-AG2034 (20 mg/kg) were evaluated for drug elimination and tissue distribution. Elimination of ^{14}C-AG2034 at 24 hr was similar between standard fed (45% urine:25% feces) and low folate fed (38.7% urine:29.3% feces) mice. Tissue (liver, kidney, spleen and small intestine) radioactivity was determined over a 7 day period. Liver, kidney and intestine exhibited the greatest amount of radioactivity. In low folate animals, the tissue levels were 2-6 fold higher than the amount detected in standard fed animals. Seven days after treatment, 5.85 nmol Eq/g and 5.03 nmol Eq/g were detected in liver and kidney extracts from low folate animals while in standard fed animals levels were undetectable (<1.0 nmol Eq/g). To determine if accumulation would occur with repeat dosing, animals were treated with two intravenous doses of ^{14}C-AG2034 (5 mg/kg), seven days apart. Following the second dose, the amount of radioactivity was determined in liver, kidney and small intestine. The amount of total radiolabel observed in these tissues was similar to the single dose study. The data suggest under these conditions the tissues sequester a saturable level of intracellular drug and repeat dosing would only offer to sustain such levels. Therefore, in this animal model, while folate status appeared to play a significant role in AG2034 tissue sequestration, there was no evidence of tissue accumulation with repeat dosing.

1511 EFFECT OF BENZOYL PEROXIDE ON SOLAR-STIMULATED ULTRAVIOLET RADIATION-INDUCED SKIN TUMOR FORMATION IN SKH1Hhhh (hr/hr) ALBINO HAIRLESS MICE.

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A photocarcinogenesis study was conducted with benzoyl peroxide at the request of the U.S. Food & Drug Administration. The study objective was to determine whether repeated, topical application of benzoyl peroxide suspended in carbopol gel enhanced solar-simulated radiation (SSR)-induced skin tumor formation in SKH1 (hr/hr) albino hairless mice. In this 12-month study, two groups of mice that received either 600 or 1200 RBU of SSR alone served as internal response calibrators for the study. Mice received benzoyl peroxide carbopol gel [0 (vehicle), 1, 15, or 50 mg/ml] applied to their back and sides before SSR (600 RBU) on Monday, Wednesday, and Friday, and after SSR on Tuesday and Thursday for 40 weeks, followed by a 12-week observation period with no treatment. The SSR received by all mice in the study was a tumorigenic dose. The time for tumors to appear (i.e., tumor latency) and the number of tumors per mouse (i.e., tumor yield) were determined at weekly intervals throughout the study. The benzoyl peroxide carbopol gels had no statistically significant dose-dependent effect on the appearance of SSR-induced skin tumor latency or yield in this study. Isolated values (i.e., sex-specific or treatment differences) that were statistically significant in multiple pairwise comparisons were small, not dose-dependent, and approximated the smallest change detectable in this test system. This study provides no clear evidence for enhancement by benzoyl peroxide carbopol gel of SSR-induced skin tumor formation in albino SKH1 (hr/hr) hairless mice. Thus, under the conditions of this rodent photocarcinogenicity study, benzoyl peroxide in a carbopol gel vehicle is not a photocarcinogenicity risk factor.

1512 PRECLINICAL SAFETY ASSESSMENT OF AG2034, AN INHIBITOR OF GLYCINAMIDE RIBONUCLEOTIDE FORMYLTRANSFERASE (GARFT), IN MICE AND DOGS.

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In support of the clinical evaluation of AG2034 for antitumor effects in humans, 5-day repeat dose intravenous (iv) toxicity studies were conducted in CD-1 mice and beagle dogs. Mice remained on study for 23 days follow-

ing the last dose and dogs remained on study for either one or 23 days after the last dose. In mice administered 1, 3, 10 or 30 mg/kg/day, deaths were observed between 8 and 11 days after the start of dosing at 30 mg/kg/day (5/10 males; 7/10 females) and 10 mg/kg/day (2/10 females). Moribund mice exhibited body weight loss, decreased food consumption, enteropathy of the small intestine, lymph node depletion, myeloid cell regeneration in the bone marrow. The NOEL in mice was 3 mg/kg/day. In dogs administered 0.05, 0.2 or 0.8 mg/kg/day, deaths were observed between 7 and 10 days after the start of dosing at 0.8 mg/kg/day (¾ males; ¾ females). Moribund animals exhibited general signs of deteriorating conditions along with emesis and unformed/watery stools, decrease in body weight and food consumption. Elevated RBC parameters, small and large intestine enteropathy (reversible after dosing cessation), decreased bone marrow cell and thymic lymphoid cell density and a decrease in peripheral lymphocytes were observed in the 0.8 mg/kg/day group. The NOEL was 0.2 mg/kg/day in dogs. Mean C_0 dog plasma levels of AG2034 on the first day of dosing were 0.046, 0.314 and 1.66 μg/mL, for 0.05, 0.2 and 0.8 mg/kg/day, respectively. The mean areas under the curve for 24 hours after dosing (AUC_{0-24}) were 0.180, 0.742 and 4.89 μg/mL-hr on the first day of dosing for 0.05, 0.2 and 0.8 mg/kg/day, respectively. The mean C0 and AUC values on day 5 were similar at each dose level in males and females.

1513 TOXICITY OF THE ANTIFOLATE, AG2034, IN LOW FOLATE MICE: EFFECT OF FOLINIC ACID RESCUE.

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AG2034 is a potent inhibitor of glycinamide ribonucleotide formyltransferase (GARFT) that exhibits antitumor activity in several mouse models. Previous studies with other GARFT inhibitors have shown that rodents are relatively insensitive to the toxicological effects of these antifolates primarily due to high folate levels. Studies were therefore initiated to evaluate the toxicity of AG2034 in animals maintained on a folate deficient diet. Female C3H mice were fed a folate deficient diet for two weeks prior to treatment with AG2034. The estimated LD_{50} in low folate animals following a single intravenous dose was approximately 20 mg/kg; 35-fold lower than the LD_{50} estimated in standard fed mice. In low folate animals the toxicity was delayed, with morbidity occurring 7 to 10 days after dosing. Clinical signs included diarrhea, decreased activity and significant loss of body weight. Hematological changes included significant decreases in hemoglobin, hematocrit, RBCs, and lymphocytes. There was a significant increase in spleen and liver weight. Histopathological evaluation of the intestine revealed dose-dependent enteropathy characterized by microvilli necrosis. In an effort to evaluate the potential rescue from these dose-limiting effects in low folate animals, a single dose of folinic acid (leucovorin) was administered intravenously three, six, or nine days after treatment with 20 mg/kg AG2034. Folinic acid reversed many of the toxicological effects induced by AG2034 with complete attenuation observed in animals treated three days after AG2034. At higher doses of AG2034 given alone (40 and 80 mg/kg), significant lethality occurred (4/6 and 5/6 respectively); however, in animals receiving folinic acid 3 days following AG2034, no deaths occurred in the 40 mg/kg group and only one animal treated at 80 mg/kg died. These data demonstrate a link between folate status and acute toxicity of AG2034 in female C3H mice, and indicate that intravenous folinic acid can attenuated AG2034-related adverse effects in low folate animals.

1514 MONITORING CELLULAR TOXICITY WITH AN INDUCED CALCIUM LOAD.

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An elevated cytosolic Ca^{2+} (Ca_i) concentration is frequently observed in cells exposed to toxic compounds. Healthy cells maintain the 10,000-fold Ca^{2+} gradient across the plasma membrane, while dying cells cannot. However, after some toxic treatments, severely compromised cells apparently can maintain their Ca_i near normal levels. In the present study we compared the effects of cisplatin and its relatively inactive isomer transplatin on Ca_i (as determined by fluo-3 fluorescence) in HepG2 cells. Published reports suggest an initial decrease in Ca_i after treatment with cisplatin (possibly due to release of free Pl^{2+} ions), followed within days by an increase in Ca_i, followed by cell death. We observed similar increases in Ca_i with higher concentrations of cisplatin (>1 μg/ml), but calibrating Ca_i in these experiments presumes similar loading of fluorescent dyes in healthy and damaged cells. A more sensitive indication of cell damage is observed by inducing a Ca_i load (e.g. by adding Ca^{2+} ionophores or dilute detergent). The cisplatin-damaged cells showed larger and/or more prolonged responses to this Ca^{2+} load than transplatin-treated or control cells. Conversely, one might expect healthy cells to respond better to phospholipase C-linked agonists which require intact phosphatidylinositol pools that may be depleted in damaged cells. As yet, we have not identified a suitable PLC-linked agonist for HepG2 cells to test this hypothesis. Monitoring the ability of cells to handle an induced Ca_i load may be useful for evaluating cellular toxicity during screening for new pharmaceutical lead compounds.

1515 A SUBCHRONIC (3-MONTH) TOXICITY STUDY OF ULTRANOX® XR-2677 IN THE RAT VIA ORAL GAVAGE ADMINISTRATION.

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The objective of this study was to assess the potential subchronic toxicity of Ultranox® XR-2677 (CAS#161717-32-4), an organophosphite-based antioxidant/stabilizer used in the manufacture of polymers, when administered for 3 months via oral gavage to Sprague-Dawley CD® rats (20/sex/group) at doses of 0, 3, 12, 20, 30, 60, 90, or 270 mg/kg/day. Physical observations, body weight, and food consumption were examined on all animals at selected intervals during the treatment period. At study termination, hematology and clinical chemistry were performed on 10 animals/sex/group. All survivors were sacrificed, selected organs were weighed, and histopathological evaluation of tissues were conducted. Results revealed no treatment-related mortality, abnormal clinical signs of toxicity, or mean body weight changes in any treated group. There were no toxicologically significant changes in any clinical chemistry parameter at any dose. Hematology results showed dose-related increases in clotting parameters, prothrombin time (PT); and activated partial thromboplastin time (APPT) at doses of 30 mg/kg/day and higher. Following necropsy, there were statistically significant increases in absolute liver weight and liver/body weight ratios at doses of 12 mg/kg/day and higher. These increases correlated with microscopic evidence of centrilobular hypertrophy and were considered the result of a metabolic adaptation to the test material and not toxicologically significant. No other organ weight changes or histopathological findings were observed, with the exception of a statistically significant increase in absolute kidney weight at 90 and 270 mg/kg/day and edema and slight thickening of the forestomach mucosa in the high dose group only. In conclusion, based on the conditions of this study, a no observed adverse effect level (NOAEL) for Ultranox® XR-2677 when administered to rats via oral gavage for at least 3 months is 20 mg/kg/day.

1516 POLYCYSTIC KIDNEY DISEASE INDUCED IN F_1 SPRAGUE-DAWLEY RATS FED *PARA*-NONYLPHENOL IN A SOY-FREE CASEIN-CONTAINING DIET.

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para-Nonylphenol (NP; CAS # 84852-15-3), an alkylphenol with a 9 carbon olefin side chain, is widely used in the manufacture of nonionic surfactants, lubricant additives, polymer stabilizers, and antioxidants. NP has estrogenic activity. The NTP elected to assess its effects on reproduction in multigenerational studies due to its wide commercial use and putative endocrine effects in humans and wildlife. To avoid known estrogenic activity of isoflavones in soy, a soy-free casein-containing diet was used in a range finding study to determine the doses of NP to be further tested. NP was fed in the diet at 0, 5, 25, 200, 500, 1000 and 2000 ppm to F_0 dams beginning on gestation day 7. Exposure of the F_1 pups continued via the dams milk until postnatal day (PND) 21 and then by feed until sacrifice on PND 50 (N =15, all groups). Terminal body weights of both males and females in the 2000 ppm dose group were approximately 25% lower than the respective control body weights. Severe polycystic kidney disease (PKD) was present in 100% of the 2000 ppm-exposed male and female rats. At 1000 ppm, 100% of males and 93% of females had minimal to moderate PKD versus only minimal cysts in 47% male and 67% female controls. Incidence and severity in other dose groups were like controls. Previous studies of comparable duration to ours also administering 2000 ppm NP, but in soy-containing diets, reported either no or only mild PKD. We conclude that the renal toxicity of NP is highly dependent on the diet on which the animals are maintained.

1517 COMPARISON OF FIVE COGNITIVE TESTS USED IN RODENT DEVELOPMENTAL NEUROTOXICITY STUDIES AFTER PERINATAL PHENYTOIN AND METHIMAZOLE.

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Tests of learning and memory are important in the assessment of potential developmental neurotoxicity for chemicals regulated by the USEPA and for the safety evaluation of new pharmaceuticals. Two critical issues for selection of tests for use in these studies are sensitivity and efficiency. There are few published studies comparing the sensitivity of the tests that are commonly used after perinatal exposures. The present study was designed to compare the sensitivity of 3 water mazes and 2 passive avoidance procedures using offspring of Sprague-Dawley dams (15/group) given sodium phenytoin in corn oil by gavage at 100 mg/kg on gestation day (GD) 6 through lactation day (LD) 21, methimazole in the drinking water at 0.1 mg/ml on GD17-LD10 or a vehicle control (corn oil) by gavage on GD6-LD21. When the offspring were young adults, they were assigned to one of five procedures: spatial M-maze (SM) - the escape ladder was always on the non-preferred side and the criterion was 5 consecutive errorless trials; visually-cued M-maze (VM) - 20 trials/day for 4 days with a light cue on the correct escape side; Cincinnati water maze (CWM) - 2 trials/day for 3 days in the forward path and 2 trials/day for 3 days in the reverse path; passive avoidance - with either one learning trial (OT) with shock or as many learning trials with shock as necessary (TTC) for the rat to avoid for 3 min. Both passive avoidance procedures had one retention trial one day later. An overall ranking of the sensitivities of the 3 tests that detected drug effects was: CWM>SM>OT. VM and TTC did not detect any deficits. The efficiencies of these procedures will be compared in terms of required resources, automation and data analysis.

1518 NINE- AND NINETY-DAY INHALATION TOXICITY STUDIES OF 4-VINYLCYCLO-HEXENE-1,2-EPOXIDE (VCMX).

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VCMX is a primary metabolite of 4-vinylcyclohexene (VCH) but also has commercial value as a reactive diluent in UV curable coatings. VCH is an ovarian toxicant in mice but not rats via the active metabolite, VCH-diepoxide (VCD). VCMX is metabolized to VCD *in vitro* but competing detoxification pathways also exist (e.g. to VCH-1,2-diol). To determine potential toxicity of VCMX, 9- and 90-day vapor inhalation studies were conducted in CD-1® mice and Sprague Dawley rats. In the 9-day study, animals (5/sex) were exposed (whole-body) to target vapor concentrations of 0, 40, 200 or 400 ppm 6 hr/d for 9 d over 2 wk. The 400 ppm level was lethal to mice but not rats and only minimal exposure-related effects were observed at non-lethal exposure levels. A 9-day NOAEL of 200 ppm was established. No ovotoxicity was observed in either species although, based on published data, absorption of VCMX with metabolism to VCD could have resulted in follicular damage/loss. For the 90-day study, animals (10/sex) were exposed to target vapor concentrations of 0, 20, 80, 200 (mice) or 400 (rats) ppm 6 hr/d, 5 d/wk for 13 wk with 4 wk recovery groups (10/sex for high exposure levels and control). Measurements included body weight, food consumption, ophthalmology, clinical pathology, urinalysis, organ weights, gross and histopathology. Except for histopathological effects in the nasal epithelium, exposure-related findings were restricted to the high exposure groups and included: Rats - minimal signs of irritation and reduced body weight in males with recovery observed; Mice - death of 3 females, minimal signs of toxicity, reduced body weight in males, decreased ovary weights, and depletion of ovarian antral follicles with recovery limited to body weight. Concentration-related irritant changes in nasal epithelium were observed in some animals from all VCMX exposed groups without complete recovery in the high concentration group. Ovotoxicity occurred in mice, but not rats, only after long-term exposure (200 ppm) that was irritant and exceeded the maximum tolerated concentration based on mortality.

1519 EVALUATION OF THE ORAL SUBCHRONIC TOXICITY OF AHTN AND HHCB IN THE RAT.

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7-Acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene (AHTN) and 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-gamma-2-benzopyran (HHCB) are the two of the polycyclic musks commonly used in fragrances. AHTN and HHCB are widely used in a variety of fragrances that are in consumer products. Because of the widespread exposure to these materials, 90-day oral feeding studies, with 4-week recovery periods for selected rats, were conducted. The materials were added to the diet at levels calculated to result in daily doses of 1.5, 5, 15 or 50 mg/kg for AHTN and 5, 15, 50 or 150 mg/kg for HHCB. On completion of the treatment period, 3 males and 3 females from each of the high dose groups and controls were maintained for a treatment free period of 4 weeks. For AHTN, a slight reduction in red cell count, hemoglobin concentration and packed cell volume was seen in the high dose group. Also a reduction of plasma cholesterol, a higher A/G ratio, a slight reduction in total protein and a lower plasma glucose concentration was seen at the two higher doses. A dose dependent increase in liver weights with no associated histopathology was seen at the two higher doses in females only. A green coloration was observed in the livers, mesenteric lymph nodes and lachrymal glands at the high dose in males and females and in the lachrymal glands of 4 females given 15 mg/kg and in 1 female given 5 mg/kg. At the end of the treatment free period, the coloring was largely reversed but one male showed green colored lymph nodes and 2 females showed colored lachrymal glands. Microscopic examination of unstained sections of frozen livers under UV illumination did not reveal any florescence that might have been consistent with porphyrin accumulation. These findings were clearly related to administration of AHTN but were not accompanied by any histopathological changes. Additional research on the significance of the green discoloration is continuing. Based on these findings, a NOAEL of 15 mg/kg/day was concluded. For HHCB, there were no significant findings at any dose level; a NOAEL of 150 mg/kg/day was concluded.

1520 ONCE-DAILY DOSING DECREASES TOXICITY OF DAPTOMYCIN.

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Daptomycin is a novel lipopeptide antibiotic with potent bactericidal activity against gram-positive bacteria, including resistant strains. Skeletal muscle has been identified as the target organ of daptomycin toxicity. To investigate the potential effects of dose fractionation on toxicity, two studies were conducted in dogs comparing the effects of repeated intravenous administration once daily (q24h) versus every 8 hours (q8h). Muscle toxicity did not appear to be directly related to Cmax, since both serum CPK increases and the incidence of myopathy observed at 25 mg/kg q8h were greater than at 75 mg/kg q24h despite the lower Cmax. A 3 to 4- fold increase in peak CPK levels was observed when the dose interval was varied from q24h to q8h at a dose of either 5 mg/kg or 25 mg/kg. In contrast, no difference in CPK elevations was apparent when the dosing interval was held constant at 24 hours and the dose was varied from 25 to 75 mg/kg. Further, 5 mg/kg administered q24h caused no change in CPK, whereas a dose regimen of q8h was associated with a 3-fold increase in CPK at a comparable Cmax. Similarly, toxicity was not related primarily to AUC_{24h}, since the toxicity observed at 25 mg/kg q8h was greater than at 75 mg/kg q24h, at approximately the same AUC. The data suggest that dosing interval had a greater influence on toxicity than did dose itself. Once daily administration is hypothesized to allow greater time between doses (at non-toxic blood levels) for repair of subclinical muscle damage associated with daptomycin. Further, studies in animal efficacy models have demonstrated that effectiveness of daptomycin is optimized by once-daily dosing since Cmax was found to be the key PK parameter associated with eradication of infection (J Legget *et al.*, ICAAC abstract, 1987). These results suggest that once-daily dosing can minimize daptomycin muscle toxicity, while optimizing its antimicrobial efficacy.

1521 STRUCTURE-BASED ASSESSMENT OF OCULAR IRRITATION.

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There is a continuing need to assess the toxicity of new chemicals for environmental, industrial and consumer exposures. To partially fulfill this need, we have developed a quantitative structure-toxicity relationships (QSTR) model for the assessment of eye irritation. The model is based on 1453 compounds previously tested in the rabbit Draize eye irritation assay. The model permits the assessment of eye irritation potential for untested organic compounds. Chemicals in the model are parametrized by powerful electropolog-

ical state (E-State) descriptors, as contrasted to presence/absence of fragments. The model is composed of 5 chemical class-specific submodels. Each submodel, in turn, consists of 3 equations: given a query, the first determines whether the compound is (non-irritating or mild) vs. (moderate or severe). If it is in the first group, then the second equation can determine whether it is nonirritating or mild. If the compound falls into the second group, the third equation determines whether the irritation grade is moderate or severe. The equation-specific sensitivity of the 15 equations ranges between 80% and 100%, with the majority falling between 88% and 93%. Specificity ranges between 74% and 100%, with the bulk of the equations in the range of 86% to 94%.These equations are installed in the *TOPKAT* program, a system for toxicity assessment of organic compounds. *TOPKAT* provides for automatic check of the coverage of the query structure by the model, as well as determination whether the query falls within the Optimum Prediction Space (OPS) of the model, i.e., whether the model is applicable to the query compound.

1522 HISTOPATHOLOGICAL BACKGROUND LESIONS IN GÖTTINGEN MINIPIGS.

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SPF Göttingen minipigs have been introduced in our laboratory four years ago as a non-rodent model for testing subchronic and chronic toxicities. The choice was based on the expectation that the similar physiology between pigs and humans would allow improved predictions in terms of safety assessment. A knowledge of the spontaneously occurring histopathological lesions is consequently essential for interpreting the drug-dependent lesions. Background lesions were evaluated in 50 male and in 50 female control minipigs from 2 to 4 weeks toxicity studies. At the end of the studies, minipigs were 4 to 8 months old and the mean body weight was around 10 kg. The full set of organs was examined. Background histopathological changes were mainly observed in adrenals, bone marrow, brain, epididymides, gall bladder, heart, kidneys, liver, lung, muscle, prostate, skin, testes, urinary bladder and vessels. The majority of these findings consisted in focal lymphoid/inflammatory cell infiltrates or small hematopoietic foci in various organs. Other findings, including lung granulomas, single vessel fibrinoid arteritis and/or periarteritis, testes tubular atrophy and bone marrow atrophy were observed in some animals. In addition, muscle myonecrosis and regeneration was described as a new finding for this strain of minipigs.

1523 MINIPIGS IN SAFETY ASSESSMENT OF A PHOSPHODIESTERASE TYPE IV INHIBITOR FOR THE TREATMENT OF INFLAMMATORY SKIN DISEASES.

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Minipigs have been considered to be a good model for dermal permeability and toxicity studies, due to their broad biological similarities to humans. Human and porcine skin are similar with regard to thickness and general morphology, epidermal cell turnover, orientation and distribution of vessels, and immunological reactivity. Therefore, PDI747, a selective type IV phosphodiesterase inhibitor with anti-inflammatory properties developed for the treatment of inflammatory skin diseases, was tested topically in Göttingen minipigs. After 2 weeks of dermal treatment, segmental vasculitis in small and medium sized vessels of the kidneys, stomach, small intestine and mesenteric lymph nodes was observed in animals of both sexes at 40 mg/kg/day with a mean $AUC_{(0\text{-}20h)}$ of approximately 10 ng.h/ml. Such lesions were not observed in minipigs after long term dermal treatment of PDI747 with similar AUC's. No cutaneous vascular lesions were observed at the application site. Finally, for the global safety assessment of PDI747, the vasculitis was of major importance taking into account that this lesion was also found in four other species (rat, dog, monkey and rabbit) and by three other routes of administration (oral, iv and inhalation).

1524 PRECLINICAL CONSIDERATIONS FOR THE DEVELOPMENT OF INTRAVITREAL DRUG PRODUCTS.

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The treatment of vitreal or retinal conditions may, in some cases, require intravitreal (ITV) administration of drug products. The pharmacokinetics, including bioavailability and ocular distribution, of ITV drugs should be defined early in the development process to assist in the design of nonclinical toxicology studies. ITV drugs should be evaluated in general toxicology, genetic toxicology, reproductive toxicology, and, if applicable, carcinogenicity studies. To fully define the toxicity profile of ITV drugs, general toxicology studies should be conducted using ITV and systemic (e.g., oral) routes. In each case, systemic toxicity should be assessed using standard measures (e.g., body weight, clinical pathology, histopathology). Toxicity at the ITV injection site should be assessed in 2 species, at least one with pigmented eyes. To support human trials during the investigational phase of drug development, the number of injections used in the nonclinical studies should at least equal the maximum number of human injections. For approval, the frequency/duration of treatment of animals should exceed the intended clinical use; however, given the intermittent nature of clinical treatment, the maximum frequency/duration of nonclinical studies will likely need to be decided on a case-by-case basis. ITV studies should be conducted with the clinical formulation and a formulation in which the concentration of drug substance exceeds that in the clinical formulation. The assessment of ocular toxicity following ITV injection should include slit lamp biomicroscopy, funduscopy, tonometry, electroretinography, and histopathology at appropriate times. Genotoxicity and *reproductive toxicity* should be assessed according to the ICH (International Conference on Harmonisation) Guidelines. However, pre- and postnatal development (Segment 3) and carcinogenicity studies may be waived unless there is significant systemic exposure or a cause for concern.

1525 EFFECT OF SINGLE SUBCUTANEOUS DOSES OF EXENDIN-4, AN ISOLATE FROM GILA MONSTER SALIVA, ON-CARDIOVASCULAR PERFORMANCE IN CYNOMOLGUS MONKEYS.

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Exendin-4 (CAS No. 141732-76-5) is a 39 amino acid peptide isolated from the saliva of *Heloderma suspectum, a* lizard commonly known as the Gila Monster. Exendin-4 is similar (53% amino acid identity) to mammalian glucagon-like peptide-1 (GLP-1) and lowers elevated plasma glucose (ED_{50} <0.1 μg/kg) in *db/db* mice. The purpose of the work reported here was to define the potential cardiovascular effects of chemically synthesized exendin-4 (AC2993) when administered subcutaneously (SC) to monkeys. Animals were surgically instrumented with radiotelemetry transmitters in the peritoneal cavity to measure blood pressure (systolic, diastolic and mean arterial), heart rate,-ECG (lead II configuration), body temperature and physical activity in freely moving conscious animals. Each of the 3 animals in the study received placebo on Day 1-followed by 30 μg AC2993/kg on Day 2; placebo on Day 3,4 and then 300 μg AC2993/kg on Day 5; placebo on Day 6,7, and then 1000 μg/kg AC2993 on Day 8. When compared to the results following the placebo dose given the day before the AC2993 dose, AC2993 at 30, 300 or 1000 μg/kg caused no changes in ECGs, arterial blood pressure and heart rate. At all dosage levels of AC2993, but in an apparent dose-level independent manner, depressions in spontaneous activity and body temperature were found. Activity depression reached a low plateau ~ 1 hr after dose administration and remained depressed for the next 3 hours when it returned to a level similar to that observed following placebo administration. The maximum temperature depression was ~0.6°C and occurred ~1 hr after dosing. The body temperature returned to pre-dose values ~4 hr after dose administration. AC2993 had no detectable effect on cardiovascular performance in the monkey at SC doses up to 1000 μg/kg.

1526 SUBCHRONIC TOXICITY AND TOICOKINETICS IN THE SPRAGUE-DAWLEY RAT OF EXENDIN-4, AND ISOLATE FROM GILA SALIVA.

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1527 SUBCHRONIC TOXICITY AND TOXICOKINETICS IN THE CYNOMOLGUS MONKEY OF EXENDIN-4, AN ISOLATE FROM GILA MONSTER SALIVA.

R A Hiles[1], K S Prickett[1] J W Noveroske[2] and L J Duggan[2]. [1]*Amylin Pharmaceuticals, Inc., San Diego, CA, USA;* [2]*Oread, Inc. Farmington, CT, USA.*

Exendin-4 (CAS No. 141732-76-5) is a 39 amino acid peptide isolated from the saliva of *Heloderma suspectum, a* lizard commonly known as the Gila Monster. Exendin-4 is similar (53% amino acid identity) to mammalian glucagon-like peptide-1 (GLP-1) and lowers elevated plasma glucose (ED_{50} <0.1 μg/kg) in *db/db* mice. The purpose of the work reported here was to define the potential toxicological properties of chemically synthesized exendin-4 (AC2993) when administered subcutaneously (SC) to Cynomolgus monkeys in 28 consecutive daily doses. Preliminary studies had shown that it could be given as a single SC dose of 5000 μg/kg without deaths. In the 28-day study, doses of 0, 10, 100 and 1000 μg/kg/d were used and each dose group consisted of 3 animals/sex. No deaths due to AC2993 occurred. Decreased food consumption and loss of body weight were observed in a dose-related manner at 100 and 1000 μg/kg/d. ECGs were normal for all animals. There were no AC2993-related changes in hematology and clinical chemistry values. There were no microscopic tissue changes in necropsy samples taken the day after the last dose. The concentration of AC2993 was determined in serial plasma samples collected after the first dose and the 27th dose using an ELISA method with a lower limit of quantitation of 63 pg/mL. The systemic load of AC2993 increased with dose level (based on both C_{max} and AUC_{0-12}), but did not increase with number of doses (no changes between day 1 and day 27). The plateau near the C_{max} concentration was more extended with time as the dosage level increased. There was no indication of significant antibody formation. It was concluded that, based on the lack of pathological changes, 1000 μg/kg administered SC for 28 days does not exceed the maximum tolerated dose.

1528 SAFETY ASSESSMENT OF COSMETIC INGREDIENTS AND COLOR ADDITIVES: EVALUATION OF A QUANTITATIVE STRUCTURE-TOXICITY RELATIONSHIP APPROACH FOR PREDICTING TOXICITY.

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Cosmetic products and their constituent raw materials (except color additives) are not required to pass any FDA premarket approval process. Cosmetic ingredients and products, however, are expected to undergo adequate safety sub-stantiation prior to being offered for retail sale. Predictive quantitative structure-activity relationship (QSAR) toxicology prediction software is increasingly being considered by regulatory toxicologists as a supplemental tool for making an informed safety decision. We initiated a study designed to evaluate six of the toxicology endpoint prediction modules available in TOPKAT® (Health Designs, Inc. (HD_i), Rochester NY). For each module chemical evaluation set, we chose known toxicity data that met specific methodology testing criteria for TOPKAT. These chemicals must not have already been included in TOPKAT training sets. The rat oral LD50 module test resulted in a concordance of 90%. Developmental toxicity potential module predictions resulted in a specificity and sensitivity of 88 and 60%, respectively. The mutagenicity module predictions gave a specificity and sensitivity of 81 and 71%, respectively. Skin and eye irritation predictions resulted in specificities of 95 and 100%, respectively, and sensitivities of 22 and 78%, respectively. Not enough data could be identified (of sufficient quality and not already used by TOPKAT) to adequately test the carcinogenicity modules. This QSAR approach may serve as a valuable tool to aid in the safety evaluation of cosmetic ingredients and color additives.

1529 A NOVEL RISK ASSESSMENT/RISK MANAGEMENT OF CHILD EXPOSURE TO FIPRONIL GEL.

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Sponsor: A K Reddy.

Hydramethylnon (HM) is an amidinohydrazone insecticide widely used by consumers and pest control operators (PCO) for the control of ants and cockroaches. Animal studies in several species have shown HM is a male reproductive toxicant. However, the mechanism as well as the exposure timeframe required to elicit adverse reproductive effects have not been well elucidated. Because of its widespread use of consumers to this insecticide, there is a concern about accidental dermal exposure to children and any possible testicular effects. To assess the potential hazard in household insecticides, a study was conducted to determine the effects of an acute exposure to young male rats. Since the dermal penetration rate of HM is relatively low (<1%), the material was administered by subcutaneous (sc) injection to assure that a higher systemic dose was achieved. Young male rats were given single sc injections of HM in an oil carrier at 100, 500, or 1,000 mg/kg of body weight. Daily clinical observations were conducted for 14 days. Clinical effects were limited to decreased body weight gain and decreased food consumption at 500 and 1,000 mg/kg. Animals were sacrificed 14 days post-exposure. Both gross and histopathological examinations revealed no apparent lesions in testes, epididymes or seminal vesicles at any dose. Relative testes to body weight ratios showed statistically significant increases only at 1,000 mg/kg dose. The study showed that single exposures even at high doses fail to elicit the testicular effects in young male rats.

1530 EFFECTS OF P-GLYCOPROTEIN (P-GP) INHIBITION WITH LY335979 ON PACLITAXEL PHARMACOKINETICS AND PHARMACODYNAMICS WHEN COADMINISTERED TO BEAGLE DOGS.

L L Truex, J L Zimmermann, J A Buben, and V R Reddy. *Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN, USA.*

LY335979 is a potent in vitro inhibitor (Ki = 59 nM) of P-gp (Dantzig et al. 1996) and is currently in development for treating human cancers demonstrating P-gp mediated drug resistance. LY335979 will be coadministered with various chemotherapeutic agents to enhance their efficacy. In a 1-month toxicity study, LY335979 was administered orally to dogs daily at doses of 2, 10, and 100 mg/kg. No important compound-related changes occurred in this study. The no-observed-adverse-effect level (NOAEL) was 100 mg LY335979/kg/day and plasma concentrations at this dose exceeded 80x the Ki for P-gp inhibition. In a subsequent study, LY335979 was given to dogs orally at doses of 10 or 100 mg/kg/day in combination with a single 4-hour continuous intravenous infusion of 60 mg paclitaxel/m^2, a dose of paclitaxel that produces moderate neutropenia. The pharmacokinetics of paclitaxel were unaffected by administration of LY335979. Decreased neutrophil, platelet, and reticulocyte counts occurred in dogs given paclitaxel alone and paclitaxel with LY335979. However, decreases in these parameters were of slightly greater magnitude when paclitaxel was given in combination with LY335979. Treatment-related lesions associated with paclitaxel administration included minimal enteropathy, slight or moderate hypospermatogenesis,

and myeloid prominence in the bone marrow. There were no histomorphologic changes to suggest an interaction or synergistic toxicity between paclitaxel and LY335979. In summary, LY335979 had no effect on paclitaxel pharmacokinetics. However, decreases in neutrophil, platelet, and reticulocyte counts were greater in dogs given both LY335979 and paclitaxel when compared to dogs given paclitaxel alone. These findings support the hypothesis that the inhibition of P-gp function in normal tissues can enhance the pharmacodynamic effects of an antineoplastic agent.

1531 SAFETY PROFILE OF THALIDOMIDE IN CD-1 MICE AND FISHER 344 RATS AFTER ORAL DOSING OVER 13 WEEKS.

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The safety profile of thalidomide was determined in CD-1 mice and F344 rats (10/sex/dose group) of both sexes after 13 weeks of oral dosing. Doses of 30, 300 and 3000 mg/kg were administered daily by oral gavage. Control animals were given 1% CMC. All animals were necropsied at the end of the study. There was no mortality in either species. In mice, thalidomide had no effect on body weight, food consumption, ophthalmic function or clinical chemistry and hematology. Oral absorption appeared to be saturable after prolonged dosing. A dose-dependent orange-red urine possibly caused by chromogenic urinary metabolites was observed. Hepatic centrilobular hypertrophy was seen in all treated males with the severity increasing with dose. Only the high dose females were affected. Liver weight was increased for the high dose mice of both sexes suggesting enzyme induction. In rats, thalidomide produced lower body weight in both sexes compared to control with a dose-response more evident in males (~ 20% decrease at 3000 mg/kg). Food consumption was also decreased in males. No treatment-related ophthalmic defects were observed. Functional observational battery tests indicated little to no persistent effects on autonomic, muscle tone/equilibrium, sensorimotor and CNS functions. At high dose, forelimb grip strength decreased for males at weeks 4, 8 and 13. Vertical motor activity increased for males at week 4 and both sexes at week 8. Drooping eyelids were observed for some males at high dose. There were some indications of mild anemia and leukopenia in males. Decreases in serum glucose, globulin and potassium and increase in globulin/albumin ratio was seen in some treated groups. A decrease in total and free T4 was more consistent in treated females than males. Gross thymus weight decreased in both sexes with no histological correlates compared to control. No treatment-related axonal degeneration of sural nerve fibers was present. No changes in histopathology were seen in distal lumbar spinal cord suggesting the absence of thalidomide-related peripheral neuropathy. In summary, some species differences in the safety profile of thalidomide were apparent after oral dosing for 13 weeks.

1532 TOXICOLOGY OF PLURONIC® F-68: A 14-DAY INTRAVENOUS TOXICITY STUDY IN HAN WISTAR RATS.

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Pluronic® F-68 (Poloxamer 188, Monolan), a surfactant that may be used to enhance the stability, absorption, and distribution of a chemical substance by micelle formation, is a possible vehicle for newly developing pharmaceuticals. The objective of this study was to determine the toxicological profile for Pluronic® F-68 in the Han Wistar rat after 14 consecutive days of intravenous treatment. Four male and four female Han Wistar rats per group were intravenously administered 0 (vehicle control), 100, 200, 400 or 750mg/kg/day of Pluronic F-68 dissolved in 0.9% sodium chloride using a dose volume of 5mL/kg for 14 consecutive days. Clinical observations, bodyweights, hematology and clinical chemistry measurements, organ weights and macroscopic and microscopic examinations (microscopic and electron microscopic examination at the 100 and 200mg/kg/day doses are currently ongoing) were performed on all animals. Treatment-related findings were noted at all doses and included a reduction in bodyweight gain (750mg/kg/day only), an increase in the lung weights (400 and 750mg/kg/day only) and kidney weights (100 and 200mg/kg/day males only and 400 and 750mg/kg/day both sexes), a dose-related increase in the accumulation of foamy macrophages in lung alveoli (400 and 750mg/kg/day), and vacuolation of the renal proximal tubules (all doses). Based on these data, the no-toxic-effect-dose was not determined and is less than 100mg/kg/day.

1533 COMPARATIVE INHALATION TOXICITY/ NEUROTOXICITY AND REPRODUCTIVE/ DEVELOPMENTAL TOXICITY OF THREE REFINERY STREAMS.

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A series of toxicity studies were performed on the gasoline blending streams, light alkylate naphtha (LAN), light catalytic cracked naphtha (LCCN) and light catalytic reformed naphtha (LCRN). Rats were exposed by inhalation to a distillate fraction of each stream which approximated the composition of the atmosphere to which humans are most likely exposed. Target concentrations were 750, 2500, 7500 ppm and exposure regimes varied with study design. Reproductive/ developmental screens were modifications of OECD Guideline 421. The 13 week subchronic toxicity study included a two week post-treatment recovery period and neurobehavioral tests consisting of motor activity and a functional observational battery, and neuropathology. Compositionally, the distillate fractions varied from 90+% isoparaffins in LAN to 60% olefins in LCCN and 58% isoparaffins and 9% aromatics in LCRN. No reproductive or developmental toxicity and no neurotoxicity were induced by any stream distillate. Systemic effects observed across all studies were increased liver weight at the high dose in both sexes without a microscopic correlate, and increased kidney weight in male rats. Increased kidney weights accompanied by increased hyaline droplet formation and alpha-2 μ-globulin accumulation in renal tubules are indicative of light hydrocarbon nephropathy (LHN), a species and sex specific toxic effect not relevant to toxicity in man. The occurrence of this event for distillates of similar composition but different proportions of specific chemical classes indicates that there are a broad range of capable of inducing LHN among the lower boiling range refinery streams. Thus, the no-observed-adverse-effects level (NOAEL) for neurotoxicity and developmental/reproductive toxicity for all three refinery stream distillates is ~7,500 ppm. Subchronic toxicity NOAEL ~2,500 ppm. (PPSC is Amoco, ARCO, BP Oil, Chevron, Mobil, Texaco and Unocal 76.)

1534 THE EFFECT OF O^6BENZYLGUANINE PRETREATMENT ON BCNU-INDUCED PULMONARY TOXICITY.

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O^6Benzylguanine (O^6BG, NSC-637037) inhibits O^6-alkylguanine-DNA alkyltransferase, a DNA repair protein. The anticancer efficacy of BCNU (Carmustine; NSC-409962), an alkylating agent, was improved with O^6BG pretreatment in preclinical animal models. In addition, O^6BG pretreatment potentiated BCNU-induced myelotoxicity. In the current study, the effect of O^6BG pretreatment on BCNU-induced pulmonary toxicity was explored in male Fischer 344 rats. Rats were given 10 mg/kg/day of O^6BG alone or in combination with 0, 1, 3 or 5 mg/kg/day of BCNU on study days 1, 5 and 9. Administration of O^6BG and BCNU in combination produced more drug-related mortality than either drug alone. Drug-related mortality occurred in 6/10 and 9/10 rats in the 10 + 3 mg/kg/day (O^6BG + BCNU) and 10 + 5 mg/kg/day dose groups compared to 0/10 and 2/10 rats given 5 and 10 mg/kg/day BCNU alone. BCNU alone at doses up to 10 mg/kg/day produced mild myelotoxicity with no evidence of bone marrow lesions. In rats treated with O^6BG/BCNU, dose-related myelosuppression occurred beginning on day 12; at necropsy all the rats had moderate to severe bone marrow cellular depletion. BCNU alone at doses of 10 mg/kg/day produced chronic inflammation of the lung (considered to be an early indicator of pulmonary injury). However, the combination of O^6BG and BCNU did not produce either a higher incidence or greater severity of pulmonary toxicity. Unlike the effect of O^6BG pretreatment on BCNU-induced myelotoxicity, O^6BG pretreatment did not appear to potentiate BCNU-induced pulmonary toxicity in rats. (Contracts N01-CM-37835 and N01-CM-87100.)

1535 THE EFFECTS OF ORALLY ADMINISTERED CHOLESTAGEL AND LOVASTATIN IN BEAGLE DOGS.

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The purpose of this study was to evaluate cholesterol values in beagle dogs administered test articles orally via gelatin capsules once daily for fourteen

days. Five groups of five male dogs each were administered either Lovastatin (L) at 5 mg/kg, Cholestagel (C) at 1000 mg/kg or a combination of L (5mg/kg) and C (100, 300 or 1000 mg/kg). An additional group of five male dogs received empty gelatin capsules and served as the control group. Evaluations were made of mortality, clinical observations, body weights, food consumption, cholesterol and triglycerides. No mortality was observed throughout the study in any dose group. There were no clinical signs of toxicity observed during the course of the study for any dose group as well as no treatment-related effects on body weights, body weight changes or food consumption values. Statistically significantly lower serum cholesterol values were observed for the 1000 mg/kg C dose group and 5 mg/kg L - 1000 mg/kg C dose group on Days 8 and 14 of the study, when compared to the control group. Cholesterol values on days 8 and 14 were all decreased when compared to baseline readings for all treated groups with the largest decreases in the 1000 mg/kg C, 5 mg/kg L - 300 mg/kg C and 5 mg/kg L - 1000 mg/kg C dose groups. All cholesterol values returned to baselines values at the day 23 evaluation and remained at that level until the duration of the study. Statistically significantly lower triglyceride values were also observed in the 1000 mg/kg C dose group (Day 8) and 5 mg/kg L - 1000 mg/kg C dose group (Days 8 & 14), when compared to controls. Based upon the results of this study, C, when administered at 1000 mg/kg or in combination with 5 mg/kg L at doses of 300 and 1000 mg/kg, significantly reduced serum cholesterol in beagle dogs when administered once daily for fourteen days orally via gelatin capsules. Although not as significant a reduction, 5 mg/kg L and 5 mg/kg L 100 mg/kg C reduced cholesterol under the same regimen.

1536 AN ACUTE SAFETY AND TOLERANCE STUDY OF AN HERBAL CONTAINING DIETARY SUPPLEMENT PRODUCT.

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In an effort to understand the potential impact of product misuse or abuse, we conducted a double-blind, placebo-controlled, dose escalation study on an herbal product combination. Nine female and 15 male normal healthy subjects were dosed 1x, 3x and 5x the recommended dosage levels of a product containing: Bilberry, Marigold, Acerola Cherry and Lemon Bioflavonoid extracts and Docosahexanoic acid. Safety evaluations, including physical and ophthalmologic examinations, 12-lead ECGs, vital signs and clinical laboratory evaluations (clinical chemistry [fasting], hematology and complete urinalysis) were performed prior to and at specified time points in the study. Adverse event information was collected throughout the study. The administration of this herbal product combination was well-tolerated and appeared to be safe at the dosages and length of administration studied. Further, none of the safety data were of a nature to preclude continuing exposure of additional individuals to this herbal product combination. This limited clinical evaluation exceeds current practices in the supplement industry. When combined with other risk assessment practices and safety surveillance programs, this type of detailed clinical evaluation provides the necessary verification of a low-safety risk with herbal combination products.

1537 AN EVALUATION OF THE POTENTIAL GENOTOXICITY OF DICHLORVOS (DDVP): FINAL REPORT OF THE EXPERT PANEL.

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An Expert Panel constituted for this purpose undertook a comprehensive evaluation of the potential genotoxicity of DDVP. The Panel of internationally known experts, using a weight-of-the-evidence approach (WOE), concluded: - DDVP should not be regarded as genotoxic *in vivo* due to a number of factors including: a) across six animals species studied (including man), DDVP favors phosphorylation over alkylation by at least 8 orders of magnitude, b) the absence of detectable alkylation of DNA *in vivo*, c) experimental data demonstrating that the metabolism of DDVP eliminates or significantly reduces the expression of genotoxicity, and d) the lack of usefulness of data generated through the use of extremely high doses and/or the IP injection route where anticipated exposure is by inhalation, and oral/dermal exposure. -DDVP is mutagenic *in vitro* but this is of minimal value in predicting either *in vivo* activity or potential risk to humans based upon what is known about DDVP metabolism and the biological activity of its metabolites. -There is no concern regarding a heritable effect of DDVP. -A WOE evaluation leads to the conclusion that a) DDVP is not genotoxic *in vivo*, b) it is an alkylating agent only at cellular concentrations that would be lethal, and c) the *in vivo* significance of the *in vitro* alkylating (DNA adduct forming) potential is biologically inconsequential for anticipated human exposures.

1538 THE MUTAGENICITY OF CAPTAN AND FOLPET.

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Mutagenicity is central to the classification of chemical carcinogens. Rodent carcinogens that are also mutagenic are considered to have non-threshold mechanisms; thus, any exposure to humans is presumed to result in increased carcinogenic risk. On the other hand, compounds that are not mutagenic *in vivo* act through epigenetic mechanisms and are generally considered having thresholds. The outcome of risk characterizations using these two approaches is profoundly different. The U.S. Environmental Protection Agency currently classifies captan and folpet as "probable human carcinogens; B2" by citing, in part, their mutagenic activity. Captan and folpet have a common mechanism of toxicity with regard to tumor induction in the mouse duodenum. This involves increased crypt cell proliferation following villi damage. The mutagenicity of these two fungicides has been studied extensively. The accumulated data are generally consistent between captan and folpet and describe a pattern of activity predicted by and consistent with their chemical and physical properties. The degradation of these chloroalkylthio fungicides involves the reaction with thiol groups and the generation of thiophosgene. Thiophosgene is a reactive acylating agent that is believed to be responsible for a variety of mutagenic findings *in vitro*. Conversely, captan and folpet are not mutagenic *in vivo* since thiophosgene rapidly reacts with a variety of chemical species including those containing thiol (-SH) groups to form non-genotoxic moieties. Similarly, this deactivation mechanism can be reproduced *in vitro* by the addition of compounds containing -SH groups (e.g., S-9, cysteine, and glutathione). The rapid degradation of thiophosgene *in vivo* prevents the transit of this agent, generated in the gut lumen, to the DNA of duodenal crypt cells. The absence of *in vivo* mutagenicity is confirmed by a variety of assays. While it is difficult to "prove" the negative with regard to *in vivo* mutagenicity, the weight of evidence is clear: captan and folpet are not mutagenic in the intact animal.

1539 SURVIVAL CURVES FROM SPRAGUE DAWLEY RATS ON CARCINOGENICITY STUDIES.

C J Perry, S B Hornby and S J Barton. *Inveresk Research, Tranent, Scotland, UK.* Sponsor: A B Wilson.

Much concern was expressed during the late 1980's and early 1990's over the apparent decrease in survival performance of the Charles River strain of Sprague Dawley rat (CD® Rats; Crl: CD® BR) which was one of the most common strains for use on carcinogenicity studies. This resulted in Charles River rederiving their stock in the mid 1990's to produce the IGS International Genetic Standard Sprague Dawley rat ((IGS)CD® Rats; Crl: CD® BR) to overcome this problem. Inveresk Research in Scotland, obtaining rats from Charles River (UK) Ltd, did not experience any problem with survival but changed to the IGS strain in 1996. These first studies have now completed the in-life phase. No changes in the diet (low calorie maintenance, ca14% protein), caging (5 per cage) and environmental conditions were made over the period of the comparison. Presented is a comparison of a sample of some of the rat carcinogenicity studies conducted at Inveresk Research over a period from approximately 1992 to1998 which shows that there is essentially no difference in survival pattern of control rats between the two derivations. At ca 110 weeks old the survival was ca 50% in males and 40% in females. Other parameters routinely collected on this type of study, such as body weight profile, are also presented for comparison between the 2 different derivations of Sprague Dawley rat.

1540 USE OF ARTEMIS II, AN ON-LINE DATA CAPTURE AND REPORTING SOFTWARE, ON PRE-CLINICAL TOXICITY STUDIES.

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The purpose of this study was to demonstrate the reliability and efficiency of the Artemis II (AII) data capture software for preclinical toxicity studies at

these laboratories. To demonstrate the suitability of this software, AII was used in a GLP environment to capture the data generated on a three month daily oral (gavage) dosing study in rats with a one month recovery period. Dose volumes were calculated by AII based on the most recent body weight. Body weights (twice weekly), clinical observations (twice daily), ophthalmology, veterinary examination were directly captured on-line. Clinical chemistry and hematology data were also directly captured at analysis. AII was sufficiently flexible to allow for the selection of any activity and for the animal processing list to be defined by the user for each activity. The time and date each activity occurred was recorded and any edit was clearly indicated by an audit trial. This user friendly system allowed for efficient data collection, and all data capture by the system was immediately available for review by the Study Director and Sponsor. Functional access to AII was limited by a security system allowing different levels of accessibility to activities such as data entry or editing. Access to AII by the technical and scientific staff was authorized only upon training completion. This study demonstrated that the use of the AII on-line data capturing system facilitates the entry, processing and reporting of data in preclinical studies.

1541 EFFECTS OF DIETARY LEAD ACETATE ON PLASMA MELATONIN LEVELS IN CHICKS.

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The administration of lead (Pb) to chicks results in increased levels of organ peroxides. Pb also provokes increased concentrations of reduced glutathione which presumably acts as part of a protective (antioxidant) mechanism against the actions of the peroxides. The hormone melatonin, like glutathione, also possesses strong antioxidant properties. Melatonin is produced by the pineal gland during the dark phase of the daily light/dark cycle. In the several experiments reported here, day-old chicks were exposed to various dietary Pb (as Pb acetate·$3H_2O$) levels ranging from 100 to 2000 ppm under conditions of continuous light (24L/0D) or of 12 h daily cycles of light and dark (12L/12D). The liver and brain levels of thiobarbituric acid-reactive substances (TBARS), an index of peroxide formation, were generally elevated by Pb. Supplementing the diet with ascorbic acid (an antioxidant vitamin not nutritionally required by chicks) or the antioxidant ethoxyquin prevented the elevation of TBARS by Pb. Exposure to 12L/12D resulted in a marked increase of plasma melatonin compared with 24L/0D. Pb consistently enhanced plasma melatonin concentrations in chicks exposed to 12L/12D but not in chicks exposed to 24L/0D. The melatonin effects were noted in chicks receiving a Pb dose as low as 100 ppm (non-growth inhibitory). Enhanced melatonin levels did not result in lower TBARS concentrations. This finding is similar to previous findings which showed that the elevated TBARS concentrations associated with Pb exposure occur despite elevated tissue levels of reduced glutathione. The results suggest that while dietary antioxidants can inhibit the production of Pb-induced TBARS, that TBARS production is unaffected by the concomitant Pb-induced increase of tissue antioxidants. Hsu (*J.Nutr.* **111**:26-33, 1981) suggested that the increase of tissue glutathione was a part of a compensatory response to ameliorate the effects of Pb-induced peroxides. The present data support that hypothesis and suggest further that, in animals exposed to diurnal light/dark cycles, melatonin may serve a similar function.

1542 EFFECTS OF SEX STEROID REPLACEMENT AND L-DOPA TREATMENT ON LEAD TOXICITY IN THE DEVELOPING RAT.

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The role of sex steroids and the GH/IGF_1 system in the pubertal effects of lead exposure were examined. Litters of rats (N = 5) were exposed to 2475 ppm lead acetate or acetic acid the drinking water beginning at gestational age 5 d. From age 30 -50 d, male and female offspring from each of the control litters were injected with saline; 7.5 mg/kg L-dopa, or fitted with minipumps delivering saline, offspring from each of the lead-exposed litters were injected with saline, L-dopa, or fitted minipumps delivering saline, 1.5 mg/kg/d testosterone; 3 mg/kg/d testosterone, 1.5 mg/kg/d DHT; 15 μg/kg/d E_2 or 45 μg/kg/d E_2. Vaginal opening was measured. Femur length was measured by X-ray, bone strength was measured by 3-point bending and plasma IGF_1 was measured by RIA. Lead exposure significantly reduced somatic and skeletal growth, bone strength and produced a pubertal delay of 4 days. L-dopa treatment produced significantly increased skeletal growth in control females ($p < 0.05$) and significantly increased bone strength in both sexes ($p < 0.05$). No effects of L-dopa were observed in lead-exposed animals. L-dopa treated, lead-exposed animals had lower plasma IGF_1, femur growth and bone strength than L-dopa treated controls ($p < 0.05$). Testosterone replacement had no effects on any of these parameters. Although DHT treatment restored plasma IGF_1 levels in lead-exposed male animals to control values no effects were observed on growth rates or bone strength. In lead-exposed female animals, estradiol replacement restored the timing of puberty but significantly further decreased somatic growth, IGF_1 and bone strength ($p < 0.05$). We conclude that lead-associated pubertal delay is a consequence of reduced sex steroid concentrations but that effects on growth and bone are either GH mediated or direct actions at skeletal targets. Supported in part by ES06684 (MJJR) and the Brooks Medical Research Fund (CKL).

1543 ESTIMATION OF CUMULATIVE LEAD RELEASES (LEAD FLUX) FROM THE MATERNAL SKELETON DURING PREGNANCY AND LACTATION.

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Recent longitudinal studies with humans and non-human primates using high precision stable lead isotopes show that lead is mobilized from the maternal skeleton during pregnancy and the post-pregnancy period. We have now calculated the cumulative lead release (lead flux, μ g) mobilized from the skeleton during these periods by analysis of monthly blood lead samples (Pb-b) from recent immigrants to Australia. Results included a statistically significant inverse relationship (p 0.006) between the lead flux and the time of conception after the arrival of the subjects in Australia. Using area-under-the-curve approaches to determine the added lead inputs to blood during pregnancy and nursing, versus a baseline, the net monitored lead release to blood varied from 0.9 to 10.1 μ g/day, equivalent to 0.3 to 4.03 mg Pb. With group Pb-b concentrations usually less than 3 μ g/dl, the observed releases imply a high skeletal turnover, greater than 10%, and possibly greater than 30% in some subjects during pregnancy and the post-pregnancy period. These elevated rates in some subjects may partly arise from low daily calcium intakes, being one-half to two-thirds that of recommended daily requirements. The lead flux calculated from a cumulative approach was compared with other approaches: 1st order kinetics, bone turnover, bone x-ray fluorescence (XRF) measurements and the International Commission for Radiological Protection (ICRP) PB-PK model. Calculated lead releases and remaining bone Pb would likely not be detectable by current XRF methods.

1544 STRUCTURAL AND MATHEMATICAL MODEL OF THE REGULATION OF PROTEIN KINASE C BY LEAD AND CALCIUM.

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Lead poisoning of the nervous, skeletal, renal, endocrine and reproductive systems is an important public health problem. Perturbations of the protein kinase C family is often identified as sensitive molecular targets for lead toxicity in affected organs systems. Pb^{2+} mimics Ca^{2+} in stimulating PKC translocation and in kinase activation in the absence of Ca^{2+}. To evaluate the interaction of Pb^{2+} and Ca^{2+} in more physiological conditions, we investigated the effects of Pb^{2+} on recombinant PKC α and β1 proteins in the presence of selected concentrations of Ca^{2+} using the PanVera assay for PKC activity. The computer program CHELATOR was used to define selected assay conditions of 0.05 to 1000 nM free Pb^{2+} in the presence and absence of 0.05 to 100 μM Ca^{2+}, buffered using ethylene glycol-bis(aminoethyl ether)-tetraacetic acid. We developed a structural and mathematical model for PKC activity in the presence of both Ca^{2+} and Pb^{2+} described by bi-substrate, bi-product, Ping-Pong kinetics involving ATP and the pseudosubstrate. In agreement with previous reports, our model demonstrates that very low levels of Pb^{2+} activate PKC α (K_a^{Ca2+} = 55.4±14.1 x10^{-9} M; K_a^{Pb2+} = 32.1±9.6 x10^{-12} M) and PKC β (K_a^{Ca2+}= 48.8±12.3 x10^{-9} M; K_a^{Pb2+} = 21.7±9.1 x10^{-12} M) activity in the presence of only one metal. However, under physiological conditions, when both metals are present, Pb^{2+} may either activate, inhibit, or have little effect on the enzymatic activity of PKCs depending on both the Pb^{2+} and Ca^{2+} concentrations. Our model assumes that Ca^{2+} and Pb^{2+} interact with PKC at multiple regulatory sites, and predicts that Pb^{2+} is a non-competitive inhibitor at high Pb^{2+} concentrations. Given

the spatial and temporal regulation of $[Ca^{2+}]_i$ and of PKCs the actions of Pb^{2+} on PKC activity is too complex to generalize. Supported by NIEHS ES04040.

1545 PKC DOES NOT MEDIATE THE EFFECTS OF LEAD ON THE VITAMIN D-DEPENEDNT PROCUDTION OF OSTEOCALCIN.

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Lead toxicity decreases serum ostecalcin levels in children and vitamin D-dependent production of osteocalcin by osteoblast-like cells, ROS 17/2.8. It is often reported that activation of PKC is a sensitive mediator of the biological activity of Pb, and that PKC exerts a negative regulatory effect on vitamin D_3-dependent processes. The objective of our study was to characterize the role of PKC activation in mediating the actions of lead on vitamin D_3-dependent osteocalcin secretion. ROS 17/2.8 cells were pretreated with Pb for 24 hr and then stimulated with 0 or 100 pM 1,25-dihydroxyvitamin D_3 in the presence of Pb and pharmacological agents to inhibit or activate PKC activity. Lead treatment (5-20 μM for 24 hr) decreased the stimulating effects of vitamin D_3 on osteocalcin secretion. Inhibition of PKC activity by Gö6976, an inhibitor of both α and β isozymes, produced similar effects as lead on vitamin D_3-dependent osteocalcin secretion, while activation of PKC by tetra decanoyl phorbol ester (TPA) did not reverse this effect of lead. Similar data with other PKC inhibitors indicate that activation of PKC is not responsible for the actions of lead on osteocalcin. We also showed that 10 μM lead does not change the cellular or secreted activity of alkaline phosphatase, another vitamin D_3-dependent gene product in osteoblasts. Direct assay of PKC activity showed that 10 μM lead does not activate or inhibit the activity of PKC in ROS cells. Western blot analysis of cytosolic and particual fractions obtained from ROS cells indicated that lead does not translocate PKCα or β1 from cytosol to membrane. Therefore, it is concluded that PKC is not the mediator of actions of lead on decreasing the vitamin D_3-dependent osteocalcin secretion in ROS cells. Supported by NIH ES04040. Several studies suggest a negative controlling effect of PKC in the actions of vitamin D.

1546 CHRONIC LEAD INTOXICATION MAY CONTRIBUTE TO OSTEOPOROSIS.

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Over 90% of the body's burden of lead is found in bone. Sequestration of Pb in bone matrix may prevent damage to sensitive cells of the brain and kidney but may not protect bone cells. During menopause, skeletal Pb is mobilized to other organs with the greatest risk encumbered by peri-menopausal baby-boomers who were exposed to the high environmental lead levels of the 1950s and 1960s. Our goal was to determine the effects of Pb on bone cell activities *in vitro* and the contribution of Pb to osteopenia in animals *in vivo*. Osteoblasts and osteoclasts from neonatal rats were used to determine the dose-dependent effects of Pb on collagen synthesis, alkaline phosphatase activity, cell proliferation, resorption pit number and pit area. Male Long Evan rats were exposed to 0, 50, 150, 1000, or 2000 μg Pb acetate /ml drinking water for 8 or 15 mo. Blood and femur Pb was determined by AAS. Bone mineral mass, area, and density were measured using a Lunar DPX-L with 'small animal' software. Meta-analysis of all cellular functions revealed that osteoblasts are more sensitive to Pb than are osteoclasts. The in vivo studies found an inverse relationship between blood Pb and total bone mass and total bone area. Moreover, 12 to 20% of the treated animals were >2.0 SD below the control mean for all skeletal parameters. PbB levels were from 10 to 100 μg/dL in the Pb groups, within the range of environmental and occupational exposure respectively. Since lead affects osteoblast functions more than osteoclast activities, and decreases bone mass, a definable proportion of osteopenia and osteoporosis may result from chronic lead poisoning. Supported by NIEHS ES08121(JEP), ES01217, ES01247 (DCS) & ES04040 (JGP).

1547 CONTRIBUTION OF LEAD FROM FORMULA AND FOOD TO LEAD IN BLOOD AND URINE OF NEWLY-BORN INFANTS AND RELATIONSHIP TO SKELETAL MOBILIZATION.

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We have estimated the contribution from formula and food to blood and urine lead in 8 newly born infants using changes in lead isotopic composition over a 6 months monitoring period. Lead concentrations in formula were generally low with a geometric mean of 1.8 μ g/kg (arithmetic median 1.99 ± 1.0) and a range from 0.36 to 4.3 μ g/kg (n=53). Lead concentrations in the baby food were up to 3 times that found in the formula with a geometric mean of 5.8 μ g/kg (arithmetic mean 6.7 ± 5.1) and a range from 1.4 to 27 μ g/kg (n=66). Differences in lead concentration between food and formula were statistically significant (p 0.003 two tailed test). The mean daily intake was 0.31 μ g Pb/kg body weight(bw)/day (geometric mean 0.25) and varied from 0.04 to 0.87 μ g Pb/kg bw/day. The contributions of infant diet to blood lead ranged from 24 to 82%, similar to those estimated for contribution of lead in breast milk to blood lead. However, multiple regression analyses showed that neither formula or food were significant predictors of urine isotopic composition or lead concentration for various time periods over the 6 months. The estimated excretion rate exceeded intake (geometric mean ratio of 2.7:1) except for two infants who had the highest daily intakes. As other sources such as air and dust contribute minimally to blood lead in these infants, we consider the increased excretion over intake and other evidence to reflect mobilization of infant skeletal lead arising from rapid bone turnover.

1548 THE USE OF LEAD STABLE ISOTOPES TO IDENTIFY HOUSEHOLD SOURCES OF LEAD EXPOSURE.

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The variations in the natural distributions of lead isotopes provide a powerful tool to investigate the sources of lead in the environment. Lead released to the environment as a result of human activities had a different isotopic composition over time depending on the source ore from where it was extracted. In many houses built before 1978, lead incorporated in lead based paints has different isotopic compositions even within the same dwelling due to the use by different paint manufacturers of lead originated in different mines. In addition, soils have accumulated lead mainly from the dry deposition of lead particles derived from the combustion of leaded gasoline. This lead has also a distinct isotopic composition. We have investigated the sources of lead in children with elevated blood lead levels (PbB >15μg/dL) by determining the isotopic composition of lead in their blood and in the potential environmental and in-house sources of lead exposure. In children living in the same house throughout their life, the lead isotopic composition of the blood is a reflection of the current lead intake and therefore it provides a reliable indication of the source(s) of exposure. In cases where the child has moved at least 6 months before the sampling time, the blood lead isotopic composition diverts from the isotopic composition of current lead intakes because it also includes isotopically different lead released from the skeleton that had been incorporated during past exposures. While this approach relies on measurable differences between the lead isotopic compositions of blood and current intake (i.e., diet, dusts, etc.) to evaluate the sources of lead exposure to the child, it also provides a unique opportunity to determine the contribution of endogenous (skeletal) lead to blood in children. (Supported by the University of California Toxic Substances Research and Teaching Program).

1549 SYSTEMATIC EVALUATIONS OF PLASMA Pb LEVELS OVER TIME IN ENVIRONMENTALLY-Pb EXPOSED SUBJECTS.

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Recently, it has been suggested the lead (Pb) in plasma may be a more relevant indicator of biologically labile Pb than Pb in whole blood. Previous

reports of the relationship of plasma-Pb (P-Pb) to whole blood-Pb (WB-Pb) indicate a several-fold variability in the relative partitioning of Pb between whole blood and plasma for a given WB-Pb level. Studies investigating the variability of the P-Pb to WB-Pb relationship of individuals over time are needed to determine if the noted variability of this relationship is real or due to methodological differences. Here, we investigated the relationship between P-Pb and WB-Pb in repeatedly sampled environmentally exposed subjects over time. Two to five plasma and whole blood samples per subject (n=14 subjects) were collected over a one year duration for [Pb] and isotopic composition analyses by inductively coupled plasma - high resolution mass spectrometry using an ultraclean method developed in our laboratory (Smith et al., Am. J. Ind. Health, 33:430). Plasma samples were analyzed for iron and hemoglobin to evaluate hemolysis and resulting plasma contamination. In subjects sampled over a four week period (n=10 subjects, 4 samples each), P-Pb levels averaged 0.29 % (± 0.01% SE, range 0.17–0.46%) of WB-Pb levels (average 5.8± 0.3 μg/dL SE, range 3.3–9.7 μg/dL). Subjects sampled over a longer time period (2–4 samples, separated by at least 5 months, n=4 subjects) were similarly invariable, exhibiting average P-Pb levels of 0.27% (± 0.03% SE, range 0.17–0.52%) of WB-Pb levels (average 9.4± 0.78 μg/dL, range 5.4–13.8μg/dL). These data indicate the importance of proper sample collection and analyses methods, and that intra-subject variability in the P-Pb to WB-Pb relationship is substantially lower than previously reported. (Supported by NIEHS #ES0782).

1550 EFFECTS OF SUCCIMER ON THE DIURESIS OF ESSENTIAL METALS IN A PRIMATE MODEL OF CHILDHOOD Pb POISONING.

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Succimer (2,3-dimercaptosuccinic acid, DMSA) is a promising chelating agent used in the clinical management of lead (Pb) poisoning in children. While succimer exhibits high relative specificity for Pb, as a metal chelating agent it may also bind and effect the diuresis of other non-target essential metals. To address this, the urinary excretion of calcium (Ca), copper (Cu), cobalt (Co), iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), phosphorus (P), and zinc (Zn) were evaluated in a juvenile primate model of childhood Pb poisoning. Twenty-two infant rhesus monkeys (*Macaca mulatta*) were exposed to Pb orally from birth to 1 year of age, then treated with placebo (n=11) or oral succimer (n=11) at a dose of 30 mg/kg BW/d for 5 days then 20 mg/kg BW/d for 14 days. Complete 24-hour urine samples were collected over the period T_{1d} - T_{5d} of treatment (6 days total), using acid-cleaned stainless steel metabolic cages and trace metal clean techniques. Sample collection blanks were similarly collected to evaluate sample contamination. Urine samples were analyzed for the above metals using a high resolution ICP-MS method developed in our laboratory. Five days of succimer treatment significantly increased the urinary excretion of Mn (by 77%) and Ca (by 63%) over controls ($P<0.05$, t-test). Excretion of Cu, Fe, Mg, Ni, and Zn also increased with succimer treatment (15 - 35%), but those increases did not reach significance ($0.2>P>0.06$). Succimer treatment had no measurable effect on the excretion of Co and P. By comparison, succimer increased the diuresis of Pb by 240% in these animals. These data may be qualified in some cases (e.g., Zn) due to possible urine contamination from monkey food — although this effect would likely diminish the observed differences between treatment groups. While the physiological significance of these data remain to be shown, they indicate that further study is warranted to evaluate the effects of prolonged succimer treatment on the excretion of essential metals. (Supported by NIEHS Grant #06918.)

1551 CHANGES IN BLOOD LEAD CONCENTRATION AND ISOTOPE RATIO IN SUCCESSIVE HUMAN PREGNANCIES.

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We measured the blood lead concentrations and the isotope ratios of blood and urine of two women living in Omaha from the first trimester of one pregnancy to 9 months after completion of a second. One was a 19 yr old primagravida who bottle fed both her infants; the other a 37 yr old breast feeder pregnant for the fourth time. During each pregnancy the blood lead concentrations showed a similar pattern, declining in the second trimester and increasing in the third, but in the second of the two pregnancies the changes and the overall blood lead concentrations were diminished. After delivery the blood lead of the bottle feeder remained elevated for 3 months, whereas that of the breast feeder continued to increase over the same period and remained elevated throughout lactation. We used seasonal variations in isotope ratio between pregnancies to infer the directions in which the isotope ratios of the cortical and trabecular bone of each woman lay. The decrease in blood lead concentration in mid-pregnancy is accompanied by a change in isotope ratio towards trabecular bone and the increase in late pregnancy by a change towards cortical bone, which in the case of both women reversed immediately after delivery. We conclude that: (1) the decline in blood lead in mid pregnancy is due more to a decrease in the flux of lead from cortical bone (an effect also observed in Cynomolgus monkeys) than to hemodilution; (2) the rise in late pregnancy results from increased resorption of cortical bone; (3) the post partum elevation of blood lead is in part due to the abrupt decrease in plasma volume (reflected in the hematocrit) that follows delivery; and (4) pregnancy strips lead from the skeleton, so that the blood lead of a multiparous woman decreases stepwise from pregnancy to pregnancy. The reason for the increase in blood lead during lactation is not clear because in our subject the isotope ratio patterns in the lactating and non-lactating states were indistinguishable.

1552 LEAD EXPOSURE IN CHILDREN FROM URBAN AREAS IN REGION LAGUNERA, MEXICO.

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The aim of this cross-sectional study was to assess lead exposure in children aged 6-9 years attending three primary schools. One of the schools is located ~500 m distant from a smelter complex which includes a lead refinery (high exposure group); the second is located ~ 900 m away from the complex and at the side of a heavy traffic road (medium exposure) in Torreón, Coahuila. The third school is located in Gómez Palacio, Durango, 5,000 m away from the smelter complex and distant from heavy vehicular traffic or industrial areas (low exposure group). Lead was measured in air, soil, dust and well water. Lead in blood (PbB) was determined in 394 children attending the above mentioned schools. Determinations were performed by atomic absorption spectrophotometry. Diet, socioeconomic status, hygienic habits and other variables were assessed by questionnaire. Average PbB (± SD) values were 8.7 ± 3.8 μ g/dL in the low exposure group, 22.4 ± 7.8 μ g/dL in the medium exposure and 28.8 ± 11.0 μ g/dL in children attending the school nearest to the lead refinery. The percentage of children having PbB ≥ 15 μ g/dL was 6.8%, 84.9% and 92.1%, respectively. In this order, the average Pb concentrations in air were 3.25 ± 2.59 μ g/m^3, 6.03 ± 2.07 μ g/m^3 and 8.16 ± 5.65 μ g/m^3. The range of Pb concentrations in soil and dust in playground areas in the medium and high exposure settings was 1,000 to 4,000 mg/kg. Pb values in drinking water were less than 6 μ g/L. Soil and dust ingestion and inhalation appear to be the main routes of exposure. Our results indicate that environmental contamination has resulted in an increased body burden of Pb, suggesting that children living in the vicinity of the smelter complex are at high risk of suffering the well known adverse effects of lead if exposure continues. Further studies are needed to evaluate the specific contributions of lead sources in the city and the presence of other metals in the environment.

1553 EQUILIBRIUM ADSORPTION OF LEAD IN WATER BY TRIOCTAHEDRAL SMECTITE CLAY.

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Lead (a commonly occurring contaminant of drinking water) has been strongly implicated in the etiology of disease in man and animals. The main objectives of the present study were to screen representative silicoaluminates (both natural and chemically modified) for their ability to reduce Pb^{2+} levels in water. Among the various clays tested, a trioctahedral smectite (TOS) was shown to significantly adsorb Pb^{2+} at equilibrium. Aqueous solutions of $Pb(ClO_4)_2$ (20 μg/mL) were shaken with clay samples (100 μg/mL) for 24 hr, and the concentration of Pb^{2+} in solution was measured using an ion-selective electrode and validated by atomic absorption spectroscopy. Preliminary data indicated that TOS was able to adsorb 99.2% of Pb^{2+} from solution. Controls (i.e., quartz and activated carbon) exhibited 11.5% and 27.7% binding, respectively. Isotherm studies showed that Pb^{2+} adsorption followed a *Langmuir-Freundlich Model* with a maximum adsorption capac-

ity (Q_{max}) of 3.18 mol/Kg, a dissociation constant (K_d) of 7.64 x 10^5, and a Gibbs free energy (ΔG^o) of −33.58 KJ/mol. These results indicated a rapid initial adsorption of Pb^{2+} with a high affinity for clay surfaces. In other studies, adult Hydra attenuata were used to confirm the ability of TOS to adsorb lead in aqueous solution and to diminish the lethality of this toxic metal. A marked reduction in the toxicity of Pb^{2+} to hydra (50 fold) was observed in the presence of TOS and correlated well with equilibrium adsorption results with this clay. Work is ongoing to further characterize thermodynamic properties and to model the mechanism of adsorption of Pb^{2+} onto the surface of TOS. High affinity, high capacity adsorbents such as TOS may facilitate the field-practical detoxification of contaminated water via effective filtration and innactivation of Pb^{2+} and possibly other hazardous trace metals (Supported by NIH P42-ES04917).

1554 SOIL CLEANUP AND OTHER PREVENTION STRATEGIES FOR LEAD: WHAT DO WE KNOW ABOUT THEIR EFFECTIVENESS FOR REDUCING BLOOD LEAD LEVELS?

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Several approaches are currently being used to reduce or prevent exposure to lead in soil and indoor dust. Primary prevention activities prevent children and other susceptible populations from being exposed to lead in soil and indoor dust. These activities include identification and control of lead sources, public education, and education and training of health care professionals. Secondary prevention activities reduce blood lead levels after exposure has occurred and include blood lead screening and follow-up care. The relationships between blood lead levels and soil or indoor dust levels, and between blood lead levels and cleanup activities are difficult to evaluate. Several studies have attempted to address these issues; however, neither unequivocal quantitative nor qualitative associations have been identified. For example, cleanup actions have been associated with reduction, no change, or only a marginal change in blood lead levels. Factors that impede an understanding of the effectiveness of strategies that prevent exposure to lead or reduce blood lead levels include failure to identify or account for all sources of lead exposure and differences in protocols among studies including criteria for subject inclusion in the study, and seasonal and geographic differences. The effect of community outreach or changes in personal activities by community members prior to study initiation may also complicate our understanding of the effectiveness of prevention or cleanup activities. (*[1]The opinions expressed are those of the authors and do not necessarily reflect the opinions or policies of the ATSDR.)*

1555 PORPHYRIN PROFILES AND CHELATOR CHALLENGE RESPONSE AFTER METHYL MERCURY EXPOSURE IN VOLES.

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Porphyrins, precursors of heme, occur in characteristic profiles and are of interest in the kidney and urine as biomarkers of Hg exposure and effect. Increases occur primarily in 4-carboxylporphyrin (copro) in voles. Mercury chelation with 2,3-dimercaptopropane-1-sulfonate (DMPS), and subsequent measurement of urinary Hg and porphyrin profiles, more accurately approximate tissue burdens of Hg and their effects. Studies were carried out to determine a DMPS dose and the time and Hg dose dependence of the DMPS challenge in the prairie vole (Microtus ochrogaster). Voles received 1 ppm methyl mercury hydroxide in drinking water for four weeks after which they were challenged with saline, 0.01, 0.1 or 1.0 mmol/kg DMPS, ip. Urine was collected for 24 hours before and after the saline or DMPS injection. Kidney, urine and brain samples were analyzed for inorganic and organic mercury. Porphyrin profile analyses were carried out on kidneys and both urine samples. Mercury reached 1.4 ppm in the brain (92% organic mercury) and 15.5 ppm (94% organic) in the kidney. DMPS reduced brain Hg to 0.98, 1.3 and 1.1 ppm and kidney Hg to 7.1, 7.6 and 9.5 ppm in the high, medium and low DMPS treatments, respectively. Urinary Hg (24 hr. total) increased from 1.63 μg, initially, to 11.7, 8.04 and 3.47 μg in the high, medium and low DMPS treatments, respectively. Urinary Hg averaged ~65% organic in pre- and post-chelation samples. The proportion of mercury in the urine from renal depletion was 26, 36 and 63 percent for the high, medium and low dose, suggesting that higher DMPS doses better accessed non-renal tissues. Methyl mercury increased renal levels and urinary excretion of copro. DMPS decreased renal and urinary copro. These data support the utility of the combined porphyrin profile and DMPS chelation techniques, establish an appropriate DMPS dose, and demonstrate that brain mercury concentrations were not increased by DMPS administration in voles. (Funded by NIH ES04696.)

1556 DIAGNOSTIC USE OF 2,3-DIMERCAPTO-1-PROPANE SULFONATE (DMPS) TO ASSESS MERCURY EXPOSURE.

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Medical toxicologists use chelating agents as "provocative" or challenge tests to aid in diagnosis of patients with potential metal intoxication. A positive test is an increased amount of urinary metal excreted over a collection period per amount chelating agent administered. However, the interpretation of a challenge test is not well defined. We hypothesize that increased urinary mercury (Hg) excretion equal to or greater than 50 ug/6-hr after an oral DMPS challenge test indicates patients with a significant history of Hg exposure. To test this, we retrospectively examined urinary Hg excretion data of patients prospectively enrolled in four oral DMPS challenge test clinical trials. Study subjects consisted of dental professionals with or without a history of Hg exposure and controls (non-dental professionals with no Hg exposure history). A history of mercury exposure was defined as extensive use of Hg amalgams in dental practice or complaints self-attributed to Hg exposure. After a baseline urine collection, subjects ingested a single oral DMPS dose (300 mg) and urine was collected for 6 hours. A positive challenge test was defined as $\geq$ 50 ug Hg/6-hr urine. Eighty-nine subjects were enrolled: 47 dental professionals with no Hg exposure history, 19 dental professionals with Hg exposure history and 23 controls. Fourteen subjects had positive challenge tests and all had an extensive Hg exposure history. No subject with complaints self-attributed to Hg exposure had a positive test. The sensitivity of the oral DMPS challenge test was 100% with 93% specificity and a negative predictive value of 100%. We propose that an oral DMPS challenge test is accurate in confirming a significant history of Hg exposure and may have clinical utility in diagnosis of Hg intoxication.

1557 EFFECTS OF 2,3-DIMERCAPTO-1-PROPANE-SULFONATE (DMPS) ON TISSUE AND URINE MERCURY (Hg) AND PORPHYRIN LEVELS IN METHYL MERCURY-EXPOSED RATS.

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Lead is known to be a potent activator of protein kinase C (PKC) and the PKC signal transduction system may modulate the proliferation rate of gliomas. This study was undertaken to investigate the ability of lead to activate PKC and its downstream events in 132 1N1 human astrocytoma cells. Treatment with 50 μM lead acetate for 1 hour was able to induce PKCα translocation as evidenced by Western Blot analysis and enzyme activity assay, while PKCε was not affected. Lead treatment (1 μM to 100 μM for 24 hour), resulted in a concentration- and time-dependent increase in DNA synthesis up to 300% of basal level, as measured by ^{3}H-thymidine incorporation assay. No cytotoxicity was observed, as measured by LDH release assay. The effect of lead was blocked by two PKC inhibitors, bisindolylmaleimide and staurosporine, and also by down-regulation of PKC by a pretreatment with the phorbol ester PMA. Flow cytometric analysis using double staining with bromodeoxyuridine(Brdu)-quenchable Hoechst 33258 and ethidium bromide showed that treatment with lead or PMA stimulated the G_0/G_1 to S/G_2 phase transition resulting in increased percentage of cells in the S/G_2 phase (30% in treated group vs. 4% in control group). These results suggest that lead stimulates DNA synthesis and cell-cycle progression in human astrocytoma cells through a PKC-dependent mechanism. (Supported in part by grant ES07033.)

1558 EFFECTS OF BILIARY LIGATION AND MODULATION OF GSH-STATUS ON THE RENAL AND HEPATIC DISPOSITION OF INORGANIC MERCURY IN RATS.

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The effects of biliary obstruction and modulation of glutathione (GSH)-status on the acute disposition of a low i.v. dose of inorganic mercury were studied in rats. Dispositional effects were studied in the kidneys, liver, small

and large intestines and blood one hour after the injection of 0.5 μmol/kg dose of mercuric chloride in control rats and rats pretreated with acivicin, buthionine sulfoximine (BSO) or diethylmaleate (DEM) that either underwent or did no undergo acute biliary ligation prior to the injection of mercury. Pretreatment with BSO or DEM was shown (in separate group of rats) to cause significant reductions in both the renal and hepatic concentrations of GSH, which is consistent with systemic depletion of GSH. Among the animals that did not undergo biliary ligation, pretreatment with BSO had no effect on the acute renal accumulation of mercury. By contrast, pretreatment with DEM or acivicin caused the renal accumulation of mercury to decrease. In general, the three chemical pretreatments used had varying effects on the disposition of inorganic mercury in the kidneys and liver. Interestingly, biliary ligation caused the net renal accumulation of mercury to decrease under all pretreatment conditions. By contrast, biliary ligation caused significant increases in the hepatic burden of mercury in all pretreatment groups except in the acivicin-pretreated animals. The additive effects of biliary ligation and chemical modulation of GSH-status on reducing the net renal accumulation of mercury indicate a likelihood of two independent mechanisms being involved in altering the renal disposition of mercury. Moreover, the data from the animals whose bile duct had been ligated indicate that at least some fraction of the renal accumulation of inorganic mercury in normal animals is linked mechanistically to the hepato-biliary (and enteric) systems.

1559 SELECTIVE ACTIVATION WITHIN THE MAPK PATHWAY BY HG(II).

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The kidneys are the primary target organ for the accumulation and toxicity of inorganic mercury. Studies have shown molecular alterations occur following a nephrotoxic insult, thus alterations in transcription factor binding and gene expression may be involved in the response of the kidney to metals. However, little is known about the molecular response of the kidney to environmentally relevant concentrations of Hg(II). Previous research in our laboratory utilizing rabbit renal cortical slices demonstrated that non-cytotoxic concentrations of Hg(II) (0-1000 nM), increased the DNA binding activity of the AP-1 transcription factor which correlated with a dose-dependent enhancement of c-jun gene expression. These results suggest a possible interference in the c-Jun NH_2-terminal protein kinase (JNK) pathway. Therefore the effects of Hg(II) on other components of this pathway were investigated. No changes were observed in the DNA binding activity of ATF2 and Elk-1, transcription factors involved in both the JNK and p38 pathways of MAP signal transduction. The specificity of Hg(II) for MAP signal transduction was also demonstrated by no changes of c-myc gene expression after exposure to Hg(II). The selectivity of alterations in molecular signaling suggests an acute response in signal transduction, specifically activation of the JNK pathway, in renal tissue following exposure to nanomolar concentrations of Hg(II). (NIH ES04940, NIEHS Training Grant T32 ES07091)

1560 INHIBITION OF NF-κ B-DNA BINDING BY MERCURIC ION (Hg^{2+}): EFFECTS OF THIOL VERSUS NON-THIOL REDUCTANTS ON Hg^{2+} INHIBITORY CAPACITY.

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Hg^{2+}, a potent thiol inhibitor, prevents expression of nuclear factor κ B (NF-κ B) by mercaptide bond formation with a critical cysteine moiety (cys62) on the p50 subunit required for DNA binding. NF-κ B-DNA binding is typically measured in reaction mixtures in which dithiothreitol (DTT) or other thiol reductants are used to maintain cys62 in the reduced state. However, the presence of thiol reductants prevents accurate assessment of the [Hg^{2+}] required to prevent NF-κ B-DNA binding because of competitive mercaptide bond formation. In the present studies we evaluated the efficacy of tris(2-carboxyethyl)phosphine-HCl (TCEP), a non-thiol reducing agent which does not bind Hg^{2+}, on NF-κ B-DNA binding, using recombinant p50 protein and a ^{32}P-labeled κ B oligonucleotide. We also measured the minimal [Hg^{2+}] required to prevent this interaction in the presence of either reagent. DTT promoted NF-κ B-DNA binding in concentrations from 0.25 to 2.6 mM in binding reactions. However, in the presence of 0.25 mM DTT, inhibition of NF-κ B binding was seen only at [Hg^{2+}] > 100 μ M and results were highly variable. The inhibitory effects of Hg^{2+} in the presence of higher [DTT] were indeterminate. In contrast, TCEP promoted NF-κ B-DNA binding in a dose-related manner in concentrations from 0.25 to 6 mM. In the presence of even 6 mM TCEP, Hg^{2+} prevented NF-κ B-DNA binding at concentrations as low as 20 μ M in binding reactions. These findings support the use of TCEP as reductant in nuclear binding reaction assays. They also demonstrate inhibition of NF-κ B-DNA binding at [Hg^{2+}] comparable to those known to eicit toxicity in vivo. (Supported by NIH grant ES07033.)

1561 ACCUMULATION OF MERCURY AND ITS EFFECT ON ANTIOXIDANT ENZYMES IN BRAIN, LIVER, AND KIDNEYS OF MICE.

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Mercury exhibits its toxicity by virtue of its interaction with proteins thereby inhibiting enzymes involved primarily in metabolism. Oxidative stress induced by various toxicants and pathological conditions in humans. Mercuric chloride ($HgCl_2$) happened to be one of the pro-oxidants that induces oxidative stress. The present study was undertaken to investigate whether oral doses of mercuric chloride alters the activities of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase) in different organs (liver, kidneys, and brain) of mice. The uptake of mercury shows that the kidneys accumulated the highest levels of mercury compare to brain and liver. The enzyme levels varied in mercury treated organs. A dose dependent increase of antioxidant enzymes occurred in the liver and kidneys. The increase in enzyme activities correlated with highest mercury accumulation in kidney and liver. Mercury is known to generate reactive oxygen species (ROS) *in vivo* and *in vitro*, therefore, it is likely that enzyme activities increased to scavenge ROS levels produced as a result of mercury accumulation. The increased antioxidant enzymes enhance the antioxidant potential of the organs to reduce oxidative stress.

1562 GENDER AND TISSUE SPECIFIC CHANGES IN GLUTATHIONE AND THE EXPRESSION OF THE CATALYTIC AND REGULATORY SUBUNIT OF GLUTAMATECYSTEINE LIGASE IN MICE EXPOSED TO METHYLMERCURY.

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The toxicity of methylmercury is known to be influenced by gender and by glutathione status. Previous studies have shown that methylmercury can deplete glutathione in some tissues while increase the level of glutathione in others in a gender specific manner. Glutamylcysteine ligase (GLCL), the rate limiting enzyme in the synthesis of glutathione, is composed of two subunits: a large catalytic subunit (GLCLc) and a smaller regulatory subunit (GLCLr), and both of them are ubiquitously expressed in mammalian tissues. Previous studies have shown that MeHg exposure can result in the induction of GLCL at the RNA and protein level. We wished to determine the influence of methylmercury on the mRNA and protein levels of this enzyme, as well as enzyme activity, and glutathione levels in various tissues in male and female mice. C57Bl/6 mice were treated with one single dose of Methylmercury hydroxide (6mg/Kg) by intraperitoneal injection, and sacrificed 24 h later. Liver, kidney, small intestine and lung were collected and analyzed for glutathione content, GLCL mRNA and protein expression, and enzyme activity. Female mice had higher glutathione levels than males in the lung, but less in the small intestine. Male mice have higher levels of GLCLr mRNA in the kidney and lung (slightly), but lower in the liver, as well as lower levels of GLCLc mRNA in the small intestine, and lower levels of GLCLc protein in the lung. In response to MeHg treatment, male mice showed glutathione depletion, and GLClr mRNA induction in the liver. Female mice showed an increase in kidney and small intestine glutathione levels, induction of GLCLr mRNA in kidney and liver, and downregulation of GLCLc in the liver. There were no singnificant changes in protein levels with MeHg treatment. GLCL enzyme activity was increased in the liver in both sexes, as well as in the female small intestine. These results show diferential regulation of the catalytic and regulatory subunits of GLCL, at least at the transcriptional level, and marked sex differences in response to MeHg treatment.

1563 LUMINAL TRANSPORT OF DICYSTEINYLMERCURY ($(CYS)_2$-Hg) IN THE RABBIT PROXIMAL TUBULE.

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Data from previous whole animal and isolated-perfused tubule experiments indicate that the lumen-to-cell transport of dicysteinylmercury ($(CYS)_2$-Hg) is via an amino acid transport mechanism. To extend these findings further we studied the luminal disappearance rate of $(CYS)_2$-Hg in isolated perfused S_2 segments of the rabbit proximal tubule. The control perfusion fluid was a balanced saline solution that contained 20μM Hg^{++} and 80μM cysteine. Replacing sodium ions with *N*-methyl-D-glucamine in both the bathing and perfusing solutions reduced the lumen-to-cell transport of $(CYS)_2$-Hg from 102 ± 4.2 to 65 ± 3.8 fmol min^{-1} mm^{-1}. To determine which amino acid transport system may be transporting $(CYS)_2$-Hg, competitive inhibition of $(CYS)_2$-Hg lumen-to-cell transport was examined by placing nine selected amino acids and two amino acid derivatives in the perfusion solution (at a concentration of 3 mM). The competitive inhibitors that had the greatest effect on the lumen-to-cell transport of $(CYS)_2$-Hg were *L*-serine, *L*-histidine, *L*-tryptophan, and 2-(-)-endoamino-bicycloheptane-2-carboxylic acid (BCH). These competitive inhibition experiments were performed in the presence and absence of sodium ions. The greatest inhibition of $(CYS)_2$-Hg transport was produced by the addition of *L*-histidine to the luminal fluid in a sodiun ion free environment, 102 ± 4.2 to 14.6 ± 1.4 fmol min^{-1} mm^{-1}. We conclude that $(CYS)_2$-Hg is primarily transported from the luminal fluid into the proximal epithelial cells by the sodium- dependent "ASC" and the sodium-independent "L" amino acid transport systems. We also conclude that $(CYS)_2$-Hg enters proximal tubular epithelial cells by a mechanism whereby this mercuric complex mimics the amino acid cystine at the site of the "ACS" and/or "L" amino acid transporters.

1564 EFFECTS OF ALUMINUM ON IMMUNE PARAMETERS IN ORALLY-EXPOSED HUMANS.

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Aluminum (Al) salts are used as adjuvants in vaccines, partly due to their immunostimulatory effects. Little is known about possible immune effects in humans during oral dosing of Al-containing pharmaceuticals. Eighteen healthy volunteers (mean age 42, 28-57 yrs) were divided into a test group (9 females, 4 males) and a control group (3 females, 2 males). Blood and urine were sampled twice during a base-line period of one month. During the following 42 days the subjects of the test group ingested 3x10 ml of antacid per day (Al-hydroxide, 59 mg Al/ml). Blood and urine were sampled on day 14, 28 and 42 in both groups. Al exposure resulted in a 20-fold increase in urine Al concentrations (base-line median: 20 μmol Al/mol creat.). The maximum Al concentration was reached already after 14 days of exposure. Nevertheless, there were no marked differences in immune parameters between Al-exposed and control groups. Both groups showed a time-dependent change in mitogen-stimulated lymphocyte proliferation, proportions of B-, T- and NK-cell populations, and immunoglobulin concentrations during the base-line and exposure periods. Therefore, seasonal changes in immune parameters may need to be considered in future immunological studies.

1565 CHANGES IN SOME ASPECTS OF CARBOHYDRATE METABOLISM IN HEPATO PANCREAS OF FRESHWATER CRAB, BARYTELPHUSA GUERINI EXPOSED TO CHROMIUM COMPOUNDS.

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Trivalent and hexavalent chromium compounds are biologically important because of their existence in the environment in significant quantities. Though literature reveals heavy metal induced biochemical changes in various aquatic species, the information pertaining to biochemical toxicology of chromium in relation to crustaceans is very less. Therefore, the present study was designed to study the *in vivo* effects of tri and hexavalent chromium compounds on certain aspects of carbohydrate metabolism in the Hepatopancreas of crab, Barytelphusa guerini. Crabs were exposed to sublethal concentration (1/8 of LC-50 value) chromium chloride (15.6ppm) potassium dichromate (11.3ppm) representing tri and exavalent chromium forms for a period of 90 days. Following the exposure, glycogen, glucose, glycogen phosphorylases, lactate, pyruvate , succinic dehydrogenase (SDH), lactate dehydrogenase (LDH)and glucose-6-phophate dehydrogenase (G6PDH) were measured on 30, 60 and 90 days .Alterations in enzyme and metabolite levels exhibited tissue specific and time dependent variations. Impairment in SDH in the tissue suggests the animal's dependance on glycolytic mechanism for the energy demands.

1566 CHROMIUM DISPOSITION IN RATS FOLLOWING CHRONIC EXPOSURE TO HEXAVALENT Cr IN DRINKING WATER.

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Hexavalent Cr (VI) is more toxic than trivalent Cr (III) because it is actively transported across plasma membranes and is reduced via unstable reactive intermediates to Cr III. Intracellular Cr III is an important mediator of Cr VI carcinogenesis and toxicity. Although the U.S. EPA drinking water standard for Cr is 100 ppb, humans have sometimes been exposed to as much as 10 ppm Cr VI in drinking water from contaminated wells. The risks to these individuals are not well-understood because the digestive tract reduces some of the Cr VI to less bioavailable Cr III prior to absorption and the disposition of the remaining Cr VI has not been well-studied. We determined tissue Cr concentrations in rats after chronic ingestion of Cr VI in drinking water at concentrations relevant to human exposure levels. Adult male and female Fischer 344 rats (N=7 per treatment) consumed *ad libitum* 0, 0.5, 3, or 10 ppm Cr VI, as K_2CrO_4 in drinking water for 44 weeks. Renal Cr concentrations varied significantly with Cr VI exposure (ANOVA, $p \leq 0.0001$) and were significantly elevated in rats that drank 10 ppm Cr VI. Similarly, bone Cr concentrations varied significantly with Cr VI exposure (ANOVA, $p \leq 0.0001$) and were significantly elevated in rats that drank 10 ppm Cr VI. Hepatic Cr concentrations were not different among rats in different Cr VI treatment groups (ANOVA, $p \leq 0.07$). Brain and whole blood Cr concentrations were below detection limits (0.1 μ g Cr/g or ml) in all exposure groups. The Cr accumulation in bone and kidney in the rats given 10 ppm Cr VI may have resulted from absorption of a greater amount of Cr III and/or less effective clearance. However, Cr III is poorly absorbed therefore the increased tissue retention may also have resulted, in part, from increased absorption of Cr VI and its subsequent uptake from the systemic circulation.

1567 EVALUATION OF EFFECTS OF SUBCHRONIC EXPOSURE TO POTASSUIM DICHROMATE (Cr-VI) AND CHROMIC CHLORIDE (Cr-III) ON TESTICULAR HISTOPATHOLOGY AND SPERM PARAMETERS IN MICE.

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Studies in the literature suggest that exposure to Cr-VI results in a reduced sperm count as well as a lower number of viable fetuses, fewer implantation sites, an increased number of resorptions and a greater number of dead fetuses (Elbetieha, 1997 and Zahid, 1990). The present study was conducted to characterize dose dependent accumulation of chromium in blood and testis as well as to determine whether histological changes occur following such exposure. Male Swiss albino mice were given drinking water containing Cr-VI (1000 ppm or 750 ppm) or Cr-III (2000 ppm) for twelve weeks. Previous studies demonstrated that the maximum tolerated dose for Cr-VI was in the range of 1000 ppm, whereas that for Cr-III was at least 2000 ppm. At the end of this study, total chromium concentrations in blood, testis, kidney, liver and spleen were monitored by atomic absorption spectrometry. Total chromium concentrations in whole blood were three-fold higher in the Cr-VI (1000 ppm) group [450 ± 80 ppb] and two-fold higher in the Cr-VI (750 ppm) group [295 ± 78 ppb] than in the Cr-III (2000 ppm) group [134 ± 48 ppb], respectively. Chromium concentrations in the testis following administration of Cr-VI (1000 ppm) and Cr-III (2000 ppm) were similar, whereas kidney concentrations in the Cr-VI (1000 ppm) group [2.27 ± 0.43 mg/g tissue] were two times that of the Cr-III (2000 ppm) group [1.19 ± 0.48 mg/g tissue]. Tissue histology and sperm parameters (sperm count, sperm motility, and sperm morphology) were also evaluated. Cr-III at 2000 ppm did not

affect sperm parameters whereas Cr-VI produced a significant reduction in epididymal sperm count to less than 40% of control, but produced no change in sperm motility or sperm morphology. No significant testicular histopathology was found with either Cr-VI or Cr-III. These studies indicate that Cr-VI may interfere with sperm production and/or viability.

1568 CHROMIUM(VI) INDUCES A HORMONE-INDEPENDENT ACTIVATION OF THE GLUCOCORTICOID RECEPTOR.

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We previously reported that the genotoxic human lung carcinogen, chromium(VI), significantly and preferentially altered expression of the model inducible gene, phosphoenolpyruvate carboxykinase (PEPCK), in whole animal and cell culture systems following a single low dose which caused no overt toxicity. These effects were closely associated with chromium-induced DNA damage and repair. Chromium exposure was shown to paradoxically increase basal PEPCK expression while blocking the normal hormone induction of this gene by dexamethasone (Dex). Mutation of the GREs or their accessory factor binding sites within the PEPCK promoter abolished both hormone induction and chromium sensitivity of a luciferase transgene in stably transfected cell lines. We now report that non-cytotoxic doses of chromium can activate the glucocorticoid receptor (GR) and increase GRE-regulated gene expression in a hormone-independent manner. Chromium treatment of H4IIE rat hepatoma cells (20 μM, 0.5-4 hr) caused an increase in nuclear GR levels comparable to levels induced by Dex, and increased in vitro binding of nuclear extracts from treated cells to a glucocorticoid receptor (GR) recognition sequence (GRE). Chromium treatment increased expression of transiently transfected luciferase transgenes consisting of tandem GREs within a minimal promoter, but blocked the normal induction of these transgenes by Dex. Thus, the glucocorticoid receptor pathway appears to be one of the principal targets for the effects of chromium on PEPCK expression. We currently hypothesize that the effects of chromium(VI) on GR are a result of a direct allosteric mechanism rather than via the hormone binding site of GR. These results suggest that chromium(VI), and perhaps several other toxic metals such as arsenic, nickel and cadmium, may also be able to act as "endocrine disrupters" at low doses through a novel mechanism. (NIH-NIEHS ES07373)

1569 Ni(II) IN ALBINO VERSUS NORMALLY PIGMENTED *XENOPUS* EMBRYOS AFTER EXPOSURE TO 63NiCl2.

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The influence of pigmentation upon 63Ni(II) uptake and distribution was studied in *Xenopus laevis* embryos exposed to 63NiCl2 according to the FETAX (Frog Embryo Teratogenesis Assay: *Xenopus*) protocol. Ten groups of 60 embryos were tested, including progeny from 4 pairs of albino male and female adults and from 6 pairs of normally pigmented male and female adults. Twelve embryos in each group were exposed to 63NiCl2 (2.1 μmol/L) in FETAX medium during one of the following periods post-fertilization: 5-9 h, 9-29 h, 29-53 h, 53-77 h, and 77-101 h. The embryos were then rinsed, depurated for 4 h in non-radioactive FETAX medium, and either assayed for 63Ni content by liquid scintillation counting, or frozen on CO2-ice, embedded in carboxymethylcellulose, and examined by autoradiography. The progeny of albino adults exhibited varying degrees of albinism; some were totally apigmented; many showed sparse punctate deposits of melanin in the retina, skin, or gut wall. Following exposures to 63Ni(II) from 5-9 h, 9-29 h, or 29-53 h post-fertilization, the contents and distribution of 63Ni in albino embryos did not differ from the normally pigmented ones. Following exposure to 63Ni(II) from 53-77 h or 77-101 h post-fertilization, the 63Ni contents were significantly lower in the albino embryos, compared to the normally pigmented ones. For example, in albino embryos exposed from 77-101 h, 63Ni content averaged 8.7 (SD ± 2.5) pmol/embryo, versus 17.1 (SD ± 3.6) pmol/embryo in normally pigmented embryos ($p < 0.05$). After 63Ni(II) exposure from 53-77 h or 77-101 h, autoradiograms showed less radioactivity in the retina and gut wall of albino embryos, compared to the normally pigmented ones; low, evenly-distributed radioactivity was evident in most other tissues of the albino and normally pigmented embryos. These observations suggest that 63Ni-binding to melanin or its precursors may play an important role in nickel metabolism and toxicology. (Supported by NIH grant ES-05331 (NIEHS).)

1570 NICKEL ACCUMULATION IN RAT PRIMARY HEPATOCYTE CULTURES.

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The present study was designed to clarify the mechanism of Ni uptake in primary cultures of rat hepatocytes. Exposure of the hepatocytes to Ni (2-50 uM; as NiCl2) for up to 6 h was not cytotoxic, as assessed by the tetrazolium-based dye (MTT) assay. Hepatocytes were treated with 10 uM NiCl2 in the absence or presence of calcium (Ca) and magnesium (Mg) (1 mM). Ni uptake was increased by 19% in medium lacking Mg or Ca and was increased by 37% in Ca- and Mg-free medium. The role of Ca channels on Ni uptake by the hepatocytes was investigated. Pretreatment with nicardipine or verapamil (200 uM), a potent inhibitor of Ca channels, decreased Ni uptake by 20%. This effect was only observed when the cells were incubated in the absence of Ca. Pretreatment with vasopressin (100 nM), a well known Ca channel agonist, significantly increased Ni uptake by the hepatocytes (24%). To determine the involvement of carrier-mediated processes on Ni uptake, the effect of temperature was also investigated. At 4C the Ni uptake was decreased by 20% compared to uptake at 37C. These results indicate that Ni uptake by the hepatocytes occurs, at least in part, through the Ca channel transport processes. Further study will be required to assess what other mechanisms are involved.

1571 INTRACELLULAR CLEARANCE OF NICKEL COMPOUNDS: AN IMPORTANT DETERMINANT OF CARCINOGENIC POTENTIAL.

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Inhalation exposure to nickel refinery dust, most of which was assumed to be nickel subsulfide, has been associated with the development of lung and nasal tumors in humans. Animal inhalation carcinogenicity studies indicate that nickel carbonyl and nickel subsulfide produce lung tumors in rats, but nickel oxide and nickel sulfate do not. The assumption has been made for some time that insoluble nickel compounds were human carcinogens, while soluble nickel compounds were not. However, evaluation of the pharmacokinetic data for nickel compounds strongly suggests that classification of these compounds based on their water solubility into soluble and insoluble species is unlikely to be predictive of carcinogenic potential. For example, the clearance of the highly potent nickel subsulfide form the lung is intermediate between the inactive soluble sulfate and insoluble oxide. Rather, both the residence time and mode of clearance, not only along the bronchial airways surface but also intracellularly, provides a more informative basis for predicting carcinogenic potential. Intracellular exposure provides a reasonable explanation for the carcinogenic differences in these three nickel compounds that may be applicable to other nickel species.

1572 TOXICITY OF DEPLETED URANIUM FRAGMENTS IN WISTAR RATS.

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The objective of this study is to assess the carcinogenic risks associated with long-term exposure to depleted uranium (DU) shrapnel in wounds. The toxicity through 15 months after DU implantation in rats is reported here. The approach is to determine the carcinogenicity of foreign-body implants of fragments of DU (containing 0.75% titanium) in tissues relative to implants of a non-radioactive metal tantalum (Ta), as a negative control, and to injections of radioactive Thorotrast, a radiographic contrast media of thorium dioxide colloid, as a positive control. The test metals, in four squares (2.5 x 2.5 x 1.5 mm or 5.0n x 5.0 x 1.5 mm) or pellets (2.0 x 1.0 mm diam) were implanted in the quadriceps muscles of the thigh in male Wistar rats, 50 rats per group. The groups received a mass of DU metal that ranged from 0.119 to 2.79 g with a surface area that ranged from 31 to 320 mm^2 per rat. Body weight gains were significantly reduced in rats with the largest mass of DU. The type or amount of implanted material did not affect survival to 15 months. Radiographs of the implants showed marked corrosion on the surface of the DU fragments as early as 3 weeks after implantation. No surface changes were observed on the Ta controls. A fibrous capsule developed

around the implanted metals, but not the injected Thorotrast. The capsules were much thicker around the DU implants. These results indicate that DU, in sufficient dose, reduces weight gain and causes a local tissue reaction, but does not affect life span or carcinogenicity to 15 months after implantation in Wistar rats. The rats will be observed for another 9 months for evidence of neoplasia related to the implants. Research sponsored by the US Medical Research Development Command under MIPR No. KFM5529 with the US DOE, under cooperative agreement DE-FC04-96AL76406, in facilities fully accredited by the AAALAC.

1573 EFFECT OF 14 DAY ZINC CHLORIDE EXPOSURE ON TISSUE DISTRIBUTION OF ZINC IN RATS.

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Male and female Sprague Dawley rats were administered either 0.0, 60.0, 80.0, 120.0, or 160.0 mg/kg/day of zinc chloride via oral gavage to determine levels of zinc accumulation in different organs. Zn accumulation in the brain, liver, kidney, pancreas, spleen, thymus, ovary, uterus, epididymis, seminal vesicle and testis were analyzed by atomic absorption spectrophotometry. Body weight comparison and Zn accumulation were not made for the 120 mg/kg/day in males and 120 and 160 mg/kg/day in the females due to the high rate of mortality. Males had 3.60, 1.60 and 1.26% reductions in body weight gain in the 60.0, 80.0, and 160.0 mg/kg/day dose groups, while females had 1.0 and 0.2% reductions in the 60.0 and 80.0 mg/kg/day dose groups, respectively when compared to controls. In males, Zn accumulation in the pancreas and spleen at 160.0 mg/kg/day and in the liver at 80.0 and 160.0 mg/kg/day were significantly higher than the control. In females, Zn accumulation in the brain, kidney, spleen and ovary at 80.0 mg/kg/day were significantly higher than the control. Zn accumulation data showed a dose dependent increase in all organs of both sexes. (Supported by MHPF/ATSDR Cooperative Agreement # 5R51/ATR398004.)

1574 PRELIMINARY COMPARISON OF TWO SOIL METAL SURVEYS IN NEW ORLEANS.

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This preliminary report evaluates similarities and differences of the quantity of Pb, Zn, Cd, Mn and Ni in soil in New Orleans between Survey I (1989-1991) and Survey II (1998-). In both surveys, three types of soil samples were collected in each census tract, streetside (9 samples) open space (3 samples) and foundation (3 samples). Streetside samples were collected from residential streets, at least one block away from the busiest road(s). All soil samples were collected from the top 2.5 cm by surface scrape. This preliminary report compares available data of Survey II (to date 55 or 19.2% of 286 census tracts) with data collected during Survey I from the same census tracts. All soil samples are air-dried, sieved (2 mm screen), shaker extracted 5:1 (volume to weight) with 1 mol L^{-1} nitric acid for 2 hours at room temperature, centrifuged (1000 x g for 15 min.), and filtered (Qualitative P4 Filter Paper). The extracts were analyzed for Pb, Zn, Cd, Mn, and Ni using Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). The data are non-parametric and median results of the two surveys were analyzed for differences and similarities for Pb, Zn, Cd, Mn, and Ni using the Wilcoxon Signed Rank Test. Pb, Zn and Mn are not significantly different between Survey I and Survey II. This is expected because these metals are generally immobile and relatively stable in soils. There are significant reductions for Cd and Ni between Survey I and Survey II. The apparent reductions of both Cd and Ni suggest previous sources of these metals have curtailed and that Cd and Ni are mobilizing from urban soils. Further study is needed to evaluate these apparent changes. (Supported by ATSDR cooperative agreement # U50/ATU398948 to Xavier University.)

1575 EFFECT OF ORAL FRENOLICIN-B TREATMENT ON ESSENTIAL TRACE ELEMENTS IN MALE RATS: RELATIONSHIP TO PROLIFERATIVE LESIONS IN SELECTED TISSUES.

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Frenolicin-B (FB), a substituted 1,4-naphthoquinone antibiotic, produces inappetance and epithelial hyperplasia (EH) in the esophagus of rats; findings that also have been induced in rats by zinc (Zn) deficiency. FB also chelates divalent cations *in vitro*. The present studies were performed to determine if Zn and/or cation chelation is associated with FB-induced EH in the esophagus and selected tissues of male rats. Male Sprague-Dawley rats (*ca.* 250g; n=20/group) were fed diets containing either adequate (40ppm), deficient (<1ppm), or fortified (100ppm) Zn concentrations *ad libitum* for 28 days. Two groups (4 and 5) fed either Zn-adequate or Zn-fortified diets also were given 100 mg . $kg^{-1}.day^{-1}$ of FB in the diet, while groups 6 and 7 fed either Zn-adequate or Zn-fortified diets served as pair-fed controls. After 28 days of treatment, blood was collected for clinical chemistry and elemental analyses, rats were sacrificed and selected tissues from 10 rats/group were processed for either histopathological evaluation or elemental analyses. Compared to pair-fed controls, FB-treated rats had significantly lower mean body weight gains (67% and 74% of groups given Zn-adequate and Zn-fortified diets, respectively). FB treatment resulted in decreased hematocrit and hemoglobin concentrations, and decreased plasma activities of alkaline phosphatase (a Zn metalloenzyme). FB treatment also resulted in increases in platelet count and plasma cholesterol and phosphorus concentrations. Marginal and inconsistent FB-induced changes in plasma/tissue Zn, iron, magnesium and calcium were observed. However, mean copper (Cu) concentrations were significantly reduced in blood (30% of control) and all tissues evaluated except the kidney from FB-treated rats (10 to 64% of control). Although mean kidney Cu concentrations in FB-treated rats were comparable to control, a significant positive correlation was observed between kidney and plasma Cu; FB-induced increases in kidney weight with tubular proteinaceous deposits also were observed. FB treatment resulted in EH of the non-glandular stomach, but not the esophagus. Data suggest that FB selectively reduces circulating/storage Cu *in vivo*, possibly by the elimination of a FB-Cu chelate.

1576 EYE IRRITATION FROM CONSUMER PRODUCTS: COMPARISON OF HUMAN EXPERIENCE AND DRAIZE EYE TEST DATA.

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Eye irritancy data were reviewed for 12 household cleaning products. Human experience data captured by the American Association of Poison Control Centers' (AAPCC) Toxic Exposure Surveillance System (TESS) for the year 1996 were compared with the results of Draize eye irritation testing (0.1 ml; no rinsing). A mathematical model was employed and linear regression analysis was performed. An Outcome Index Factor (OIF) or weighted average of AAPCC TESS data was calculated for each product according to the equation: 7 (# of incidents x outcome)/(total # of ocular calls), where outcomes were assigned values of 0 for no effect, 1 for a minor effect, 5 for a moderate effect, or 10 for a major effect. The OIF was plotted against the Maximum Average Draize Score (MAS) for each product. There was poor correlation between the OIF and MAS (0.56), indicating that the 0.1 ml Draize test results generally overestimate the outcome of eye exposures. The results of this analysis are consistent with the findings of Freeberg et al (J Toxicol-Cut & Ocular Toxicol 1(3), 53-64 (1984).

1577 DISTRIBUTION OF LEAD (Pb) AND TRANSTHYRETIN (TTR) IN HUMAN EYE.

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The retinal pigment epithelium serves as a defensive barrier to the retina in the same way that the choroid plexus (CP) functions in the brain. Previous studies have shown that the CP sequesters Pb, which results in reduced production and secretion of TTR. This study was undertaken to examine whether Pb was accumulated within human ocular tissues and to learn about associated levels of TTR. Eight pairs of human eyes, aged 19-90, not suitable for corneal transplantation, were obtained from the NYC Eyebank within 24 hr of postmortem. The eyes were dissected to obtain aqueous, vitreous, retina, and choroid. The samples were stored at -80°C until the time of the assays. Pb and TTR concentrations in ocular tissues and liquids were determined by AAS, for Pb, and radioimmunoassay, for TTR. Our results showed that Pb accumulated in the retina at concentrations that were 166, 739, and 5 times higher than those in aqueous, vitreous, and choroid, respectively. Pb

disposition in ocular tissues or intraocular liquids, however, did not seem likely to be age-related. The average concentration of TTR in vitreous humor, 14.4 ± 5.1SE μg/ml, was twice as much as in aqueous humor, 7.0 ± 1.1SE μg/ml. We also found that TTR concentrations in retina were strongly associated with vitreous TTR levels (correlation coefficient = 0.9081, $p<0.005$), but not aqueous TTR levels. This preliminary study suggests that Pb accumulates in human ocular tissues and particularly in retina. The biochemical, physiological, and clinical implications of Pb disposition in human eyes are currently under investigation. (Supported in part by private funds and NIEHS ES08146.)

1578 ELECTRORETINOGRAM (ERG) RECORDING AND INTRAVITREAL INJECTIONS IN RABBITS AND MONKEYS.

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Background data for Dutch-belted rabbits and cynomolgus monkeys were obtained using an EPIC 2000 ERG recorder and a Grass flash stimulus with the background light off (scotopic conditions). Under anesthetic, following 30 minutes dark adaptation, rabbit eyes were tested independently (due to the lateral position of the eyes) and monkey eyes simultaneously. ERG protocol consisted of 4 tests: Blue filter (rod only response), red filter (rod and cone response), white (maximal response) and 30 Hz white flicker (cone only response). An average of 5 single flashes with 5 seconds between flashes comprised the blue, red and white tests. White flicker stimulus consisted of an average of five 5 second periods of white light stimulus at 30 Hz. Parameters measured from the waveforms were b-wave amplitude and latency. ERGs were recorded in untreated rabbits (n=63) and monkeys (n=22) and monthly or every other week for up to 32 weeks following single or multiple intravitreal injections of 50 μL of 0.9% NaCl or phosphate buffered saline. In untreated animals, only small differences between left and right eyes were apparent in mean results, though larger differences were occasionally observed for individual animals. Amplitude and latency in serial ERGs following single or multiple intravitreal injections generally remained consistent, with an occasional small transient decrease in b-wave amplitude shortly following intravitreal injection, which resolved by the subsequent dose/ERG measurement. The coefficient of variation (CV) for b-wave amplitude in untreated animals (a measure of individual variation) was approximately 20-30% within a given parameter. In treated animals, CV over time was 6 - 10% for single flash stimuli and 15-19% for flicker stimuli, sufficiently low to permit the onset of a retinal change to be ascertained. Thus combined with ophthalmology examinations, tonometry and histopathological examination, ERG recording is an important aid in the overall evaluation of changes in the retina.

1579 13-WEEK FEEDING STUDY WITH AN AROMATIC DIAMINE IN ALBINO RATS.

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Groups of 10 male and 20 female Crl:CD albino rats were fed diets containing 0, 100, 500, 1000, or 1500 ppm of 1,3-bis(4-aminophenoxy) benzene. Ten rats/sex/group were killed after 13 weeks of treatment and remaining groups of 10 female rats were killed after a 13-week recovery period. After 13 weeks, a slight anemia was seen in females at ≥ 500 ppm and in males at 1500 ppm; anemia was not found after a 1-mo recovery. Hepatic hypertrophy and eosinophilic foci were found in male and female rats at ≥ 1000 ppm. One of 10 males at 1500 ppm had a thyroid follicular cell carcinoma; this change was not accompanied by similar changes in any other treatment or recovery group rats, nor by proliferative changes such as hyperplasia or adenoma. In the eyes, retinal pigment epithelium hypertrophy, retinal photoreceptor degeneration/necrosis, and choriocapillaris atrophy were seen in females at ≥ 500 ppm and in males at ≥ 1000 ppm. Following 13 weeks of recovery, minimal hepatocellular eosinophilic foci were seen at ≥ 100 ppm. In addition, increased mitotic figures were detected in the liver of 1500 ppm females. Retinal injury, of comparatively greater severity than that noted after 13 weeks of exposure, was seen in females at 1000 and 1500 ppm. In mesenteric lymph nodes, macrophage aggregates (a common change in aged rats) were seen at all dose levels. The no-observed-adverse-effect level was 100 ppm based on the retinotoxicity observed in female rats at 500 ppm.

1580 SPONTANEOUS OCULAR FINDINGS IN THE COMMON MARMOSET (*Callithrix jacchus*).

O Loget, G Chevalier and R Forster. *CIT (Centre International de Toxicologie), Evreux, France.* Sponsor: R Glomot.

There is currently great interest in the use of common marmosets in toxicology and teratology. The present study was undertaken to provide background data on the normal pattern of ophthalmological findings in the marmoset. The examinations performed were those generally required for toxicity studies in non-rodent species. The following examinations were undertaken on a population of marmosets: the ocular reflexes (corneal, palpebral, direct and consensual pupillary reflexes) were evaluated. Macroscopic examination of the eye and adnexae was performed and more detailed examination was made using the head light source of the ophthalmoscope. After administration of a mydriatic, examinations were performed using a binocular indirect ophthalmoscope and a slit lamp biomicroscope. Sedation was required only for photography of the fundus, which was performed in a few instances. The reflexes were found to be normal in all animals. The principal ophthalmological findings were of developmental origin or of non determinate etiology and were: hyaloid artery remnants in the vitreous body, tigroid fundus, pupillary membrane remnants, posterior cortical pinpoint opacities and posterior capsular opacities of the lens. Other minor findings were observed at a lower incidence. The eye of the marmoset showed anatomical similarities with the human eye as regards the retina, the structure and curvature of the lens, and the holangiotic vascularisation of the fundus. These characteristics suggest that the eye of the marmoset will be a good experimental model for the identification of ocular adverse effects in general toxicology studies.

1581 EVALUATION OF CORNEAL DAMAGE USING CONFOCAL MICROSCOPY.

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Area and depth of corneal injury have been shown to be diagnostic parameters for corneal response to surfactant-based substances *in vivo*. In the current work, an *ex vivo* method has been developed to examine damage to the cornea by surfactants and surfactant-based product formulations using confocal microscopy. Rabbit corneas, derived from commercial abattoir tissues, were exposed to test substances on the epithelial surface for up to 30 seconds. The corneas were washed, then incubated with fluorescent stains to differentiate live and dead cells and provide clear visualization of corneal cell damage by confocal microscopy. Several stain combinations were evaluated, including Calcein AM with Ethidium Homodimer-1 (C-AM/EthD-1), and Syto® 10 with Dead Red™ (Syto/DR). The C-AM/EthD-1 method results in live cells with green cytoplasm and dead cells with a red nucleus. The cytoplasmic staining also provides information about the size of the cell. Syto 10/Dead Red staining results in a field of fluorescent nuclei, live stained green and dead stained red. Confocal microscopy allowed determination of the distribution of cell damage in the corneas in three dimensions. The x, y, and z position of each nucleus and cell was calculated using image analysis, and mosaics were created of adjoining 3-D images. Using commercially available software (JMP), the x, y, and z data were plotted and evaluated for patterns of damage. Using prototype anionic and cationic surfactant-based formulations and ingredients, it was possible to distinguish substances and test conditions producing mild (epithelium alone), moderate (epithelium and stromal), and strong (full-thickness) damage to the corneas. It is proposed that the use of *ex vivo* rabbit corneas and quantitative confocal microscopy provide an alternative method to assess corneal responses that is linked directly to the *in vivo* mechanism of response (area and depth of injury).

1582 AN *IN VITRO* HUMAN CORNEAL MODEL FOR OCULAR IRRITANCY TESTING.

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The cornea is the major target of concern in the anterior segment of the eye following accidental exposures to chemicals or product formulations. The current work is focused on development of human corneal cultures as alter-

native models for assessment of corneal injuries. We have developed human corneal cells lines from the three main cell layers (epithelium, stroma and endothelium). These cell lines have been used in monolayer cultures to examine the effects of chemical irritants on mRNA expression of early response genes, cytokines and extracellular matrix molecules. We have also used the cell lines in combination with a mixture of native and/or chemically modified extracellular matrix molecules as scaffolding to reconstruct an artificial cornea *in vitro*. The reconstructed corneas contain the three cell layers (epithelium, stroma and endothelium) and resemble human eye bank corneas that have been maintained in tissue culture. Prototype chemical irritants have been applied to the epithelial surface of the reconstructed corneas to model *in vivo* exposures. The expression of early response genes, cytokines and extracellular matrix genes that are thought to have a role in the ocular irritancy response and in wound healing have been determined. Preliminary results indicate that there are differential patterns of gene expression with different chemicals. Experiments comparing responses to irritant chemicals in the monolayer cultures vs. the reconstructed corneas are in progress. The knowledge obtained to date is being used to further refine the reconstructed human corneas and their use as an alternative to animal tests for ocular irritancy. It is proposed that reconstructed human corneas will be a mechanistically sound model for the tissue and species of concern. Further, the base technology can be used to reconstruct other *in vitro* human tissues for toxicity testing.

1583 THE EPIOCULAR PREDICTION MODEL: A REPRODUCIBLE IN VITRO MEANS OF ASSESSING OCULAR IRRITANCY POTENTIAL.

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The EpiOcular™ tissue model (OCL-200) is an organotypic model of the human corneal epithelium (HCE) cultured from normal human keratinocytes using serum free medium. Paraffin embedded, H & E stained histological cross-sections show the structure of EpiOcular closely parallels that of the HCE. During 1996 and 1997, 47 and 82 lots, respectively, of the OCL-200 model were produced and monitored using the MTT assay. The common surfactant, Triton X-100 (0.3%), was used as a positive control and the Effective Time-50 values (time of exposure after which viability is reduced to 50%, or ET-50) was determined for each lot of tissue. The ET-50 average of 24.9 ± 6.3 minutes (± 1 standard deviation) for 1996 (n=47 lots) was not statistically different ($p < 0.05$) than the 1997 average of 22.9 ± 4.7 minutes (n = 81 lots). Stability of the EpiOcular model for international shipment was probed using these standard quality control tests following packaging and storage of the tissue for 4 days; both positive and negative controls and histology of the tissue remained constant. Interlaboratory testing also confirmed the reproducibility of the tissue. Coefficients of variation (c.v.) for 3 materials tested in 3 laboratories were all below 10%. ET-50 values were measured for 19 water-soluble chemicals from the ECETOC database and 41 materials including final formulation shampoos, off-the-shelf cosmetic/personal care products, and surfactants. Draize data for these materials were correlated to ET-50 values and a prediction model was constructed: Draize = 4.735+101.65/(ET-50)^0.5 (correlation coefficient, r = 0.90). Thus, the EpiOcular tissue model is reproducible and appears to be an accurate *in vitro* means of predicting *in vivo* ocular irritancy.

1584 COMPARATIVE CYTOTOXICITY OF MITOMYCIN C AND 5-FLUOROURACIL IN IMMORTALIZED RABBIT CORNEAL ENDOTHELIAL CELLS.

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Mitomycin C (MMC) and 5-fluorouracil (5FU) are drugs currently used to stem cell proliferation following glaucoma filtration surgery. Their respective cytotoxic mechanisms on the corneal endothelium, however, differ. In *in vitro* experiments using immortalized rabbit corneal endothelial (RCEndo) cells, MMC elicited significant cytotoxicity (Neutral Red Uptake) after 1 h with 50 μg/ml and after 4h with 2 μg/ml. Higher doses of 5FU, 20 mg/ml after 1 h and 4 mg/ml after 4 h exposure were required to elicit significant cytotoxicity. Both drugs produce significant increases in protein carbonyl content, which suggest drug-induced oxidative insult. MMC produced a dose-dependent loss of GSH, a rise in GSSG and a reduction of the ratio of GSH/ GSSG. MMC reduced GSH S-TR by nearly 40% and GSH-PX by 28 % after 4 h of exposure but did not affect GSSG reductase activity. However after 4 h, 5FU produced a dose-dependent decrease in corneal endothelial cell GSH as well as increases in GSH-PX (4.2-fold, 20mg/ml) and GSSG Reductase (1.6-fold, 20 mg/ml) activities. DT-diaphorase was inhibited in a dose-dependent manner by MMC but was significantly increased by 5FU after 4 h. These data indicate that both drugs can induce oxidative-injury to corneal endothelial cells but that the differences in the toxic potency between 5FU and MMC may be due to effects on the glutathione redox cycle and DT-diaphorase in corneal endothelial cells.

1585 REVIEW OF THE LONG-TERM IN-HOUSE USE OF AN *IN VITRO* TEST BATTERY FOR PREDICTING SEVERE OCULAR IRRITANTS.

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In 1994, we reported the development of an *in vitro* test battery for use within a stepwise approach to the assessment of ocular irritancy *in vivo* (Lewis *et al*, Tox *In Vitro*, 8, 865-6, 1994). The battery comprised a cytotoxicity assay using the K562 cell line followed by the isolated rabbit eye test. In contrast to the use of data from rabbit skin irritation tests, the *in vitro* battery was significantly more predictive; 83% of severe eye irritants were predicted compared to 23% by the use of *in vivo* skin irritation data; the battery was also highly specific (85%). This was based on more than 200 compounds, covering a wide range of chemical classes and formulations. This paper reports the extended use of this *in vitro* battery from 1991-1997. Using the same methods and prediction model, 1,136 compounds have now been assessed *in vitro*; 832 of these materials were also tested *in vivo* using CTL's approach of initial testing in one rabbit (using a "low volume" - 0.01ml/0.01g - following a prediction of severe irritant potential) with no further *in vivo* testing following a severe response. Correct predictions were made for 580 (70%) of these materials; sensitivity and specificity were both approximately 70%, with 19/65 confirmed severe eye irritants being under-predicted; the over-prediction rate was 233/767. Of the 19 false negatives, 14 were either surfactants or surfactant-containing pesticide formulations; however, many similar materials were correctly predicted or even over-predicted and no clear structure-activity relationship emerged from the analysis. Although this represents a reduction in performance of the battery compared to its initial use in CTL, it still allowed a reduction in the percentage of severe ocular irritants which were actually tested *in vivo* - only 2% (19/832) rather than 8% (65/832) - a significant refinement. In addition, many of the 304 compounds which did not proceed to *in vivo* testing were assessed *in vitro* to select the least irritating chemicals or formulations in a series, thus giving substantial reduction in animal use.

1586 CORRELATION OF THE BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) ASSAY WITH THE RABBIT DRAIZE EYE IRRITATION ASSAY USING TRADITIONAL AND HISTOPATHOLOGICAL ENDPONTS.

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The ability of the BCOP to predict the outcome of the Draize eye irritation assay was examined for laundry and home cleaning formulations. Based on screening experiments, the contact time of the products in the BCOP was reduced from 10 minutes to one minute to better differentiate between moderate and severe irritants. Two Draize parameters were used; the Maximum Average Draize Score and Days to Clear. These were compared to the standard BCOP score as well as the opacity and permeability scores. The best overall linear correlation (r=0.85) was between the calculated BCOP score and the maximum average Draize score. The second best correlation was between the BCOP opacity value and the maximum average Draize score (r=0.82). In addition to traditional BCOP endpoints, the corneas were also examined via histopathology. Injury severity was graded based on the structures of the cornea that were injured, their importance in recovery after eye injury and their depth in the cornea. Based on these factors, a "histopathology" score was assigned. These data were correlated versus the recovery time in the Draize eye irritation assay.

1587 HISTOPATHOLOGY ASSOCIATED WITH OPACITY AND PERMEABILITY CHANGES IN BOVINE CORNEAS IN VITRO.

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The Bovine Cornea Opacity and Permeability (BCOP) assay has been used

to evaluate the ocular irritation potential of a broad range of chemicals. However, the mechanisms by which opacity and permeability are induced are poorly understood and may well be different among chemical classes. We have used histopathological evaluation of corneas treated in the BCOP assay to obtain a concurrent assessment of the degree and depth of injury, and other qualitative tissue changes, associated with the in vitro opacity and permeability measurements. Corneas were fixed after completion of the fluorescein permeability step, bisected, embedded, sectioned, and stained with hematoxylin and eosin. Increased opacity readings were associated with two general types of damage, epithelial cell condensation (typical of acids and some organic solvents) and stromal swelling (typical of cationic surfactants and highly alkaline materials). If epithelial condensation was present, there was generally relatively low fluorescein permeability even when the epithelium was badly damaged. Stromal swelling, either microscopic (separation of the collagen fibrils progressing from the anterior to the posterior) or macroscopic (visible increases in overall cross sectional thickness), was usually accompanied by epithelial damage and increased permeability. In general, increased permeability reflected progressive damage to the epithelial cell layers, particularly the basal cell layer that accounts for the majority of the permeability barrier.

1588 A LONGITUDINAL ANALYSIS OF BENCHMARK AND CONTROL VALUES IN THE BCOP ASSAY.

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The Bovine Corneal Opacity and Permeability (BCOP) assay has been employed internationally to evaluate the ocular irritation potential of a wide range of test materials: surfactant-based formulations, organics, and fragrance materials. To evaluate the reproducibility of the assay over time, a longitudinal analysis of the scores from a concentrated fragrance benchmark and the assay positive control was made to ascertain possible changes over time. The benchmark was a moderately irritating complex fragrance mixture used as a comparative standard for evaluating prototype air fresheners. Ethanol exposures of ten minutes constituted an internal positive control for monitoring performance of the assay system. Over sixty benchmark values obtained in the assay over a five year period were plotted for both ten and thirty minute exposure times. The analysis showed that: 1) The assay was capable of significantly distinguishing a minor change in benchmark preparation ($p<0.001$); 2) A relocation of the testing laboratory led to no significant change in the studied values; 3) The degree of variance was significantly greater for the benchmark than for the positive control ($p<0.001$); and 4) The assay demonstrated internal consistency between exposure times for the benchmark ($r^2=0.75$). Studying the benchmark and control values over time in the BCOP Assay can be a valuable tool in monitoring variability and change in the assay system and testing environment.

1589 CORNEAL THICKNESS AS A MEASURE OF CORNEAL HYDRATION IN THE BOVINE CORNEAL OPACITY AND PERMEABILITY (BCOP) ASSAY.

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The Bovine Corneal Opacity and Permeability Assay (BCOP) currently employs measurements of those two parameters to assess eye irritation potential. The concept of corneal hydration was presented in a poster session at the 1998 SOT Annual Conference as a third possible measurement tool when using the BCOP. The purpose of this study was to determine whether corneal thickness correlates with measurements of corneal hydration. With good correlation, this key piece of information could be the trigger for development of a fast and accurate, yet practical, way to measure corneal thickness during routine BCOP procedures. All corneas were mounted in standard BCOP holders and incubated for one hour at 32°C in Eagle's Minimum Essential Medium (MEM). Following this equilibration period, irritant-treated corneas were dosed for 10 minutes with either acetone, 30% sodium lauryl sulfate (SLS), isopropyl alcohol (IPA) or 1% sodium hydroxide (NaOH) and incubated for an additional two hours in MEM. After incubation, the lower opacity corneas were dyed with methylene blue in MEM for 60 seconds to enable the thickness-measuring device to detect the corneal surface. Higher opacity corneas needed no such staining. Four 5mm punches were cut from each cornea and the thickness of each was measured at nine different sites using Smart Scope, manufactured by Optical Gauging Products, Inc., Rochester NY and its accompanying Master Mind Plus software. Thickness differences between treated and untreated corneas were detectable. Average thickness of corneas were 1) untreated = 0.9857mm 2) 30% SLS = 1.0225mm 3) IPA = 1.4158mm 4) acetone = 1.4731mm and 5) 1% NaOH = 1.7657mm. We conclude that measuring hydration via thickness has the practical potential to become part of the BCOP.

1590 AN INTERAGENCY EVALUATION OF THE MURINE LOCAL LYMPH NODE ASSAY (LLNA) IN THE REGULATORY PROCESS.

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The LLNA, an alternative method to guinea pig (GP) tests for the identification of chemicals with potential to produce contact hypersensitivity, was the first test method to undergo independent peer review using the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) process. Although the LLNA has been extensively evaluated and reported in peer reviewed journals, it has not been accepted in the regulatory community as a stand-alone alternative to GP tests. Using validation and acceptance criteria recently developed by the Federal government, an expert peer panel conducted a comprehensive and rigorous review to reach consensus on the usefulness and limitations of the LLNA. The panel concluded that the LLNA was a satisfactory method for evaluating dermal sensitization and that it provided acceptable intra- and inter-laboratory repeatability and reproducibility. Furthermore, the LLNA exceeded the GP tests in method transferability, cost-effectiveness, reduced assay time, quantitative/objective endpoint and refinement of animal use. Based on this review, ICCVAM will make recommendations to relevant agencies concerning the usefulness and suitability of the LLNA. The LLNA review provided insight into the process that ICCVAM has established to evaluate new methods proposed for improved hazard identification and risk assessment with relevance to regulatory agencies. In addition to conserving resources, cost and time involved in the evaluation/acceptance process among agencies, the ICCVAM review process emphasizes the protection of human and environmental safety while attending to animal refinement, reduction and replacement. This organized and consistent approach may protect against premature acceptance of test methods and yet provide a mechanism to facilitate the adoption of scientifically valid and innovative alternatives. (This abstract does not reflect EPA, FDA or NIEHS policy.)

1591 TUMOR CLASSIFICATION FOR EVALUATION OF LONG-TERM RODENT BIOASSAYS.

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Evaluation of long-term rodent cancer bioassays requires analysis of many different organs, tissues and cell types. The manner in which tumor types and sites are combined for statistical analysis can have a significant influence on bioassay interpretation. In this study, we evaluate the influence of several alternative tumor classification schemes for the more than 4000 tumor types in the Cancer Bioassay Data System (CBDS) database of carcinogenesis test results on conclusions about carcinogenic or anticarcinogenic effects of specific chemicals. We compare our standard classification system, based on classification by tumor type, with an alternative classification system developed by Dr. Kenny S. Crump and colleagues. In addition, we test a classification system based on tumor sites, rather than types. We go so far as to evaluate all tumors as one classification, analyzing response by total tumors in control and treated groups. We compare the fraction of chemicals in the database classified as carcinogenic, or anticarcinogenic, under the different classification schemes. There are clear differences in the conclusions that would be drawn using the alternative classification systems. Of course, this would have significant influence on risk assessment and regulation. Often neglected in the past, formulation of tumor classification systems to most appropriately interpret the rodent bioassay will require cooperation of toxicologists, pathologists, and biostatisticians.

1592 MONONUCLEAR CELL LEUKEMIA (MNCL) IN F-344 RATS: IMPLICATIONS FOR HUMAN CANCER RISK ASSESSMENT OF PHTHALATES AND OTHER NONGENOTOXIC CHEMICALS.

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The biological significance of F-344 rat MNCL for humans remains controversial and has confounded recent carcinogenicity studies of several chemicals, including dialkyl phthalate esters. Thus, a survey of the published literature was conducted to assess the occurrence of this finding in F-344 rats and to evaluate its potential significance to humans. MNCL occurs in untreated, aged F-344 rats at a high and variable rate, and is uncommon in most other rat strains. The background incidence of MNCL in F-344 rats has increased over time, which presents problems in retrospective data interpretation. MNCL has not been found in other mammalian species. No histologically comparable tumor is found in humans. Most leukemiogens are genotoxic and this is likely their mode of action. However, phthalates are not genotoxic as a class. Furthermore, in the case of phthalates, there is an apparent threshold dose that must be exceeded before MNCL is seen. The occurrence of a common tumor in the F-344 rat, which is not observed in other species, is an insufficient basis for determining that a chemical presents a carcinogenic hazard to humans. When these considerations are collectively evaluated, it can be concluded that a finding of increased MNCL in F-344 rats exposed for a lifetime to a particular chemical is not biologically relevant to humans, even when MNCL is observed at an increased incidence that is statistically significant. Thus, classification of a chemical as a carcinogen based on increased MNCL in F-344 rats is not scientifically supported. The increased incidence of MNCL observed in F-344 rats exposed to some alkyl phthalates is likely a strain-specific effect of little or no relevance for humans.

1593 CHRONIC BIOASSAYS FOR LIVER TUMORS IN $B6C3F_1$ MICE USING IDEALIZED WEIGHT CURVES PRODUCED BY CONTROLLED FEEDING.

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Use of dietary control (DC) to manipulate body growth in $B6C3F_1$ mice was used in an ongoing chronic bioassay. An idealized weight curve for male $B6C3F_1$ mice, which predicts a terminal background liver tumor incidence of 15%, was constructed and tested using a known mouse hepatocarcinogen. This approach successfully maintained cohorts of mice at weights approximating their idealized target weights throughout the two-year study. These mice exhibited less individual body weight variation than did their *ad libitum*-fed (AL) counterparts (e.g. BW ranged from 25-35g and 25-50g in the DC and AL groups respectively at 1 year of age). This was associated with a decreased variation in liver to body weight ratios, which allowed the demonstration of a statistically significant dose response to the test chemical in the DC, but not the AL test groups. The test chemical, in AL and DC diet groups, increased terminal liver tumor incidence but a statistical significant dose-response was observed only in the DC mice. Furthermore, this dose response positively correlated with markers of peroxisomal proliferation in the DC mice only. This increase in sensitivity did not appear to be due to diet effects on pharmacokinetics since there was no significant difference in the clearance (area under the curve) of the compound or its major metabolite between the DC and AL mice. Thus, DC not only improves terminal survival and decreases inter-assay variation, but can also increase assay sensitivity by decreasing intra-assay variation.

1594 RODENT CANCER BIOASSAYS COULD BE TERMINATED AT 18 MONTHS.

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One of the problems with the rodent cancer bioassay is that it produces "too many positive results" (about 50%). It is often difficult to decide which of these positive results poses a real cancer risk to humans. Clearly, fewer positive results would result from the use of lower dose levels or a shorter duration of study. We evaluated the carcinogenicity studies for chemicals reviewed in IARC Monographs Volumes 1 to 61 to determine the time to onset of treatment-related carcinogenicity (by clinical or pathological examination) and to assess the effect of using an 18 month study duration. Only study reports where chemicals were administered by the oral route, or by other routes not involving the production of site of administration tumors, were included. Several hundred study reports were reviewed on a total of 194 chemicals. Of these, 129 (66%) showed evidence of carcinogenicity in either rats or mice at or before 52 weeks, 48 (25%) between 52 and 80 weeks, and for only 17 (9%) was a study of longer than 80 weeks required to reveal a carcinogenic response. In this last group, most chemicals caused tumors of the liver, forestomach or endocrine organs. These tumors, depending upon the circumstances, have often been cited to be of limited relevance to humans. All of the Group 1 human carcinogens were found to be detected in studies of 15 months or shorter duration. The present analysis indicates that termination of bioassays at 18 months would not result in loss of information relevant to human cancer risk assessment. It would also improve survival and alleviate difficulties associated with the analysis of tumors within a background of age-related pathology.

1595 INTEGRATION OF CANCER AND NONCANCER RISK ASSESSMENT WITHIN A QUANTITATIVE FRAMEWORK OF UNCERTAINTY AND VARIABILITY.

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The current system for evaluating cancer and non-cancer risks used by regulatory agencies differ in their use of models, assumptions and produce different descriptions of risk. Toxicological criteria such as the Reference Dose (U.S. EPA), Minimal Risk Level (U.S. ATSDR), and Allowable Daily Dose (WHO) are establish using safety factors and estimate a dose associated with a minimal risk. The carcinogenic risks are evaluated using a linear dose response model that is intended to characterize the lower confidence limits of the true dose response and a model of interspecies extrapolation. The results of the two approaches differ with cancer risks being described in terms of a dose response slope and non-cancer in terms of a single toxicological criterion. However, both approaches must deal with the same issues including extrapolating to humans from animal results, high-dose to low-dose extrapolation, and management of uncertainty. We believe that both approaches can be placed into a single toxicological framework that describes dose response assessment as an attempt to characterize the lower confidence limit of the uncertainty in the human dose response. Under this framework, the non-cancer toxicological criteria are viewed as lower confidence limits estimates of zero or minimal risk level for compounds with non-zero thresholds. The dose response slope for a carcinogen is defined as the lower confidence limit of the dose response slope for a compound with non-threshold effects. This framework allows the comparison of the strengths and weaknesses of the various steps of the cancer and non-cancer assessment process. In addition, it provides an improved description of the uncertainty in current regulatory criteria.

1596 PCB TUMORIGENICITY IN RODENTS: HOW SHOULD IT BE MEASURED?

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In the past 30 years, several studies in rodents have been interpreted to suggest that PCBs are potentially carcinogenic for humans. More recently, the most comprehensive and detailed rat tumorigenicity study of PCBs ever conducted has been reported, and this study confirms the capacity of PCBs to increase the expression of liver tumors in Sprague-Dawley rats (described in Mayes *et al., Toxicol. Sci.* 41, 62-76, 1998). In 1996, using all available rodent carcinogenicity data (including data from the study cited above), the U.S. EPA reassessed the evidence for tumorigenicity and assigned revised (decreased) PCB cancer slope factors (q1*), based on hepatic tumorigenicity. In addition to hepatic tumors, the recently completed SD rat study allows for the numeration of the total tumor incidence across all sites, an important consideration for determining the true risk of cancer ascribable to PCBs. Total tumor counts, for all primary tumors (benign or malignant) at any site of origin, were determined during 24 months of exposure for the following dose groups: controls (0 ppm) and Aroclors 1016 (200 ppm), 1242 (100 ppm), 1254 (100 ppm) and 1260 (100 ppm). The average total number of tumors per rat for males was 1.35, 1.36, 0.92, 0.98 and 1.68, and for females was 1.83, 2.0, 2.02, 1.82 and 2.12, respectively. For males, decreases in total tumor incidence were identified for Aroclors 1242 ($p=0.024$) and 1254 ($p=0.054$) compared to control (Chi-square test). There were no statistically significant differences for females between any of the Aroclor-dosed groups and control. These data show that although there was an increased expression of hepatic tumors in rodents (especially females) chronically exposed to high doses of PCBs, the incidence of *total* tumors was not increased, and in

two instances, was actually decreased. Current risk assessment paradigms, by focusing on selected, isolated endpoints, can overestimate the actual total risk associated with PCB exposure. Therefore, a scientifically-based risk assessment should include evaluations for the overall incidence of tumors, in addition to counts at selected target organ sites.

1597 CENTRO DE INFORMACION TOXICOLOGICA: A RETROSPECTIVE STUDY OF NINE YEARS OF SERVICE.

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The Toxicological Information Center was founded in 1989 and is situated in the Department of Pharmacology and Toxicology of the Medicine School of the Universidad Autónoma de Nuevo León. It has 24-h information service 7 days a week given to the general public and health professionals. The present is a report of calls received by the center during 9 years of activities. Our center had 9320 telephone calls (1989-1997 period), of which 61% were real intoxications and 39% were calls regarding toxicologic information about antidotes, adverse drug effects or asking for analysis. Sixty two percent of the calls came from hospital physicians and practitioners, 20% from general public and 18% from industries. Children were involved in 53% of the cases, adults in 47%. The children were mainly preschoolers (71%). Among adults, women were slightly more represented than men. Among the products involved, medicinal drug were the most important group(30%), household products and cleaning agents were second in frequency (20) followed by pesticides(15). Accidental was the predominant situation (78%) and the main route of exposure was oral (89%).

1598 RISK COMMUNICATION REGARDING DIOXIN EXPOSURES TO INFANTS FROM MOTHER'S MILK: KEY UNCERTAINTIES RELATING TO STEADY STATE MODELING ASSUMPTIONS.

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Accumulation of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs and PCDFs) in the adipose tissues and mother's milk of humans is an important risk communication issue. In regulatory risk assessments the breast-fed infant dose model by Smith and colleagues is typically used to evaluate the incremental dose from a specific industrial source. This model infers that long term body burdens of 2,3,7,8-PCDDs and PCDFs accumulate according to an exponential function until "steady state" is reached, i.e., until the daily dose absorbed equals the daily dose excreted. The underlying logic of this model is probably not clear to most risk assessors or risk managers, yet is important both for accurate dose estimation and risk communication. Our presentation illustrates the following key aspects: 1) plausible maternal exposure time and rate are key because steady state for TCDD is not likely reached until after 25 or more years of continuous exposure; 2) modeling TEq can be misleading because each dioxin and furan congener has different pharmacokinetics; 3) to understand the potential for infant toxicity, the infant's body burden vs. time profile is most important; and 4) the use of multiple upper bound parameters can greatly overstate the plausible dose attributable to the source(s) at issue. Use of conceptually similar steady state modeling for source-related dioxin accumulation in human foodstuffs further magnifies the likely overestimation of the plausible infant dose. We recommend that these significant uncertainties be explained in all risk assessments attempting to estimate breast-fed infant exposures to PCDDs/PCDFs and other chemicals.

1599 URANIUM HEALTH ADVISORY.

A T Bathija. *U.S.EPA, OW/OST, Washington, DC.* Sponsor: E V Ohanian.

Uranium (U) is a naturally occurring element that makes up about 2% of the earth's crust. Uranium is naturally released into the air and water through weathering of rocks. The principal isotopes of concern in drinking water are U-234 and U238. In 1991, EPA proposed a Maximum Contaminant Level Goal (MCLG) for U of zero based on emission of alpha radiation known to be carcinogenic in humans. EPA based the Maximum Contaminant Level (MCL) of 20 *ug* /L based on kidney effects seen in a 30-day rabbit study by Maynard and Hodge (1949). The kidney effects have been identified as the more sensitive end point of concern; the kidney effects are seen at very low levels of exposure to U. Also, carcinogenicity due to oral exposure to U has not been observed. The relative Source Contribution (RSC) used in the proposed MCL was 20%; EPA policy for water is to use a default factor of 20% RSC in the absence of specific data. Since the 1991 proposal, a new study has been published which has identified histopathological effects in rat kidneys (Gilman et al, 1998). In June of 1998, EPA sponsored a Uranium Issues Workshop (WS) to share research information and technical expertise on effects of U in drinking water. At the WS it was decided that the Gilman et al, 1998 study provided the most scientifically sound basis for risk assessment. Since the 1991 proposal, EPA has gathered data to show that the RSC from water is 77%. The details of the rat study and the procedure for deriving health-based levels and the new data to calculate the RSC will be presented. (The opinions expressed are those of the authors and do not reflect the Agency policy).

1600 IMPLICATIONS OF REGULATIONS IN THE CONTEXT OF CALIFORNIA'S PROPOSITION 65: IS IT REALLY NECESSARY TO LIMIT EXPOSURE TO BACKGROUND CONCENTRATIONS OF CHEMICALS?

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Under the Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65), Cal-EPA has developed a list of chemicals considered by the State to be carcinogens and/or reproductive/developmental toxicants. Cal-EPA has also established No Significant Risk Levels (NSRLs)-daily intakes that would result in one theoretical excess cancer in 100,000, for many of these chemicals. The NSRLs for N-nitrosodimethylamine (NDMA) and bis-diethylhexylphthalate (DEHP) are 0.04 and 80 μ g/day, respectively. In this study, we analyzed some major contributors to background exposures to these chemicals, and found backgrounds to range between 5-100 times the NSRLs. The criteria for NDMA are stringent for a chemical that is rare as an environmental contaminant, yet common in our diet. Cancer risks from consumption of two meals of bacon a week from adolescence to age 70, or consumption of 3 bottles of beer or malt whiskey drinks a week (NDMA concentration of 2.5 ppb) for 40 years are approximately 1/10,000. The estimated daily dietary intake of NDMA (1 μ g), may pose a cancer risk of approximately 1/1,000, and the total dietary dose of all nitrosamines is about 100 times the daily dose of NDMA. Further, lack of epidemiological data regarding excess cancers in humans associated with NDMA exposure suggests that it is unlikely that background exposures to NDMA, although significantly in excess of the NSRL, pose a genuine cancer risk. The DEHP NSRL is based on increased incidence of adenomas and carcinomas in the rat liver. Alternative analysis of the same animal data indicates the NSRL could be substantially higher, given the likely epigenetic mechanism of action, and still be health-protective. Given the stringent regulation of carcinogens by Cal-EPA, the risks from dietary and other background exposures are likely to be high relative to risks due to contaminated soil, water or other sources. We suggest that Cal-EPA and other regulatory agencies consider background concentrations while legislating and regulating chemicals. The recent EPA position on dioxin from incinerators using a Margin of Incremental Exposure Approach is a good starting point in this direction.

1601 RISK ASSESSMENT OF ATRAZINE, BENTAZON, DIBROMOCHLOROPROPANE, 1,2-DICHLOROPROPANE and 1,3-DICHLOROPROPENE FOR DETERMINATION OF CALIFORNIA PUBLIC HEALTH GOALS IN WATER.

R Tomar, D Rice, J Bankowska, J Polakoff, J Wisniewski, J Faust, M DiBartolomeis and A Fan. *California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Pesticide and Environmental Toxicology Section, Berkeley and Sacramento, CA.*

Derivation of proposed California Public Health Goals (PHGs) for atrazine, bentazon, dibromochloropropane (DBCP), 1,2-dichloropropane (1,2-DCP) and 1,3-dichloropropene (1,3-DCP) is described. Risk assessments on each pesticide estimated the concentrations in drinking water that would pose no significant health risk to individuals consuming the water daily over a lifetime. Critical studies were selected, and exposure scenarios, relative source contribution, and body weight were considered. The proposed PHGs are based on mammary tumors in rats for atrazine (0.15 ppb), reduced body weight, hematological changes and intestinal disturbances in dogs for bentazon (200 ppb), squamous cell carcinoma of the forestomach in mice for DBCP (1.7 ppt), hepatocellular adenoma and carcinoma in mice for 1,2-DCP (0.5 ppb), and bladder transitional cell carcinoma in mice for 1,3-DCP (0.2

ppb). The animal cancer potencies for atrazine, 1,2-DCP and 1,3-DCP were calculated by fitting the multistage model to the tumor incidence data and the lower 95% confidence limit on dose was used to estimate the 10% incidence. The cancer slope factors (CSFs) were calculated as $0.1/LED_{10}$. For DBCP, q1* was based on 95% upper confidence limit on the linear term (q1) calculated using the standard likelihood procedures. The PHG for bentazon was based on a non-cancer endpoint using the uncertainty factor approach.

1602 CALIFORNIA PUBLIC HEALTH GOALS FOR ENDRIN, HEPTACHLOR/HEPTACHLOR EPOXIDE, LINDANE, AND METHOXYCHLOR.

R Howd, J Brown, and L Jowa. *California Environmental Protection Agency, Berkeley and Sacramento, CA.*

The Office of Environmental Health Hazard Assessment conducted risk assessments to determine Public Health Goals (PHGs), based exclusively on public health considerations, for endrin, heptachlor/heptachlor epoxide, lindane, and methoxychlor. PHGs are concentrations of chemicals in water that are not expected to produce adverse effects in humans ingesting the water for a lifetime. In this process, published scientific studies as well as unpublished documents submitted for pesticide registration were reviewed. The rationale for the existing state and federal drinking water standards and current recommendations on risk assessment methods were also evaluated and considered. The proposed health-protective levels were peer reviewed and discussed in public workshops. The proposed PHG for endrin is 1.8 ppb based on convulsions and liver toxicity in dogs. The proposed PHGs for heptachlor and heptachlor epoxide are 0.008 and 0.006 ppb, respectively, based on mouse liver tumors. For lindane, the proposed PHG of 0.008 ppb is based on immunotoxicity in mice. The proposed PHG for methoxychlor of 30 ppb is based on reproductive system toxicity in female rats. Once adopted in December 31, 1998, the final PHG values are intended to guide California risk managers in establishing and interpreting state drinking water standards.

1603 CALIFORNIA PUBLIC HEALTH GOALS FOR CADMIUM, CHROMIUM, INORGANIC MERCURY AND THALLIUM.

L Jowa, D Morry, and J Polakoff. *California Environmental Protection Agency, Berkeley and Sacramento, CA.*

California Public Health Goals (PHGs) are concentrations of chemicals in water that are not expected to produce adverse effects in humans ingesting the water for a lifetime. The Office of Environmental Health Hazard Assessment conducted risk assessments to determine PHGs (based exclusively on public health considerations) for cadmium, chromium, inorganic mercury and thallium. In this process, current scientific literature was reviewed along with the documentation for the existing drinking water standards, and current recommendations on risk assessment methods were incorporated whenever applicable. The proposed health-protective levels were subjected to peer review and discussed in public workshops. The proposed PHG value for cadmium is 0.07 ppb based on renal tubular toxicity observed in an epidemiological study. Two PHG values were developed for chromium, 2000 ppm for trivalent chromium based on no effects seen in a chronic rat study, and 0.02 ppb for hexavalent chromium developed from evidence of carcinogenicity in female mice. For inorganic mercury, the proposed PHG of 1.2 ppb is based on renal pathology in a 6-month rat study. The proposed PHG for thallium of 0.02 ppb is based on lacrimation, exophthalmos, alopecia, and other changes to the coat and skin of rats. Once adopted in December 1998, the final PHG values are intended to guide California risk managers in establishing and interpreting state drinking water standards.

1604 CALIFORNIA PUBLIC HEALTH GOALS FOR 1,2-DICHLOROETHANE, 1,1-DICHLOROETHYLENE, HEXACHLOROCYCLOPENTADIENE, TOLUENE, 1,2,4-TRICHLOROBENZENE, AND TRICHLOROETHYLENE.

D Ting, J Brown, T Parker, J Polakoff, D Rice, and A Fan. *California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Berkeley and Sacramento, CA.*

The Office of Environmental Health Hazard Assessment conducted risk assessments to determine Public Health Goals (PHGs), based exclusively on public health considerations, for 1,2-dichloroethane, 1,1-dichloroethylene, hexachlorocyclopentadiene, toluene, 1,2,4-trichlorobenzene, and trichloroethylene. PHGs are concentrations of chemicals in water that are not expected to produce adverse health effects in humans using the water for drinking and other purposes for a lifetime. In this process, up-to-date scientific studies were critically reviewed and current risk assessment procedures were evaluated and applied. The proposed PHGs of 0.4 ppb for 1,2-dichloroethane and 0.8 ppb for trichloroethylene were based on cancers in rodents; they were developed considering the pharmacokinetic, metabolic, and other mechanistic data. The proposed PHGs for the other four chemicals were based on non-cancer endpoints in test animals; they were developed considering relative source contributions, exposure routes in addition to drinking water consumption, and interspecies as well as intraspecies variability. The proposed PHGs for 1,1-dichloroethylene, toluene, hexachlorocyclopentadiene, and 1,2,4-trichlorobenzene are 10, 150, 50, and 5 ppb, respectively. The proposed health-protective levels will be peer reviewed and discussed in public workshops. Once adopted on December 31, 1998, the final PHG values are intended to guide California risk managers in establishing and interpreting state drinking water standards.

1605 PRELIMINARY TOXICOLOGICAL EVALUATION OF POTENTIAL STAGE II DISINFECTION BY-PRODUCTS.

O Conerly. *USEPA, OST/OW, Washington, DC, USA.* Sponsor: C O Abernathy.

To control pathogens and prevent the spread of waterborne disease, water is treated with disinfectants. However, byproducts form when the disinfectant reacts with organic matter in water. Some disinfection byproducts have produced tumors or developmental effects in animals. In 1994, the Environmental Protection Agency (EPA) proposed regulations for a group of disinfectants and their byproducts (Stage I). These regulations are expected to be finalized by the end of 1998. The Stage I Disinfectant/Disinfection Byproducts Rule focused on total trihalomethanes, haloacetic acids and bromate. In preparation for Stage II, EPA collected toxicological data for thirteen other byproducts. Genotoxicity, systemic toxicity, carcinogenicity, mutagenicity, reproductive and developmental data were collected, analyzed and summarized for: acetaldehyde, bromoacetonitrile, bromochloroacetic acid, bromodichloroacetic acid, chlorate, cyanogen chloride, dibromoacetic acid, 1,1-dichloropropanone, glyoxal, hexachloropropanone, propanal and 1,1,1-trichloropropanone. There are data for positive genotox responses for 54% and data for 38% suggest potential for carcinogenicity. However, significant data gaps exist for the majority of the contaminants. (The opinions therein are those of the authors and do not necessarily reflect the opinions or policies of the EPA.)

1606 AMBIENT WATER QUALITY CRITERIA METHODOLOGY REVISIONS.

D R Borum, J Y Du, J M Donohue, Y M Patel, K G Sappington, R E Cantilli, C O Abernathy, W J Morrow, and S Gilbertson. *US EPA, Washington, DC.*

EPA published draft revisions to the Methodology for Deriving Ambient Water Quality Criteria (AWQC) for the Protection of Human Health pursuant to Clean Water Act requirements. States and Tribes may use the revised methodology to develop water quality standards. The revisions will substantially enhance science policy guidance for setting water quality criteria by incorporating scientific advances that have occurred during the past 18 years since the original guidance was published and, thus, increase the scientific defensibility of recommended criteria. It also provides more flexibility for decision-making at the State, Tribal and EPA Regional level. EPA is promoting greater involvement by States and Tribes in making risk assessment decisions and in adapting criteria to local conditions. The revisions recommend consideration of the Agency's guidance on assessing noncarcinogenic effects of chemicals, including integrating reproductive/developmental, immunotoxicity, and neurotoxicity data. EPA also recommends the use of quantitative dose-response modelling for RfD derivations. For cancer risk assessments, more sophisticated methods to comprehensively determine the likely mechanism that causes human carcinogenicity are recommended, consistent with EPA's 1996 proposed revised cancer guidelines. In the area of exposure assessment, the use of local studies on fish consumption is encouraged and EPA provides default values for the general population, recreational fishers and subsistence fishers. The methodology can also account for other sources of exposure when setting criteria to ensure that an individual's total exposure does not exceed a threshold level. The revisions also emphasize the use of bioaccumulation factors for estimating potential exposure to contaminants via the consumption of contaminated fish and shellfish, since they reflect the accumulation of chemicals by aquatic organisms from all surrounding media (water, food, sediment).

1607 PATHOGENICITY OF MAN-MADE VITREOUS FIBERS AFTER LONG-TERM INHALATION.

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A summary is given of the pathology results after long-term inhalation in rats of a newly developed man-made vitreous fiber, MMVF 34 (HT stone wool), and the pathology results were compared with previously conducted long-term inhalation study with MMVF 21 (traditional stone wool). MMVF 34 is characterized by a relatively high content of aluminum, and a relatively low content of silica compared to MMVF 21. With regard to in-vitro dissolution rate measurements, MMVF 34 has a high dissolution rate at pH 4.5, and a relatively low dissolution rate at pH 7.5. Laboratory rats were exposed at one exposure level of 30 mg/m^3 by nose-only inhalation to a well-characterised fiber test atmosphere that had been selected to be largely rat respirable. The negative control group was filtered air. The exposure duration was 6 hours/day, 5 days/week for 104 weeks, with a subsequent non-exposure period lasting until approximately 20% survival in one of the groups. Interim sacrifices were performed at weeks 13, 26, 52, 78, and 104. Biopersistence was determined after 26, and 52 weeks exposure. Effectively the main protocol for the previously conducted chronic study with MMVF 21 was the same, except that there were three fiber exposure groups (3, 16, and 30 mg/ m^3). The incidence of proliferative lesions for the MMVF 34 fiber was comparable to the control group, which corresponds to the results from the long-term study with MMVF 21. However, MMVF 21 was more inflammatory than MMVF 34, and resulted in Wagner grade 4 fibrosis.

1608 EFFECTS OF 2-YEAR INHALATION EXPOSURE TO GLUTARALDEHYDE IN RATS AND MICE.

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Whole-body inhalation toxicology and carcinogenicity studies were performed with the widely used fixative and cold-sterilant glutaraldehyde. Groups of 50 male and female F344/N rats and B6C3F1 mice were exposed to glutaraldehyde (rats: 0, 250, 500, and 750 ppb; mice: 0, 62.5, 125, and 250 ppb) for 6 hours/day, 5 days/week, and 104 weeks. Concentrations were based on results of 13-week studies. Survival of 500 and 750 ppb female rats was less than that of controls. Mean body weights of all exposed groups of male rats, 500 and 750 ppb female rats, and 250 ppb female mice were generally less than those of controls. No exposure-related neoplastic lesions were observed in either rats or mice. Exposure-related non-neoplastic lesions were limited to the nasal cavity and primarily to the most anterior region. In rats, hyperplasia and inflammation of the squamous epithelium; hyperplasia, goblet cell hyperplasia, inflammation, and squamous metaplasia of the respiratory epithelium; and hyaline degeneration of the olfactory epithelium were observed. In mice, the nasal lesions were quantitatively similar to those in rats. Squamous metaplasia of the respiratory epithelium was observed in both sexes of mice while female mice also had inflammation and hyaline degeneration of the respiratory epithelium. The toxicity of glutaraldehyde prevented the use of exposure concentrations compared to those which induced nasal cavity tumors with formaldehyde. However, exposure to glutaraldehyde resulted in considerable non-neoplastic lesions in the nose of rats and mice.

1609 HIGH DOSE EXPOSURE TO DINITROTOLUENE ASSOCIATED WITH CARCINOGENIC EFFECTS IN HUMANS?

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Dinitrotoluene (DNT) is suspected to be carcinogenic in humans. It is toxicologically closely related to aromatic amines, which may cause urothelial cancer. In order to investigate possible carcinogenic effects of technical DNT (t-DNT) we carried out a retrospective cohort study in miners with high exposure to explosives containing t-DNT. 14 kidney tumour cases and 6 urothelial tumour cases could be identified among 500 miners formerly exposed to t-DNT out of a total group of 3000 underground miners. A representative cohort of 183 workers with former occupational contact with t-DNT was interviewed and divided into 4 exposure categories. All identified tumour patients were genotyped by PCR of lymphocytic DNA regarding NAT2, GSTM1 and GSTT1. Genomic DNA of pathological tumour tissue was isolated and submitted to sequential analysis within the p53 gene (exon 5-8). The categorization of the 14 kidney tumour cases revealed no dose-dependent distribution over the 4 exposure categories, whereas the urothelial tumour cases were mainly found within the „high" and „very high" exposure categories. Genotyping of the tumour patients showed no remarkable distribution except for the urothelial tumour cases which were exclusively identified as „slow acetylators". Preliminary results of the p53 mutational analysis are a high mutation frequency (detection of missence mutations in all 6 urothelial cancer cases, among them 2 double mutations). Identified mutations were almost exclusively G>A transitions, which also have been reported in urothelial tumours from workers highly exposed to aromatic amines. Our findings corraborate the suspicion of a carcinogenic effect of t-DNT with regard to the human urothelium.

1610 MATHEMATICAL MODEL OF EFFECTS OF CARCINOMAS ON AIRFLOW PATTERNS IN THE LUNGS OF CHILDREN.

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We investigated how tumors within lungs affect airflow patterns. In particular, we were interested in how tumor characteristics (i.e., sizes and locations) and ventilatory parameters (i.e., breathing rates and tidal volumes) influenced (1) bulk flow distribution within the lung and (2) localized velocity profiles within airways. We employed a computational fluid dynamics (CFD) package, FIDAP, to define flow conditions within lungs. Our model is based on the adult airway network proposed by Weibel, but scaled to be appropriate for a four-year-old child. Original physiologically realistic skewed velocity profiles were developed in our work. When a tumor was sufficiently developed (r/D > .4, where r=tumor radius and D=airway diameter), maldistribution of flow occurred (i.e., flow was directed away from the region containing the tumor). When the inspiratory flow rate was increased, these tumor effects were enhanced. At increased respiratory intensities, localized circulation currents developed downstream from the tumors. This work indicates that inhaled particle deposition is complicated by the existence of tumors but that the effects are systematic and predictable and therefore can be integrated into drug delivery protocols for the treatment of lung cancers. Disclaimer: This is an abstract of a proposed presentation and does not necessarily represent EPA policy. (Acknowledgements: RAS is funded by the NCSU/EPA Cooperative Training Program in Environmental Sciences Research, Training Agreement CT826512010 with NCSU.)

1611 CARCINOGENICITY OF INHALED BUTADIENE DIEPOXIDE.

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The greater sensitivity of mice to 1,3-butadiene (BD) may result from mice metabolizing more of the BD to butadiene diepoxide (BDO_2) than rats. Female B6C3F$_1$ mice and Sprague-Dawley rats, 10-11 weeks old, 56/group, were exposed by inhalation to 0, 2.5, or 5.0 ppm BDO_2, 6 hrs/day, 5 days/wk for 6 wks to compare tumor incidence between mice and rats under identical exposure conditions. Previous studies in rodents indicated that blood levels would be ~100 and 200 pmole/g at the two exposure concentrations in the rat and twice those levels in the mouse. During the last week of exposure, the 5 ppm mice showed signs of labored breathing, and 4 died; the respiratory symptoms disappeared after exposures ended. Rats showed no clinical signs of toxicity during exposure, but developed labored breathing after the exposure leading to the death of 13 rats in 3 mos. At the end of the exposure, necrosis, inflammation, squamous metaplasia of the nasal mucosa, and atrophy of the turbinates were present in both species. Within 6 mos, necrosis and inflammation had subsided, but squamous metaplasia remained. In rats, squamous metaplasia extended beyond the areas of earlier inflammation with time. At the end of 18 mos, there was a dose-dependent increase in neoplasms of the nasal mucosa in the rat (0/47, 12/48, and 21/48 for the control, 2.5 ppm, and 5.0 ppm exposures, respectively), but not in the mice. Neoplastic lesions in the mice occurred in reproductive organs, lymph nodes, bone, liver, Harderian gland, and pancreas. Although there was a statistically significant increase in total neoplastic lesions as a function of dose (10/40,

7/42, and 16/36 for control, 2.5 ppm, and 5.0 ppm, respectively), there were no increases in any individual organ except the Harderian gland (0/44, 2/44, and 5/41). These findings indicate that BDO_2 is carcinogenic in the upper respiratory tract of rats. The lack of respiratory tract tumors in mice suggests that the site of formation of BDO_2 from BD is an important factor in the tumorogenicity of BDO_2 in mice. (Research supported by the Health Effects Institute, DE-FI04-95AL86988.)

1612 MOLECULAR DOSIMETRY OF N-7-GUANINE DNA ADDUCT FORMATION IN MICE AND RATS EXPOSED TO 1,3-BUTADIENE.

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The molecular dosimetry of N-7 guanine adducts was studied in B6C3F1 mice and F344 rats exposed to 0, 20, 62.5, or 625 ppm of 1,3-butadiene (BD) for 4 weeks. The adducts, racemic and meso form of *N*-7-(2,3,4-trihydroxy-but-1-yl)-guanine (THB-Gua), *N*-7-(2-hydroxy-3-buten-1-yl)-guanine (EB-Gua I), *N*-7-(1-hydroxy-3-buten-2-yl)-guanine (EB-Gua II), were quantitated by LC/ESI+/MS/MS. THB-Gua adducts were more abundant (6-27 times) than the EB-Gua adducts in rats and mice. Liver, lung and kidney had similar numbers of adducts, with THB-Gua adducts (/10^6 guanine) of 6.3±1.2, 8.2, and 5.0±1.2 in mice exposed to 20 ppm BD and 5.5±1.1, 3.8±0.7 and 3.7±0.1 in rats, respectively. EB-Gua was 0.3, 0.8 and 0.4±0.2 in mice and 0.3, 0.5 and 0.3±0.2 in rats, respectively. THB-Gua was 15.4±1.2, 13.7±3.6 and 16.2±3.0 in mice exposed to 62.5 ppm BD and 13.7±3.3, 7.7±0.6 and 7.5±3.7 in rats. EB-Gua was 0.3±0.1, 0.5±0.3 and 0.6±0.2 in mice and 0.8±0.6, 0.3±0.2 and 0.5±0.1 in rats exposed to 62.5 ppm BD, respectively. Following exposure to 625 ppm BD, mice had 64.1±6.1, 59.1±8.2 and 62.5±13.9 THB-Gua, while rats had 11.8±6.6, 13.0±2.3 and 16.6±2.9, respectively. EB-Gua values in 625 ppm mice were 5.4±0.3, 3.6±0.5 and 3.7±0.6, while rats had 2.1±1.0, 2.1±0.5 and 2.9±0.7, respectively. EB-Gua adducts had a linear dose response relationship in both species, whereas THB-Gua adduct formation was supralinear. The THB-Gua data demonstrate that saturation of metabolic activation occured at 62.5 ppm in rats, while mice reached saturation at higher exposures. Assuming proportionality between tissue 3,4-epoxybutene and EG-Gua and between 1,2,3,4-diepoxybutane (DEB) and the fraction of THB-Gua adducts arising from DEB, we conclude that most of the THB-Gua adducts are formed by 1,2-epoxy-3,4-butanediol. This electrophilic metabolite has not been measured *in vivo*.

1613 ANALYSIS OF MUTATIONS IN *HPRT* cDNA OF SPLENIC T-LYMPHOCYTES FROM MICE EXPOSED BY INHALATION TO 1,3-BUTADIENE.

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Experiments were conducted to define mutations occurring in the *Hprt* gene of T-cells in female mice exposed for 4 weeks (6 hr/day, 5 days/week) to BD (625 ppm). In mice necropsied at 6 weeks post exposure, *Hprt* mutant frequencies (M/s) in exposed animals were 4.1-fold greater than control values. *Hprt* mutant colonies were expanded to ≥ 400,000 cells and analyzed by RT-PCR to produce *Hprt* cDNA for sequencing. To date, 47/52 *Hprt* mutant clones from BD-exposed mice were found to have mRNA by RT-PCR of β-actin. No *Hprt* cDNA was produced in 15 of these 47 mutants (32%), presumably due to whole gene deletions. The remaining mutations were base substitutions (12/47, 26%), internal exon deletions (10/47, 21%), frameshifts (9/47, 19%), and one complex mutation (2%). The base substitutions included 3 AT → CG transversions, 8 GC → AT transitions, and one GC → TA transversion. Additional mutants from control and BD-exposed mice are being analyzed; nonetheless, the M/ values suggest that about 75% of the mutations in exposed mice should be BD-induced. These preliminary data from BD-exposed mice suggest that the carcinogenicity of BD in the mouse may be more related to the production of large scale DNA damage than to the induction of point mutations. (Supported by Health Effects Institute and NIH grants.)

1614 LOSS OF DIFFERENTIATION IN THE PHENOTYPICALLY ALTERED PROGENY OF ALPHA-PARTICLE-EXPOSED NORMAL HUMAN BRONCHIAL EPITHELIAL CELLS.

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Pulmonary deposition of alpha-particle-emitting radon daughters is estimated to account for 10% of all lung cancer deaths in the USA. However, the nature and timing of early (preneoplastic) genetic alterations in radon-associated lung cancer are still relatively uncertain. We have previously shown that exposure of normal human bronchial epithelial (NHBE) cells to fractionated doses of ^{238}Pu alpha-particles results in the formation of foci of phenotypically altered cells (PACs) (Kennedy et al. Carcinogenesis 17: 1671, 1996). Although these PAC cultures are not immortalized, they do exhibit an extended population-doubling potential relative to unexposed NHBE cells. The purpose of the current investigation was to determine if these PACs exhibit altered responsiveness to differentiation-inducing signals. Retinoic acid (RA)-deficiency induces squamous terminal differentiation in NHBE cells; therefore, we compared the effect of RA-deficiency on NHBE cells and PACs by measuring the expression of three genes involved in differentiation: bcl-2, p21 (WAF1/CIP1) and small proline-rich protein (SPR1). The results of Northern and Western analyses showed no significant difference between cell phenotypes with regard to expression of either bcl-2 or p21 in PACs. However, SPR1 mRNA and protein levels were significantly lower in the PACs relative to NHBE cells. These data suggest the following: 1) SPR1 expression is uncoupled from RA regulation in PACs; and 2) this uncoupling occurs via a mechanism that is independent of bcl-2 and p21 expression. Because altered control of differentiation is a hallmark of lung cancer cells, elucidation of the mechanism by which PACs fail to induce SPR1 following RA deprivation should provide insight into the early genetic changes that occur during the development of radon-associated lung cancer.

1615 THE EFFECT OF TRICHLOROETHYLENE CARCINOGENIC METABOLITES, TRICHLOROACETIC ACID (TCA) AND DICHLOROACETIC ACID (DCA), ON PEROXISOMAL ENZYME ACTIVITY AND DNA SYNTHESIS IN CULTURED HUMAN LIVER CELLS.

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Trichloroethylene (TCE), a common environmental contaminant, causes hepatocarcinoma in the $B6C3F_1$ mouse. The hepatic neoplasia is species and strain specific; the risk to humans is not yet defined. TCE and its metabolites, trichloroacetic acid (TCA) and dichloroacetic acid (DCA) are considered epigenetic carcinogens. Hepatic tumors are thought to arise directly as a result of excessive peroxisome-dependent active oxygen production or secondary to enhanced cell replication. We have previously demonstrated the peroxisome proliferative activity of TCA and DCA in mouse and rat hepatocytes in vitro. The current studies are aimed at isolating and characterizing human hepatocyte cultures, and subsequently studying the effects of TCA, DCA, and classic peroxisome proliferators such as clofibrate or WY-14,643, on peroxisomal function, DNA synthesis, and cell replication in human liver cell cultures. Both human primary and long-term cell cultures were characterized for liver-specific functions and used to study the potential of TCA and DCA to induce peroxisomal enzymes and modulate DNA synthesis/cell replication. For peroxisomal studies, cells were kept in serum-free medium and treated for 72 hours with either 0.5 mM clofibrate or 5 μM WY-14,643, 0.5-2 mM TCA, and 0.5-2 mM DCA. Peroxisome proliferation was assessed by measuring palmitoyl-CoA oxidation, selective staining for catalase activity, and Northern blot analysis of peroxisomal enzymes. Cultured human liver cells do not appear to be sensitive to clofibrate-, WY-14,643-, TCA-, or DCA-induced peroxisome proliferation. For DNA synthesis/cell replication studies, cells were kept in growth factor-free, serum-free medium for 24 hours before treatment with epidermal growth factor (EGF;positive control), 5 μM WY-14,643, 0.01-1 mM TCA, or 0.01-1 mM DCA. The cultured human liver cells did not display an increase in either ^{3}H-thymidine incorporation or cell number following treatment with WY-14,643, TCA, or DCA. The comparison of these human hepatocyte experiments to similar rodent hepatocyte studies can lead to a better assessment of the potential risk of TCE exposure in humans.

1616 IS PERCHLOROETHYLENE (PERC) A PROBABLE CARCINOGEN IN HUMANS?

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Classification of perc as a probable human carcinogen by EPA is primarily based on positive findings in rats and mice in cancer bioassays. In human populations, positive findings have been inconsistent, even in populations receiving the highest exposures to perchloroethylene - drycleaners. To resolve this discrepancy, we evaluated species differences in type of cancer (*i.e.*, liver cancer in mice and kidney cancer in rats), and linked cancer site to species differences in perc metabolism. In mice, perc is metabolized to trichloroacetic acid, resulting in peroxisome proliferation, which in turn leads to hepatic cancer. Peroxisome proliferation is the result of a gene recently discovered to be specific to mice. In rats, peroxisome proliferation does not occur, so hepatic tumors are not observed in rats. In rats, metabolism of perc *via* the glutathione pathway results in byproducts that result in kidney cancer, an endpoint not observed in mice, which do not metabolize perc *via* the glutathione pathway. Humans, like mice, do not metabolize perc *via* a glutathione pathway, and unlike mice do not exhibit peroxisome proliferation. Therefore there is no metabolic basis for perc-induced cancer in humans. These findings are consistent with those observed in epidemiology studies in populations most exposed to perc (*e.g.*, drycleaners), which show inconsistent increases in certain types of cancers. However these cancers are known to be associated with confounders (*e.g.*, smoking, alcohol consumption, income), that could not be evaluated in the studies. Based on this analysis, we conclude that perc is not likely to be a human carcinogen.

1617 HEALTH RISK ASSESSMENT/CHARACTERIZATION OF THE DRINKING WATER DISINFECTION BYPRODUCT CHLOROFORM.

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Chloroform causes the development of cancer in several animal species by a mechanism that is believed to be non-linear in the low dose range, and a Margin of Exposure (MOE)-based dose of 0.02 mg/kg-day is proposed. Although chloroform will likely be carcinogenic to humans by all routes of exposure if a sufficient dose is administered, human data are insufficient to determine chloroform's potential carcinogenicity. Experimental animal studies are somewhat variable in both tumors evoked and magnitude of response. The principal studies for the determination of chloroform's tumorigenic effect and low dose extrapolation are NCI (1976) and EPA sponsored Jorgenson et al. (1985) in the Osborne-Mendel rats. Both linear and nonlinear approaches to the assessment of chloroform's cancer risk are presented, with emphasis on the nonlinear approach as being more relevant for chloroform, based on the substantial amount of data from oral and inhalation studies in rats and mice that cytotoxicity is necessary, although not sufficient, for chloroform carcinogenesis. For noncancer toxicity the use of the existing Reference Dose (RfD) of 0.01 mg/kg-day is proposed. This RfD is based on a Lowest-Observed-Adverse-Effect Level (LOAEL) for minimal liver injury of 15 mg/kg/day identified in a 7.5-year study in dogs (Heywood et. al., 1979). The critical study was of chronic duration, used a fairly large number of dogs, and measured multiple endpoints. Confidence in critical study is considered medium, and in its supporting database is considered medium to low. Overall confidence in the RfD is also considered medium to low, meaning that additional data may more likely change the value of the RfD when compared to a high confidence RfD.

1618 EXPOSURE OF JAPANESE MEDAKA (ORYZIAS LATIPES) EMBRYOS TO BENZENE, CHLOROFORM, AND BROMOFORM FOR ASSESSMENT OF CARCINOGENIC POTENTIAL.

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Validation of *in vivo* short-term bioassays for screening chemicals such as disinfection by-products for carcinogenic potential using alternative species such as the medaka is needed for risk assessment. In this research, embryos in the 64 cell to early high blastula stage (critical period of embryogenesis) were exposed in groups in sealed vials for ten consecutive days to varying concentrations of benzene (0-150 mg/L), chloroform (0-100 mg/L) and bromoform (0-50 mg/l). After exposure, the embryos were washed in embryo rearing solution, grown-out for six months and one-year post hatch, and evaluated histologically. In the benzene study, at six months hepatic preneoplastic lesions included basophilic, eosinophilic, and clear cell foci. After one year the combined incidence of neoplasms in the liver (adenoma and cholangioma) increased with increasing concentration. There was one case of cholangioma in the controls. The one-year tumor incidence for benzene fit a linearized multistage model. In the chloroform studies, based on evaluation of 1/3 of the fish (20/60 per dose) at six-month, there were no neoplasms noted. Preneoplastic lesions included one case of clear cell foci in the highest exposure concentration. Non-neoplastic lesions in the liver included cysts. In the bromoform studies, based on evaluation of 1/3 of the fish (20/60 per dose) at six-months, one case of cholangioma was observed in the high concentration. Preneoplastic lesions including hepatic clear cell and altered foci were observed at 10 mg/L and 50 mg/L of bromoform. Non-neoplastic lesions observed in animals exposed to bromoform include hepatic cysts, spongiosis hepatis and cystic bile duct. Complete data on chloroform and bromoform exposed fish will be presented. These initial results indicate that a medaka embryo exposure method may be useful for efficient screening of chemicals for carcinogenic potential and other applications in risk assessment. (Abstract may not reflect U.S. EPA policy). (Funded in part by USEPA Cooperative Agreement.)

1619 THE INDUCTION OF ABERRANT CRYPT FOCI (ACF) IN THE COLONS OF RODENTS ADMINISTERED TRIHALOMETHANES (THMs) IN THE DRINKING WATER.

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THMs administered by corn oil gavage increased cancer in various target organs of rodents. Among these, bromodichloromethane (BDCM) enhanced large bowel tumor incidence in both male and female F344 rats. Our recently completed 2-year bioassay for BDCM in the drinking water of male F344 rats failed to demonstrate large bowel tumor induction (manuscript in preparation). The objective of this study was to evaluate the ability of BDCM in the drinking water to induce ACF in the colons of male F344 rats and B6C3F1 mice. Chloroform (TCM), dibromochloromethane (DBCM), and bromoform (TBM) were also examined. Rats and mice (N=6) were given deionized water containing 0.25% emulphor and isomolar THM concentrations of 0.5 g/l TCM, 0.7 g/l BDCM, 0.9 g/l DBCM, and 1.1 g/l TBM for 13 weeks. Azoxymethane (AOM, 30 mg/kg ip) was the positive control; emulphor and deionized water served as controls. ACF incidences (percent) and number (foci/colon±SEM) in rat colons were: emulphor and water controls (0), AOM (100%; 41.3±14.3), TCM (17%; 0.2±0.2), BDCM (83%; 1.5±0.6), DBCM (50%; 1.2±0.7), and TBM (67%; 1.2±0.4). BDCM, DBCM, and TBM significantly ($p \leq 0.05$) increased ACF above the water and emulphor values. ACF (86-100%) occurred in the rectal 1/3 of the colon (segment 3). These studies demonstrate that brominated THMs given via drinking water induce ACF in the colon of rats though the preneoplastic lesions do not appear to progress to cancer at least in the case of BDCM. The effect of dietary fat and other promoting regimens are being investigated. No ACF in the mouse colon were observed for any of the THMs. (*This abstract does not necessarily reflect the opinion of the USEPA.*)

1620 AN EVALUATION OF THE POTENTIAL CARCINOGENICITY OF DICHLORVOS (DDVP): FINAL REPORT OF THE EXPERT PANEL.

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An Expert Panel was convened to evaluate the results of the large database of cancer studies available on DDVP. The Panel included biochemical & genetic toxicologists, animal & human pathologists, molecular biologists, oncologists, and physicians. Using the weight-of-the evidence the Panel made the following conclusions: -The only endpoint currently given any consideration by regulatory agencies is monocellular leukemia (MCL) in the Fischer 344 rat. MCL is species-, strain- and in the case of DDVP, sex- specific. It is not an appropriate endpoint to estimate potential risk to humans. - The incidence of MCL (NTP, 1989) does not support a conclusion of carcinogenicity in part because 1) corn oil gavage was a confounder, 2) DDVP did not alter the relative severity of the disease, time-to-tumor latencies or percentage of rats surviving to study termination, 3) there was no dose-

response, 4) the results are not replicated in a very large number of carcinogenicity studies, 5) inhalation and/or drinking water are more appropriate routes than gavage for estimating human risk, and 6) the incidences are similar to normal background rates that are increasing over time. -Other potential carcinogenic endpoints provided neither substantive evidence of carcinogenicity in the rat or the mouse; nor were they useful for extrapolation to human risk. -DDVP is not a direct-acting, genotoxic carcinogen. -Based upon these data, the Panel concluded it is unlikely that DDVP poses a carcinogenic risk to humans.

1621 ALIETTE: AN EXAMPLE OF THE USE OF MECHANISTIC DATA IN THE CARCINOGENIC CLASSIFICATION.

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Aliette, a fungicide compound, was re-evaluated for carcinogenic potential by the Environmental Protection Agency's Office of Pesticide Programs Cancer Peer Review Committee taking into consideration mechanistic and other information bearing upon the relevance of the tumor findings in rodents to man. Aliette is not genotoxic and the primary urinary metabolite of aliette, monosodium phosphite, is not carcinogenic when administered in the diet to rats. Tumor induction is not observed at any site in mice. However, the induction of transitional cell urothelial tumors (papillomas and carcinomas) is observed in male rats after chronic dietary administration of aliette at a concentration of 30000/40000 ppm. Humans are not likely to be exposed to levels of the chemical that produce the urinary tract response that was determined to be a required precursor of the carcinogenicity. It also appears that the human bladder may be less sensitive than that of the rat to the carcinogenic stimulus. Anatomical, biochemical and physiological factors cause to male rat to be unusually sensitive to this form of tumor induction. The OPP Cancer Peer Review committee concluded that aliette is unlikely to pose a carcinogenic hazard to humans under the anticipated conditions of exposure. The conclusions of the EPA re-evaluation are consistent with the general approach to the use of mechanistic data that is proposed in the 1996 draft EPA Carcinogen Risk Assessment Guidelines and is exemplary of the use of the new guidelines.

1622 THE KEY RISK FACTORS IN DICHLOROMETHANE CARCINOGENICITY.

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The inter-species differences in sensitivity to the carcinogenic effects of dichloromethane (DCM) has been a challenging issue to toxicologists for over a decade. Following exposure to DCM, there is an increase in liver and lung tumours in mice but not in rats or hamsters. The enzyme responsible for the activation of DCM is class Theta glutathione S-transferase T1-1 (T1). In this study the activity, expression levels and localisation of the mouse and human homologues were compared. Heterologously expressed native mouse and human T1 were purified and the activity towards DCM shown to be 1.8 fold higher with the mouse transferase. Immunoblotting of hepatic extracts using antibodies raised against human T1 demonstrated that mice express two orders of magnitude greater levels of T1 than humans. The tissue distribution of T1 in mice and humans was also compared. Interestingly, it was noted that the mouse kidney had similar levels of expression to the liver and lung, however this is not a DCM target organ. Immunohistochemical analysis demonsatrated that T1 is frequently present in the nuclei of certain mouse liver and lung cells but not in kidney cells. In comparison with human tissue a number of hepatocytes were observed that had the presence of T1 in the nucleus. In summary, humans express lower levels of an enzyme that has less activity towards DCM than the mouse. Nuclear localisation of the enzyme appears to be the major risk factor associated with DCM carcinogenicity. Since the human enzyme may be localised in the nucleus of certain cells, there is a finite carcinogenic risk associated with human exposure to DCM.

1623 THE EFFECTS OF AD LIBITUM (AL) OVERFEEDING AND MODERATE OR MARKED DIETARY RESTRICTION (DR) ON BODY WEIGHT, CARCASS COMPOSITION, CLINICAL PATHOLOGY, AND DEGENERATIVE LESIONS IN CD-1 MICE.

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Two 3-month studies were conducted with single-housed Charles River CD-1 mice (40/sex/group) to evaluate the effects of Purina (PMI) Rodent Chow (precision particles) fed AL or at moderate (70-75% of AL) or marked (50% of AL) DR to select appropriate measured feeding regimens for toxicity/carcinogenicity studies. As expected, body weight gain and body fat were proportional to food consumption. Average food intake and body weight were significantly different between and within most female and male groups. Food wastage was approximately 7-11% for each group. The mice in the marked DR group had transient paleness, tail tip necrosis, were cool to the touch, and had slight decreases in erythroid parameters and total leukocyte counts, compared to the AL and moderate DR groups. The marked DR group had lymphoid depletion in the thymus and hypoplastic ovaries and uteri that were not observed in the other groups. However, the lowest incidence of all other degenerative lesions was seen in the marked DR group, and the highest incidence and severity was seen in the AL group. No diet-related adverse changes in clinical signs, serum biochemical or hematologic parameters were observed in the moderate DR groups. The AL group had the highest hepatic oxidative stress (lipid peroxidative damage with low antioxidative capacity) which was significantly decreased by dietary restriction. The moderate (70-75% AL) DR groups did not have the adverse findings of the marked (50% AL) groups, but did have decreases in degenerative lesions seen in the AL groups. These data indicate that moderate DR (70-75% of AL) under single housing conditions is optimal for CD-1 mouse toxicity and carcinogenicity testing.

1624 CHRONIC TOXICITY OF N-METHYL-PYRROLIDONE (NMP) IN A TWO-YEAR FEEDING STUDY WITH RATS.

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The toxicity and potential oncogenicity of NMP was evaluated by administration of diets containing 0, 1600, 5000, or 15000 ppm NMP to Crl:CD®BR male and female rats for approximately 2 years. Body weights were determined weekly for the first 3 months, and every other week thereafter. Food consumption was determined at the time of weighing. At 12, 18, and 24 months, blood samples were collected for determination of leukocyte differential counts. A morphological evaluation was conducted at the end of the 2-year feeding period. NMP was not oncogenic in males or female at 15000 ppm or below. Decrements in body weight and weight gain occurred in 15000 ppm males and females, which correlated with decrements in food consumption and food efficiency. Survival was decreased in 15000 ppm males compared to control males due to an increased incidence of severe chronic progressive renal nephropathy. Although chronic progressive nephropathy is a common, age-related lesion in this rat strain, administration of NMP exacerbated the severity of the lesion in 15000 ppm males. There were no adverse test substance-related effects on clinical observations, ophthalmic observations, or differential leukocyte counts in males or females, or on female survival and tissue morphology at any dietary concentration. The NOEL was 5000 ppm for males and females.

1625 A 24-MONTH DIETARY CARCINOGENICITY AND TOXICITY STUDY OF MICROENCAPSULATED METHYL BROMIDE IN SD RATS.

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Methyl bromide (MeBr), an important soil fumigant, has been studied extensively. However, there was no acceptable rodent chronic toxicity study using an oral route of exposure that does not involve bolus dosing. Thus, in the current dietary study microencapsulated MeBr was used. Test diets containing 0.5, 2.5, 50 and 250 ppm MeBr and control diets containing placebo

microcapsules or basal diet were offered daily. Each group consisted of 70 Crl:CDR(SD)BR rats/sex; 20 rats/sex/group were assigned to the chronic portion (sacrificed after 52 weeks) and 50 rats/sex/group were assigned to the carcinogenicity portion (sacrificed after 104 weeks). There were no adverse effects on survival (52%, 34%, 58% and 40%, 38%, 58% for basal diet, placebo, 250 ppm males and females, respectively), clinical condition, clinical pathology, and organ weights, nor were there any test article-related findings observed at the ophthalmic, macroscopic or microscopic examinations. Food consumption in the 250 ppm animals was decreased during the first 18 months, resulting in lower body weight gains relative to the start of the study of 10% and 13% for the males and of 9% and 15% for the females when compared to the diet control and placebo control groups, respectively. Thus, the no-observed-effect-level (NOEL) for systemic toxicity was 50 ppm. The low-observed-effect-level (LOEL) was 250 ppm. There was no treatment-related increase in tumor incidence in either sex; MeBr was not carcinogenic.

1626 ORAL BUCCAL AND TONGUE CARCINOGENESIS IN SENCAR MICE.

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Oral cancer is the most prevalent form of cancer in some regions of the world, but the mechanisms producing this lesion are not well established. In the present study, we developed a mouse model which should aid in understanding the development and progression of oral cancer. Female SENCAR mice were used to examine the carcinogenic effects of 7, 12-dimethylbenz[a]anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) or 4-nitroquinoline-1-oxide (4NQO). One group of mice were initiated with 200 nmol DMBA followed by promotion with 5 nmol TPA. Another group (n=5) was treated with 0.5% 4NQO alone thrice weekly for 28 weeks. In the mice that were initiated with DMBA for one (n=6), two (n=12) or six (n=12) weeks and then promoted with TPA, an average of 0.5, 0.92 and 0.83 papillomas/mouse developed in the oral buccal mucosa, respectively, and the incidence of papillomas was 50% in all sub-groups. More than 50% of tumors produced with DMBA/TPA contained a c-Ha-ras A182 → T mutation. Similarly, 30% of mice initiated with DMBA for two or six weeks developed invasive oral squamous cell carcinoma (SCC), although none of the mice treated with DMBA for one week had SCC. In the mice that were initiated and promoted with 4NQO, an average of 0.6 papillomas/mouse developed in the tongue, and the incidence of papillomas was 60%. 67% of the tumors in these mice contained a c-Ha-ras A182 → T mutation. 4NQO produced a spectrum of lesions, ranging from papillomas with dysplasia to SCC. Collectively, DMBA/TPA and 4NQO reliably produced preneoplastic and malignant oral lesions. These results indicate that the SENCAR mice are a sensitive strain of mice useful for studies on the mechanism of oral carcinogenesis.

1627 DETECTION OF TUMOR PROMOTING ACTIVITY OF TWO COMBINATION PESTICICDES (CHLORPYRIPHOS 50% + CYPERMETHRIN 5% AND PROFENOS 40% + CYPERMETHRIN 4%) BY SHORT TERM *IN VIVO* BIOASSAY.

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It has been stated that upto 90% of cancers are attributable to environmental factors. The identification of the major environmental factors linked to human cancer is important for prevention and intervention efforts. Hence there is an urgent need to develop reliable and efficient screening systems for detecting potential carcinogens. Nurella-D, a combination of chlorpyrifos 50% and cypermethrin 5%, and Polytrene-C, a combination of Profenos 40% and Cypermethrin 4% were tested for its cancer promoting potential in wistar rats. Eight groups of rats (5/sex/group) were selected and to groups 1, 3, 5 and 7 diethyl nitrosamine (DEN) (200 mg/kg b.w. i.p) was administered on day-1. To groups 2, 4, 6 and 8 saline was administered (i.p). On day 14, 2-acetaminofluorine (2-AAF) (200 ppm i.p) was given to groups 3 and 4, Neurella-D (250 ppm) to groups 5 and 6 and polytrene-C to groups 7 and 8 in feed daily, for 7 weeks. Group 2 remained as vehicle control. On day 21, rats belonging to groups 1-8 were subjected 70% hepatectomy.At the end of week 9, blood was collected from the rats for estimating ALT, AST, GGT, AChE, protein and glucose, thereafter the animals were euthanised. Organs (liver and brain) were collected for biochemical (GGT in liver and AChE in brain) estimations and histopathology (liver and kidney). GGT showed significant increase in liver homogenate of group 3 and AChE showed significant inhibition in blood and brain of groups 5, 6, 7 and 8. Histopathological studies showed more number of altered hepatic foci in group 3 compared to other treated groups. The present study shows that the above combination pesticides are non carcinogenic.

1628 A NOVEL ELECTROPHORETIC PATTERN OF MICROSOMAL AND CYTOSOLIC POLYPEPTIDES FROM HEPATIC TUMORS IN RAINBOW TROUT.

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There is compelling evidence to suggest that many of the major liver diseases including cancer are of environmental origin. Fish have been used as nonmammalian vertebrate surrogates for understanding mechanisms of human cancer and its prevention. In a recent study from this laboratory, it was demonstrated that hepatic tumor (HT) in rainbow trout (RT) are different from humans and rodents, in phase I and phase II drug metabolizing enzymes. This observation prompted us to study the elecrophoretic pattern in HT of microsome and cytosolic polypeptides by SDS-PAGE. Tumors were induced in male and female RT by dietary exposure of aflatoxin B_1. Liver and HT were excised from RT for the preparation of microsome and cytosol. HT of male and female RT microsomes showed a marked decrease in the mol. wt. of approximate 45-55 kDa, a cytochrome P-450 isozyme region. The male and female HT exhibited a similar pattern and so did the controls. This was further confirmed by estimating the total cytochrome P-450 content. Cytosolic polypeptide also showed a series of differences in HT. Most bands showed a decreased intensity. A prominent difference was the reduction in the polypeptide of mol. wt. 26 and 24 kDa, a glutathione s-transferase (GST) region. This was confirmed by determining GST activity using CDNB as a substrate. A consistent difference in all HT was a significant reduction in the amount of polypeptide of 14 kDa. In conclusion, HT of male and female RT showed a marked decrease in microsomal as well as cytosolic protein profile. The identification and expression of these proteins at the molecular level are under investigation. (Supported by The Linus Pauling Institute.)

1629 KINETICS OF ABERRANT CRYPT FOCI FORMATION IN MICE OF DIFFERING SUSCEPTIBILITY TO AZOXYMETHANE-INDUCED COLON TUMORIGENESIS.

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Aberrant crypt foci (ACF), putative preneoplastic lesions, are the earliest morphological change induced by the colon carcinogen, azoxymethane (AOM). Although inbred mice differ markedly in their susceptibility to AOM tumorigenesis, we have found that ACF develop in both resistant and sensitive mouse strains after AOM treatment. The purpose of this study was to evaluate the kinetics of ACF formation and regression in sensitive and resistant mice. SWR/J, A/J (both susceptible) and AKR/J (resistant) mice were treated with 10 mg/kg AOM or saline *i.p.* once a week for six weeks, and were sacrificed at 1, 2, 4, 6 and 9 weeks following the last injection. The number of ACF and adenomas was determined in distal colons. At one week following AOM administration, a comparable number of ACF was found in the SWR/J and AKR/J mice (5.9/cm colon vs. 4.8/cm; $p<0.05$). The A/J mice, however, developed a significantly greater number of ACF (10.6/cm; $p<0.05$) than the other strains, a finding that was consistent at each of the time points examined. At two weeks after treatment, the number of ACF was significantly higher in SWR/J than AKR/J mice(10.4/cm vs. 5.3/cm; $p<0.05$), but there was no significant difference between the two strains at each of the remaining time points. Adenomas were present as early as four weeks after AOM exposure in SWR/J and A/J mice and increased in frequency throughout the study in both strains. No adenomas developed in the AKR/J mice. Based on the presence of ACF and absence of adenomas in the resistant mice, our data suggest that the biological fate of ACF is governed in part by genetic background. (Supported by NIH Grant DK49805-01 and NIEHS Training Grant 1T32ES07163.)

1630 DIFFERENTIAL EXPRESSION OF p16INK4a IN AZOXYMETHANE-INDUCED MOUSE COLON TUMORIGENESIS.

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Alterations in the p16INK4a gene have been implicated in the pathogenesis of a variety of human cancers and animal tumor models. We postulated that alterations in the expression of p16INK4a may also be involved in mouse

colon tumorigenesis induced by the chemical carcinogen, azoxymethane (AOM). In the present study, SWR/J mice were given AOM by i.p. injection at a dose of 10 mg/kg once a week for 8 weeks, and colon tissue and tumors were isolated 18 weeks later. The expression and localization of p16INK4a were examined by reverse transcription-polymerase chain reaction and immunohistochemical analyses. p16INK4a mRNA levels were low, and in some cases undetectable, in control colon tissue. However, colon tumors exhibited an 8-fold increase in p16INK4a mRNA level when compared with control colon tissue ($p<0.01$). While control colon epithelium was uniformly negative for p16INK4a immunoreactivity, p16INK4a immunoreactive cells were markedly increased in preneoplastic lesions and in adenomas isolated from AOM-treated mice. Immunohistochemical analysis also revealed a reciprocal relationship between p16INK4a and Rb protein expression. These findings suggest that alterations in the p16INK4a /Rb pathway may play an important role in AOM-induced mouse colon tumorigenesis. (Supported in part by NIH grant DK49805-01 and NIEHS Training Grant 1T32ES07163.)

1631 IDENTIFICATION OF A CANDIDATE UPREGULATED GENE IN TRANSFORMED C3H10T1/2 MOUSE EMBRYO CELL LINES INDUCED BY CARCINOGENIC NICKEL COMPOUNDS.

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Nickel compounds have been implicated in causing nasal and lung cancer in workers exposed to these compounds. Certain nickel compounds induce a variety of tumors in experimental animals and induce neoplastic transformation of cells in culture, and also have shown to cause chromosomal aberrations. Nevertheless, the exact mechanisms of nickel carcinogenesis are still unknown. Identification of the genes that are specifically expressed in nickel transformed cells but not in normal cells or genes that are expressed in normal cells but not in nickel transformed cells, is important for understanding the molecular basis of nickel carcinogenesis. We used the mRNA Differential Display to identify the mRNAs that are differentially expressed from normal and nickel transformed cell lines. Ten cDNA fragments that are differentially expressed were subcloned and sequenced. Subclones that are truly differentially expressed were identified by differential screening using composite cDNA probes. The expression of one cDNA fragment designated R3-2, is upregulated in nickel transformed cell lines compared to its expression in nontransformed 10T1/2 cells. Northern analysis of the fragment R3-2 showed a transcript size of 6.1 Kb. Sequence searches have revealed a very high sequence identity (93-98%) to calnexin gene. The isolation of this gene (fragment R3-2), that is upregulated in nickel transformed cell lines, will allow us to evaluate the functional consequences of this gene, to study its involvement in relation to tranformed phonotype.

1632 COMPARATIVE HEPATOCARCINOGENICITY OF HEXA CHLOROBENZENE, PENTACHLOROBENZENE, 1,2,4,5-TETRACHLOROBENZENE AND 1,4-DICHLOROBENZENE: APPLICATION OF A MEDIUM-TERM LIVER FOCUS BIOASSAY AND MOLECULAR AND CELLULAR INDICES.

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Due to the ubiquitous nature of chlorobenzene exposure in humans, a thorough understanding of potential adverse health effects induced by chlorobenzene exposure is needed. In the studies presented here, we measured the ability of 1,4-dichlorobenzene (DCB), 1,2,4,5-tetrachlorobenzene (TeCB), pentachlorobenzene (PeCB) and hexachlorobenzene (HCB) to promote glutathione s-transferase π (GSTP1-1) positive preneoplastic foci formation in rat liver following diethylnitrosamine (DEN) initiation. Within this same initiation/promotion study design, we also measured induction of CYP1A2, c-Jun, c-Fos ,and non-focal GST-P protein expression in liver. Liver reduced and oxidized glutathione (GSH) levels were also measured in response to chlorobenzene exposure. Results show that TeCB, PeCB and HCB all promote the formation of GST-P positive foci and that DCB does not. TeCB, PeCB and HCB treatment also led to perturbations in liver glutathione levels, whereas DCB does not. Induction of CYP1A2 was seen following treatment with TeCB, PeCB and HCB, and changes in c-Jun and c-Fos expression were consistent with GST-P expression. The results from these studies help elucidate the sequelae of chlorobenzene-induced preneoplastic foci promotion and applied to biologically-based modeling methodologies to predict the hepatocarcinogenicity of other chlorobenzene isomers. This work was supported by AFOSR (F49620-94-1-0304) and NIEHS Superfund Basic Research Program (P42 ES05949).

1633 ANTAGONISM OF HEPATIC PRENEOPLASTIC FOCI BY AN ARSENIC-CONTAINING CHEMICAL MIXTURE: ROLE OF APOPTOSIS.

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Previously, we reported the antagonistic effects of a 4-chemical mixture containing arsenic, 1,2-dichloroethane, vinyl chloride, and trichloroethylene on the development of rat pulmonary and hepatic preneoplastic/neoplastic lesions in a Multiple Organ Carcinogenicity Bioassay (Pott *et al.*, The Toxicologist 42(1-S):75, 1998). To determine if the antagonistic effects in the liver could be due to increased apoptosis and/or decreased proliferation of hepatocytes, we immunohistochemically evaluated hepatocellular apoptosis using the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) method. Apoptotic cells were evaluated in 10 random fields of each liver section (≥2000 total hepatocytes), and labeling index (LI) was expressed as the percentage of labeled nuclei. Compared to controls (LI=1.115±0.251), 4-chemical mixture treatment was associated with a significant decrease in the occurrence of hepatocellular apoptosis (LI=0.708±0.137, $p<0.01$). The decrease in apoptosis is contrary to what we anticipated and cannot account for the antagonistic effects of the 4-chemical mixture on the development of hepatic preneoplastic foci. Analysis of hepatocyte proliferation is currently underway. Co-localization of apoptotic and proliferating hepatocytes with preneoplastic hepatic foci is also planned. (This project is funded by the NCI (K08 CA72396-02), ATSDR (Cooperative Agreement No. U61/ATU881475), and NIEHS Superfund Basic Research Program (P42 ES05949).)

1634 DIETARY GLYCINE PREVENTS THE DEVELOPMENT OF LIVER TUMORS CAUSED BY THE PEROXISOME PROLIFERATOR WY-14,643.

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Previous studies demonstrated that dietary glycine prevents elevated rates of cell proliferation following treatment with WY-14,643 (WY). Since increased cell replication is associated with the development of hepatic cancer caused by peroxisome proliferators, glycine may have anti-cancer properties. Therefore, experiments were designed to test the hypothesis that dietary glycine would inhibit the hepatocarcinogenic effect of WY. Male Fischer 344 rats were fed 4 different NIH 07-based diets: 5 % glycine; 5 % valine for N_2 balance (control); 0.1 % WY + 5 % valine (WY); 0.1 % WY + 5 % glycine (WY + glycine). Food consumption did not differ among the 4 groups, but WY-fed rats weighed 10 to 25 % less as expected. Serum glycine levels were elevated 4-5 fold by glycine-containing diets. After 22 weeks, livers from rats fed WY had a similar incidence and multiplicity of proliferative lesions (foci and adenomas) as those fed WY + glycine. Moreover, cell proliferation in the surrounding "normal" parenchyma (labeling index ≅ 4 %) and the foci (labeling index ≅ 50 %) did not differ between WY and WY + glycine-fed rats. However, after 52 weeks of dietary exposure to WY, glycine prevented formation of small (0-5 mm diameter) tumors by 23 % and inhibited the development of medium size (5-10 mm) tumors by 64 %. Moreover, glycine prevented the formation of the largest tumors (> 10 mm) nearly 80 %. Therefore, glycine did not inhibit early foci formation; however, it did significantly decrease tumors at 52 weeks, and the inhibitory effect of glycine was greater with increasing tumor size. These studies demonstrate that dietary glycine prevents the development of hepatocarcinoma caused by WY consistent with the idea that it may be an effective chemopreventive agent.

1635 DIETARY GLYCINE INHIBITS THE GROWTH OF B16 MELANOMA TUMORS IN MICE.

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Dietary glycine has been shown to blunt increases in cell proliferation which

are associated with tumor development. However, the effect of glycine on tumor growth *in vivo* is unknown. Therefore, these experiments were designed to test the hypothesis that dietary glycine would inhibit the growth of experimental tumors in mice. C57BL/6 mice were fed diets containing 5 % glycine or control diet (5 % casein for nitrogen balance) for 3 days prior to *s.c.* implantation of B16 tumor cells (7 x 10^5/mouse). Weight gain and food consumption did not differ between the control and glycine diet-fed mice for the 14 days of the study. As expected, tumor size, measured with digital calipers, increased rapidly in mice fed control diet. In mice fed glycine diet, however, tumor size was 50 to 75 % less, and at sacrifice on day 14, tumors from glycine-fed mice weighed about 65 % less than tumors from mice fed control diet. However, glycine (0.01mM – 10 mM) did not effect rates of growth of B16 cells *in vitro* consistent with the hypothesis that the mechanism of action of glycine was not via a direct action on cell growth. Moreover, tumor size and mitotic index of B16 tumors *in vivo* did not differ 2 days after implantation. All tumors were less than 2 mm diameter at this time and therefore were not dependent on vascular supply. After 14 days, tumors from mice fed dietary glycine had 70 % fewer arteries than those fed control diet ($p<0.05$). Further, glycine (0.01- 10 mM) inhibited the growth of endothelial cells *in vitro* in a dose-dependent manner (IC_{50} = 0.05 mM). These data are consistent with the hypothesis that dietary glycine prevents tumor growth *in vivo* via inhibition of angiogenesis by blocking proliferation of endothelial cells.

1636 TYROSINE AND PHENYLALANINE DEPRIVATION DECREASES B16BL6 MELANOMA CELL INVASION BY DECREASING PLASMINOGEN ACTIVATORS AND INCREASING PLASMINOGEN ACTIVATOR INHIBITOR-1.

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The incidence of malignant melanoma is rapidly increasing in the United States. Once this cancer becomes invasive, it is very aggressive and can readily metastasize. Diet and nutritional factors are important to the development and progression of human cancers. We previously reported that B16BL6 melanoma cells isolated from mice fed a low tyrosine (Tyr) and phenylalanine (Phe) diet are less able to establish metastases when re-inoculated into mice fed a complete diet. Herein, we showed that invasion through Growth Factor Reduced Matrigel™ is significantly reduced by Phe (22%), Tyr (67%), or Tyr and Phe (76%) deprivation, respectively. Successful invasion requires destruction of basement membranes. Plasminogen activators (PAs) are enzymes necessary for local invasion of tumor cells. Zymographic analysis showed that Phe or Tyr deprivation and to a greater extent Tyr and Phe deprivation decreased urokinase PA and tissue PA activity. These results were verified by Western blot analysis. PA protein expression was decreased in melanoma cells cultured in Tyr- and Phe-free medium. PAs are regulated by plasminogen activator inhibitors (PAIs). Western blot analysis showed that Tyr and Phe deprivation increased PAI-1. Together, these data suggest that the decreased invasion observed in B16BL6 melanoma cells cultured in Phe-, Tyr-, or Tyr- and Phe-free medium is partially explained by decreased PAs and increased PAI-1. (Supported by NIH grants RO1 CA42465 and F31 CA70130 and AICR grant 97A158.)

1637 GENISTEIN STIMULATES GROWTH OF MALE HUMAN PANCREATIC TUMOR CELLS *IN VITRO*.

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Extensive research has focused on the anticarcinogenic effects of phytoestrogens. Genistein has been one of those most studied. A number of studies have shown growth inhibition of gastric cancer, prostate cancer, breast cancer, and lymphoma. Epidemiological studies have suggested this compound as a potential chemoprotective agent. However, our data in the present study suggest that this compound may have adverse effects on pancreatic cancer in males. This study demonstrated that genistein, at concentrations of 1 and 10 μ M, stimulated (>60%) the growth of human pancreatic adenocarcinoma cells *in vitro* from males and had no effect on pancreatic tumor cells from females. Equol, which is structurally similar to genistein, demonstrated inhibitory effects on the growth of female pancreatic tumor cells. Biochanin A also had inhibitory effects on tumor cells from females. This study further showed that genistein increased expression of K-*ras* in both male and female pancreatic tumor cells. Increased K-*ras* expression is associated with increased proliferation. Data in the present study also revealed that genistein upregulates the multidrug resistant gene, *mdr*-1, in pancreatic tumor cells from males. This study has shown gender differences in the response of pancreatic tumor cells to genistein. Because of the increased use of dietary supplements, including genistein, by consumers and non-regulation of these products by FDA, more studies are needed to ascertain their effects in humans under normal and cancerous conditions, as well as potential gender differences.

1638 DEVELOPMENTAL EXPOSURE TO GENISTEIN RESULTS IN LONG TERM ADVERSE EFFECTS.

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Exposure to synthetic estrogenic compounds results in permanent alterations in the reproductive tract if exposure occurs during critical stages of development. Reports from our lab have shown that perinatal exposure of mice to diethylstilbestrol (DES) results in impaired fertility, structural malformations and tumors in the genital tract. Thus, the developing reproductive tract appears to be exquisitely sensitive to perturbation by estrogenic compounds. The isoflavone genistein, which is found in soy products including soy-based infant formulas, is known to have estrogenic activity. The nutritional and pharmaceutical use of this compound has increased over the last few years because of its potential beneficial effects. We have investigated the possible risks posed by genistein if exposure occurs early in development. Mice were exposed to genistein (1-100 μ g/pup/day) on neonatal days 1-5. Early markers of estrogenicity including estrogen receptor alpha and beta expression were examined on day 5. Increases in uterine epithelial cell height and proliferation, and lactoferrin (LF) expression were demonstrated following neonatal genistein exposure similar to DES. Neonatally exposed genistein mice were allowed age to 18 mo. Lesions following genistein exposure include multi-oocyte follicles, uterine epithelial hyperplasia, and hypoplastic uteri. Many long-term effects observed after DES treatment, including uterine adenocarcinoma, were observed after developmental exposure to genistein. Similarities between DES and genistein point to the need for further mechanistic studies on phytoestrogens and their role in long-term effects following developmental exposure.

1639 EFFECTS OF NATURALLY-OCCURRING COUMARINS ON 7,12-DIMETHYLBENZ[A]ANTHRACENE DNA ADDUCT FORMATION IN SENCAR MOUSE EPIDERMIS.

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Coumarins are widely distributed in nature, present in citrus fruits, herbs, and vegetables, and represent an important part of the human diet. We have previously shown that naturally-occurring coumarins can inhibit P450 activity, block DNA adduct formation by 7,12-dimethylbenz[a]anthracene (DMBA), and inhibit skin tumor initiation in mice by this polycyclic aromatic hydrocarbon. Imperatorin, which preferentially inhibits pentoxyresorufin o-dealkylase activity, effectively blocks skin tumor initiation by DMBA whereas bergamottin, which preferentially inhibits ethoxyresorufin o-deethylase activity, does not. The current study was designed to determine the mechanistic basis for these differences. Female SENCAR mice were pretreated topically with coumarins or acetone (vehicle control) 5 min prior to topical treatment with 10 nmol [3H]DMBA. At 24 hr, epidermal DNA was isolated and DNA adducts determined using liquid scintillation spectroscopy. Adduct levels in mice that received no pretreatment were 4.5±0.6 (mean±SE, n=3) pmol/mg of DNA. Adduct levels (expressed as % control) in mice pretreated with coumarins (100, 400, 800 and 1600 nmol), were as follows: bergamottin, 124±37, 141±17, 70±29, and 67±23; imperatorin, 55±19, 42±15, 29±9, and 18±5; and isopimpinellin, 58±6, 45±9, 47±1, and 31±5, respectively. HPLC analysis of the above DNA hydrolysates revealed differences in the ratio of DNA adducts arising from syn-:anti-DMBA diol-epoxides.This ratio in control mice was 0.40±0.02. Pretreatment with the coumarins (100, 400, 800, and 1600 nmol) resulted in the following ratios: bergamottin, 0.71±0.08, 0.93±0.03, 0.94±0.06, and 0.98±0.08; imperatorin, 0.53±0.04, 0.65±0.03, 0.70±0.03, and 0.96±0.08; and isopimpinellin, 0.45±0.02, 0.49±0.02, 0.51±0.06, and 0.49±0.07, respectively. The differences in ability of specific coumarins to block DMBA DNA adduct formation and skin tumor initiation may be related to their specificity toward

inhibiting specific cytochrome(s) P450. Metabolism experiments in progress will specifically address this hypothesis. (ES06014)

1640 ANTIPROLIFERATIVE AND CYTOTOXIC EFFECTS OF XANTHOHUMOL IN HUMAN PROSTATE CANCER CELLS.

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Xanthohumol (XN), the major prenylated flavonoid found in hops (Humulus lupulus), was examined for its potential chemopreventive activity for prostate cancer in humans. Out of 12 flavonoids from hops tested, XN was found to be most effective in inhibiting the growth of the human prostate cancer cell line, LNCaP. The antiproliferative activity of XN was accompanied by inhibition of DNA synthesis using the [3H]-thymidine incorporation assay, with IC50s of 5.1 and 3.8 μM, after 2 and 4 days of treatment, respectively. Xanthogalenol (XG), a flavonoid structurally similar to XN with an O-methyl substituent at 4' as compared to 6' in XN, was much less growth-inhibitory than XN in LNCaP cells. At 5 μM or greater, XN was cytotoxic as indicated by the tetrazolium dye (MTT) and trypan blue exclusion assays. Analysis by flow cytometry showed that XN, at 40 μM, caused apoptosis in LNCaP cells after 4 days of treatment. This concentration of XN also produced an increase in the number of cells in S phase with a decrease in cells at the G1 phase. The findings from these in vitro studies suggest that XN may potentially act as a chemopreventive agent for androgen-dependent prostate cancer in humans. However, further in vivo animal studies are needed to confirm this hypothesis. (Supported by the Hop Research Council and NIEHS grants no. ES00210 and ES03850).

1641 A *MU*-CLASS GLUTATHIONE S-TRANSFERASE (GST) IS RESPONSIBLE FOR HEPATIC CYTOSOLIC AFLATOXIN B1-8, 9- EPOXIDE (AFBO) CONJUGATING ACTIVITY IN THE NON-HUMAN PRIMATE *MACACA ASCICULARIS(MF).*

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Large species differences exist in sensitivity to aflatoxin B1 (AFB1)-induced liver cancer. Constitutive expression of certain forms of *alpha*-class GST are important determinants of resistance of rodents to AFB1-induced liver cancer. Mice are resistant to AFB-induced liver cancer because they express an *alpha*-class GST (mGSTA3-3) that has high activity towards AFBO. Rats do not constitutively express a GST with high AFBO activity and are sensitive to AFB1-induced hepatocarcinogenesis, but an inducible *alpha*-class GST (rGSTA5-5) can confer resistance in rats. In contrast to rodents, human hepatic *alpha*-class GST have little or no AFBO detoxifying activity. Recently, our lab has found that liver from the nonhuman primate *Mf*, has significant constitutive hepatic GST activity toward AFBO. Previous cDNA cloning from a *Mf* hepatic cDNA library identified three different *alpha*-class GST, but none had measurable AFBO activity. To determine which class of GST is (are) responsible for this activity, hepatic *Mf* GST were affinity purified, and individual proteins identified by reverse-phase HPLC and chromatofocusing. Specific *Mf* GST cDNAs were also cloned by RT-PCR. Western blot analysis of purified GST proteins revealed that the AFBO activity is due to *mu*-class GST. Two distinct *mu*-class *Mf* GST cDNAs were cloned- mfGSTM1 and mfGSTM2. cDNA-derived amino acid sequences revealed that mfGSTM1 shares 97% identity with hGSTM4 and mfGSTM2 shares 96% homology with hGSTM2. Activity analysis of cDNA-expressed proteins found that mfGSTM1-1 had no AFBO activity whereas mfGSTM2-2 had an AFBO activity of 333 pmol/min/mg, which could account for most or all of the hepatic cytosolic AFBO activity. Northern blot results demonstrated that mfGSTM2 is expressed in liver but not in lung and kidney. These results indicate that, unlike rodents, it is a *mu*-class GST that is responsible for the hepatic cytosolic GST activity toward AFBO in the nonhuman primate, *Mf*. (Supported by R01ES-05780, P30ES-07033 and P30RR00166.)

1642 THE ROLE OF DNA DOUBLE-STRAND BREAKS IN A NOVEL DNA REPAIR PROCESS.

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DNA repair is vital for maintaining genomic stability. The role of DNA repair in assuring normal development and preventing carcinogenesis is demonstrated in individuals with DNA repair disorders such as xeroderma pigmentosum and Cockayne syndrome. We have discovered a novel, inducible DNA repair response in cells treated with ionizing radiation (IR) at the G1/S border of the cell cycle. This process differs from constitutive repair processes by producing very long repair patches (VLRP) of at least 150 nucleotides in addition to constitutive repair patches of 3-5 nucleotides. The aim of these studies is to examine the signal that induces VLRP and the proteins involved in their production. We examined whether VLRP are induced in response to a DNA lesion specific to IR. Cells were treated with agents that produce subsets of the damage induced by IR: hydrogen peroxide which produces predominately base modifications and single-strand breaks, or bleomycin which produces localized, multiple lesions, resulting in DNA double-strand breaks. The repair patch size was analyzed by alkaline cesium chloride density gradients. Treatment of cells at the G1/S border with hydrogen peroxide resulted in only short repair patches, while bleomycin treatment produced VLRP of 170 bases and constitutive short patches. Because the damage produced by bleomycin is concentrated in a manner similar to that produced by IR, our results indicate that the production of double-strand breaks, possibly as discrete clusters, may be the signal for the induced repair. These findings have led to an examination of the role of double-strand break repair proteins in the production of VLRP. We hypothesize that the ataxia telangiectasia (AT), DNA-dependent protein kinase, and XRCC4 gene products will be involved in the production of VLRP. Fibroblasts with mutant AT protein were synchronized to the G1/S border, treated with IR, and subjected to gradient analysis. The AT fibroblasts produced both VLRP of 170 nucleotides and constitutive repair patches. While this data does not support our hypothesis, it does not preclude a role for VLRP production in the repair of double-strand breaks. The components of the DNA-dependent protein kinase and XRCC4 gene product are currently under investigation. (Supported by NCI CA62059.)

1643 NEW CYCLIC ADDUCTS FORMED IN REACTIONS OF 2-CYANOETHYLENE OXIDE AND GLYCIDAMIDE WITH dA AND dC.

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We have previously demonstrated that several mutagenic and carcinogenic epoxides including ethylene oxide, propylene oxide, epichlorohydrin, glycidol and 2-cyanoethylene oxide (ANE, the mutagenic epoxide metabolite of acrylonitrile) react with dC and DNA under physiological conditions to form 3-hydroxyalkyl-dU (3-HA-dU) lesions which can be mutagenically bypassed during *in vitro* DNA replication. These lesions are formed following epoxide-induced hydroxyalkylation at N3 of dC and is followed by rapid hydrolytic deamination. This deamination is intramolecularly catalyzed by the sidechain hydroxyl group via the formation of a cyclic intermediate. During our investigations of the *in vitro* reactions of the mutagenic epoxide metabolite of acrylamide, (glycidamide, GA) with nucleosides, we found that in addition to the expected 3-HA-dU adduct with dC, relatively stable cyclic adducts were formed in reactions of GA with dC and dA. The cyclic adducts were spectroscopically characterized by UV and MS. These cyclic adducts can be hydrolyzed under physiological conditions to form adducts which are unstable and undergo deamination or Dimroth rearrangement to form 3-(2-hydroxy-2-carboxyethyl)-dU (3-HCE-dU) and N6-HCE-dA, the same adducts that form in the *in vitro* reactions of ANE with DNA (pH 7, 37oC, 3h). Mechanistic studies on the formation of these adducts will be presented. (Supported by grants from NIEHS (ES05694) and NIH Center grants ES00260 and CA16087.)

1644 IRON AND/OR AROCLOR 1254 DO NOT INDUCE MUTATIONS OF THE *CII* GENE OF LIVER DNA FROM LAMBDA/*LACI* C57BL/6 TRANSGENIC MICE.

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Iron is a human hepatocarcinogen. ß2-Microglobulin gene and hemochromatosis gene knockout mice accumulate hepatocellular iron with postulated increase in liver carcinogenesis by an oxidative mechanism. Previously, we have shown massive synergistic interaction between iron overload and the polychlorinated biphenyl mixture Aroclor 1254 (AR) in C57BL/10ScSn mice leading to oxidative DNA damage and hepatocellular cancer. As one approach to study mutagenesis in this system, groups (n=4) of male lambda/*lacI* C57BL/6 transgenic mice were given iron-dextran (600mg/kg s.c.)

and some, after one week, AR in the diet (0.01 %) for 7 weeks. Hepatic ethoxyresorufin de-ethylase was determined as an estimate of CYP1A1 activity i.e. control 107 (&πλυσμν;25), iron 44 (&πλυσμν;9), AR 2714 (&πλυσμν;268), iron/AR 1418 (&πλυσμν;429) pmol/min/mg protein (&πλυσμν;SD). The lower levels of activity with iron have been observed before and may reflect increased CYP1A1 turnover. Western blotting confirmed the marked induction of both CYP1A1 and 1A2. No significant changes in mutational frequencies (MFs) of *lacI* were observed between the treatment groups. In an alternative assay, MFs of the *cII* gene of the lambda phage shuttle vector were determined i.e. control 8.0 &πλυσμν; 2.3, iron 10.5 &πλυσμν; 1.6, AR 8.2 &πλυσμν; 2.7, iron/AR 9.6 &πλυσμν; 2.4 per 10^5 plaques. In contrast, 14 days after 5 daily doses of N-nitrosodimethylamine as a positive control gave 24.9 &πλυσμν; 5.2. In conclusion, these studies show that oxidative processes with either iron overload alone or in combination with PCBs, do not induce simple point mutations and small insertions or deletions detected by this assay. However, other mutation processes such as homologous recombination require investigation.

1645 CYP1B1 SELECTIVELY MEDIATES BIOACTIVATION OF DIBENZO[A,L]PYRENE AND DIBENZO[A,L]PYRENE-11,12-DIHYDRODIOL IN PRIMARY HUMAN BREAST EPITHELIA.

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Human primary breast epithelial cells [HBEC] convert the potent carcinogen, dibenzo(a,l)pyrene [DB[a,l]P], to dihydrodiol epoxides [DE] that modify adenine and guanine bases of DNA. Primary HBEC constitutively express cytochrome P450 [CYP], CYP1B1, which is induced by DB[a,l]P and by Ah-receptor [AhR] ligands, such as 3,4,5,3',4',5'-hexachlorobiphenyl [HCB] and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin [TCDD]. Constitutive expression of CYP1A1 is absent in these cells. This study demonstrates that the formation of DB[a,l]P-DNA adducts in HBEC from three donors is consistent with metabolism by CYP1B1 rather than by CYP1A1. Relative distribution of adducts was constant, while the absolute level of adduct formation varied in proportion to the magnitude of CYP1B1 induction. The putative precursor, DB[a,l]P-11,12-dihydrodiol [DB[a,l]P-DH], produces syn- but not anti-DE adducts, which, however, form from DB[a,l]P [1.0 μM]. The magnitude of adduct formation following treatment with 0.1 μM DB[a,l]P [24 h] was 2- to 4 times lower than for 1.0 μM DB[a,l]P and showed a loss of syn-PAHDE adduct formation, consistent with exclusive metabolism through the DH. The bioactivation of DB[a,l]P-DH was highly induced by TCDD, while being extensively inhibited by HCB pretreatment. This is consistent with previous observations that HCB is a potent inhibitor of CYP1B1. This is consistent with previous observations that HCB is a potent inhibitor of CYP1B1. p53 was elevated approximately in proportion to adduct formation, was substantially increased two days after removal of DB[a,l]P or DB[a,l]P-DH, but declined five days later. p21 responded in parallel to p53, while also exhibiting independent regulation [high levels in confluent, untreated HBEC and a late response to DB[a,l]P and DB[a,l]P-DH]. DB[a,l]P and DB[a,l]P-DH each effected cell cycle arrest of HBEC in G2. (Supported by NIEHS 144EN46, NIH 144-FR43, and DOD DAMD17-94-J-4054.)

1646 7, 12-DIMETHYLBENZ[A]ANTHRACENE METABOLISM AND DNA ADDUCT FORMATION IN ISOLATED JAPANESE MEDAKA (*Oryzias latipes*) LIVER CELLS.

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Some fish species are being used as supplemental animals for carcinogenicity studies; however, basic research is required to determine if mechanisms of carcinogenesis in these teleosts reflect the carcinogenic process in mammals. The object of this study was to compare 7, 12-dimethylbenz[a]anthracene (DMBA) metabolism and DNA adduct formation in liver cells from the teleost, Japanese medaka, with that in mouse embryo fibroblasts (MEF). Cells were isolated by enzymatic digestion and maintained in monolayer culture. Following exposure to DMBA, culture medium was removed and analyzed by high pressure liquid chromatography for phase I DMBA metabolites. The rate of formation of phase I metabolites was significantly lower in medaka liver cells (MLC) than in MEF. Differences in the types of phase I metabolites formed were also apparent: DMBA 3,4-dihydrodiol (important for mammalian carcinogenesis) was barely detectable in MLC, but was a significant metabolite in MEF; DMBA 5,6-diol and hydroxymethyl DMBA were major metabolites in MLC, but were minor metabolites in MEF. 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin pretreatment resulted in 5 to 6 fold increases in formation of total DMBA phase I metabolites in both types of cells. Furthermore, ^{3}H-DMBA-DNA adducts were formed at lower levels in MLC than in MEF, and C_{18} chromatography demonstrated the DNA-adducts formed in MLC were much more polar than most of the adducts formed in MEF. These findings indicate significant differences in DMBA metabolism and adduct formation between MLC and MEF. Although DMBA is a potent carcinogen in both mammalian and teleost systems, the mechanisms that bring about carcinogenesis may be quite different.

1647 ANALYSIS OF N-OH-NAABP INDUCED MUTATIONS AT THE HPRT GENE.

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Interaction of environmental chemicals with biological macromolecules can result in genotoxic and cytotoxic effects. 4-ABP, a human bladder carcinogen present in cigarette smoke, can be metabolized to N-OH-N-acetylaminobiphenyl (N-OH-AABP) a strong electrophile capable of reacting with DNA. In this study the mutational spectrum of N-OH-AABP exposed human lymphoblasts were analyzed using the HPRT gene as the target gene. Cultures of 2 x 10^8 TK6 cells were treated with N-OH-AABP, incubated for four days and treated with the HPRT selective agent 6-TG for two weeks. Control cultures (no treatment) were also exposed to 6-TG. DNA was then extracted for mutational spectra analysis of the HPRT exon 3, from both control and exposed cultures. HPRT sequences were separated from wild type sequences by denaturing gradient gel electrophoresis (DGGE). DGGE analysis of the HPRT exon 3 clearly indicated that exposure to N-OH-N-AABP can increase the level of induced mutations when compared to control cultures. Sequencing analysis showed the high specificity of the 4-ABP metabolite mutations induced. Background mutation detected both in control and exposed cultures were at base pair 197 with A to G transition. From the exposed mutants the detected changes were mainly G to T transversions (30%), followed by an insertion and repeat at position 178 (20%) and G to A transition (5%). In conclusion, all the mutations observed readily translated into structural changes that would alter or inactivate the HPRT enzyme.

1648 DETERMINATION OF RELATIVE GENOTOXICITIES OF SPECIFIC NICKEL COMPOUNDS IN SHORT-TERM *IN VITRO* ASSAYS USING 10T1/2 CELLS.

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To evaluate carcinogenic potentials of specific nickel compounds, we conducted short-term in vitro assays for genotoxicity, cytotoxicity, and phagocytosis using C3H 10T1/2 clone 8 (10T1/2) mouse embryo cells. We studied six insoluble nickel compounds and two soluble nickel compounds. All insoluble nickel compounds were phagocytosed by 10T1/2 cells in the following order: NiO (black) > NiO (green) > Ni_3S_2 (INCO) > Ni_3S_2 (NTP) > Metallic Ni (<1 m Nm) > Metallic Ni (3-7 m 7m). All compounds caused cytotoxicity in the following order: Ni_3S_2 (INCO) = NiO (black) > Metallic Ni (<1 mIm) > NiO (green) > Ni_3S_2 (NTP) > $NiSO_4$ (Aldrich) > $NiSO_4$ (NTP) > Metallic Ni (3-7 m(m). Most of the compounds studied increased the micronuclei frequencies in the following order: Ni_3S_2 (INCO) ~ Ni_3S_2 (NTP) > NiO (black) ~ Metallic Ni (<1 m Mm) > $NiSO_4$ (Aldrich) ~ $NiSO_4$ (NTP) > Metallic Ni (3-7 m(m) > NiO (green). Ni_3S_2 (INCO), $NiSO_4$ (Aldrich) and Metallic Ni (<1 m(m) caused increases in chromosomal aberrations, while Metallic Ni (3-7 mrm) did not. All compounds studied caused morphological transformation, in the following order: NiO (green) > NiO (black) > Ni_3S_2 (NTP) > Ni_3S_2 (INCO) > $NiSO_4$ (Aldrich) = $NiSO_4$ (NTP). Our results for the genotoxicities of these nickel compounds were generally consistent with their carcinogenicity in animal studies. The efficiencies with which these nickel compounds induce morphological transformation in 10T1/2 cells broadly correlates with their carcinogenicity in animals. This transformation assay should be further validated with additional nickel compounds to determine its ability to predict the carcinogenicity of nickel compounds in general.

1649 STUDY ON METABOLISM OF ARSENIC AND FLUORIDE IN THE CHRONIC ARSENIC POISONING IN INNER MONGOLIA, CHINA.

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Chronic arsenic poisoning in China occurs as a result of arsenic as well as the mixture of arsenic(As) and fluoride(F) in well water. This studies on the metabolism of As and F in patients with chronic arsenic poisoning from Inner Mongolia, China. It is found a significant correlation between As and F in the well water. In some patients with chronic arsenic poisoning dental fluorosis was also observed patients with chronic arsenic poisoning. The mean urinary total As (iAs + metabolites) and F level in the 36 subjects was 2.39 ± 0.67 mg As/L and 2.1 ± 1.1 mg/L, respectively. There was a significantly higher concentration of inorganic arsenic(iAs), methylated arsenic and dimethylated arsenic in urine in the patients with chronic arsenic poisoning as compared to the control group. On the other hand, F concentrations were 2 times higher in the As group than control. Comparisons between male and female revealed higher concentrations of arsenicals and F in the urine of male patients with chronic arsenic poisoning in comparison to the female patients. The characteristic accumulation of iAs in the hair was confirmed in our study with a higher level found in the male patients versus the female patients. The level of F in the hair was 2 times the concentration found in controls. These data show no significant correlation between As and F concentrations in the hair. The cytotoxicty study using normal human liver cells exposed to As and F showed that fluoride reduced the cytotoxicty of arsenic. The importance of such data will be the focus of future studies.

1650 MONTE CARLO ANALYSIS OF INORGANIC ARSENIC EXPOSURE IN THE US.

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Daily inorganic arsenic (As_i) exposure of adults in the conterminous US from air, food, soil, and drinking water has been estimated. Existing databases of arsenic in air, soil, and drinking water were linked with data from a study of food commodities expected to account for at least 90% of the As_i in the US diet. A Monte Carlo simulation estimates that the range of daily As_i exposure (5th to 95th percentile) from the four exposure sources is 0.6 to 13.2 μg for women and 0.8 to 15.9 μg for men. The mean daily As_i exposure from the four sources is 5.2 μg for women and 6.3 μg for men. At the mean ratios, food and drinking water contributed most to the total daily As_i exposure. Food contributed 67.7% for women and 70.7% for men whereas drinking water contributed 28.8% for women and 26.4% for men. Contributions to the total daily As_i exposure by air and soil at the mean ratios are estimated to be negligible: air contributes 0.9% for women and 0.8% for men and soil contributes 2.7% for women and 2.0% for men. Variations in the mean daily exposure to As_i among the West, South, Midwest, and Northeast regions of the US appear to result primarily from differences in drinking water As_i levels. The results of simulation indicate that, for most adult Americans, the highest exposure to As_i comes from food and the next highest exposure comes from drinking water. The majority of adults in the US receive little exposure to As_i from air and soil.

1651 CONTROLLED ARSENATE EXPOSURES OF HUMAN SUBJECTS: KINETICS OF ELIMINATION.

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The health effects of arsenic containing drinking water have been of concern because inorganic arsenic is a potential carcinogen or cancer promoter. Previously, we developed a physiologically based pharmacokinetic (PBPK) model of ingested, inhaled and injected arsenate (AsV); arsenite (AsIII), monomethylarsonic acid (MMA) and dimethylarsenic acid (DMA) using studies in mice. The PBPK model has been scaled to humans and the urinary elimination was optimized from literature values that are from foreign populations exposed at 5-10 times the US standard. To our knowledge this is first controlled human exposure study at the current EPA standard. To validate the model and to investigate the balance between inorganic arsenic and arsenic metabolites, five subjects (2 female; 3 male) were given a single dose of 100 μg sodium arsenate dissolved in deinoized water. A 24 hr pre-exposure and repeated individual urine samples were collected to 96 hr post exposure. Hydride generation and atomic absorption spectrometry quantified the urine arsenic. The pre-exposure values varied between 2.5-9.0 μg/L. The pattern of metabolites was essentially the same for all five subjects. The total arsenic processed to urine was about 55% of the dose. Clearance rates also varied between subjects. We conclude that diet has a major impact on the pre-exposure dose as reflected by diet recall. Methylation also varies as does clearance rates. Therefore, arsenic PBPK models should be optimized by the Monte Carlo method to account for population variations. (EL is supported by a Department of Education Graduate Assistance in Areas of National Need Program Grant. Supported by ARCO.)

1652 METABOLISM AND TOXICITY OF ARSENICALS IN PRIMARY RAT HEPATOCYTES.

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The metabolism and toxicity of arsenite (iAs^{III}), arsenate (iAs^{V}) and trivalent and pentavalent methylated arsenicals have been examined in primary hepatocytes isolated from adult male Fischer 344 rats. The uptake and methylation of iAs^{III} by hepatocytes cultured in phosphate-rich medium was six to eight-fold greater than the uptake and methylation of iAs^{V}. At concentrations of 0.1 to 1 μ M, iAs^{III} was converted mainly to dimethylarsenic (DMAs). DMAs was quickly released from the cells. Little methylarsenic (MAs) was detected inside the cells. At 4 to 10 μ M iAs^{III}, the portion of MAs in cells increased and a significant amount of MAs was released into the medium. The total methylation yield increased in the range of 0.1 to 4 μ M iAs^{III}. At 10 to 20 μ M iAs^{III}, inhibition of methylation occurred and the DMAs/MAs ratio decreased dramatically. Metabolism of MAs also depended on valency of arsenic. Methylation of 1 μ M MAs^{V} yielded little DMAs; in contrast, more than 90% of 1 μ M MAs^{III} was converted to DMAs. The cytotoxicities of arsenicals were examined over a 24-hr incubation period using the MTT assay. Pentavalent arsenicals (iAs^{V}, MAs^{V}, and $DMAs^{V}$) were not toxic at concentrations up to 20 μ M. MAs^{III} and $DMAs^{III}$ were significantly more toxic (IC_{50} ~ 2.5 μ M) than iAs^{III} (IC_{50} > 5 μ M). Addition of 2.5 mM GSH into the culture did not change methylation yield from iAs but stimulated the release of MAs and DMAs from cells, decreasing the intracellular retention of arsenicals. GSH completely protected cells against toxicity of trivalent arsenicals. Simultaneous addition of 2 μ M sodium selenite reversibly inhibited methylation and increased retention of iAs^{III} in cells. Selenite potentiated the cytotoxicity of iAs^{III}, MAs^{III}, and $DMAs^{III}$ and interfered with the protective effect of GSH. These results indicate that the capacity for the methylation of arsenicals by primary rat hepatocytes is concentration dependent, influenced by the valency of the arsenical, and affected by the presence of GSH and selenite.

1653 ACTIVATION OF NF-κ B AND AP-1 BY SODIUM ARSENITE IN HUMAN KERATINOCYTES.

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Arsenic exposure is associated with a variety of human cancers. Although the mechanism of arsenic carcinogenesis remains elusive, inorganic arsenic has been suggested to act through epigenetic mechanisms such as interaction with intracellular signaling pathways. We examined the effect of arsenic on the transcription factors nuclear factor kappa b (NF-κB) and activator protein-1 (AP-1). We report here the time-course and concentration-response relationship for the activation of NF-κB and AP-1 by sodium arsenite in human keratinocytes. HaCaT cells were exposed to arsenite, whole cell extracts were prepared, and NF-κB and AP-1 activity were analyzed by the electrophoretic mobility shift assay. Activation of both NF-κB and AP-1 by arsenite occurred at concentrations as low as 0.1 μ M. Supershift analyses using polyclonal antibodies to various NF-κB and AP-1 subunits were performed to identify the proteins in the shifted complexes. The inducible form of AP-1 was found to be a heterodimer of c-Jun and c-Fos while the inducible form of NF-κB contained p50 but not p65. The involvement of reactive oxygen species in arsenic-induced activation of the studied transcription factors was also investigated by pretreatment of cells with the

antioxidants butylated hidroxyanisole (BHA), pyrrolidine dithiocarbamate (PDTC), and N-acetyl cysteine (NAC). BHA and PDTC inhibited arsenite-induced activation of NF-κB whereas NAC inhibited AP-1 activation. These studies suggest that arsenic activates these two signaling pathways by different mechanisms. MV holds a NIEHS Individual Predoctoral Fellowship.

1654 ARSENITE [As(III)] AND ARSENATE [As(V)] ENHANCE DNA BINDING OF THE AP-1 AND Elk-1 TRANSCRIPTION FACTORS AND c-jun AND c-fos GENE EXPRESSION IN PRECISION-CUT RENAL SLICES.

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Although arsenic is recognized as a nephrotoxicant, little is known about the molecular impact of low concentrations of various arsenic species on the kidney. Our previous studies have shown that nanomolar concentrations of arsenite [As(III)] or arsenate [As (V)] do not elicit overt cytotoxicity, but are associated with a concentration- and time-dependent induction of heat shock protein 32 (Hsp 32; heme oxygenase-1). Given that Hsp32 is regulated, in part, by the activator protein-1 (AP-1) transcription factor, we hypothesized that As(III) and As(V) would activate the MAPK pathway. Three transcription factors lie downstream of the stress-activated c-jun N-terminal kinase and p38 kinase: AP-1, ATF2 and Elk-1. Using electrophoretic mobility shift analysis, the impact of As(III) and As(V) on the DNA binding of these factors was investigated (0–1000 nM; 2-6 hr). Both species enhanced the DNA binding of AP-1 and Elk-1, but did not effect DNA binding of ATF2. Since expression of the early-immediate genes c-jun and c-fos are driven by AP-1 and Elk-1, respectively, expression of these genes was examined by RT-PCR. After 2 hr of exposure, 100 nM As(III) or As(V) enhanced c-jun gene expression 1.32±0.07 and 1.20±0.04 fold, respectively. c-fos gene expression was enhanced 1.44±0.12 and 1.78±0.16 fold by 100 nM As(III) and As(V), respectively, at 2 hr. These results demonstrate that both As(III) and As(V) elicit a specific activation of molecular signaling pathways in renal tissue at low concentrations. (Supported by NIH ES04940 and an SOT Colgate-Palmolive Fellowship.)

1655 MODEL FOR ARSENITE RELATED SKIN CARCINOGENESIS INVOLVING THE TUMOR SUPPRESSOR PROTEIN P53.

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The genetic toxicity of arsenic compounds has been rigorously evaluated and, based on the available data, arsenic and its metabolites do not seem to act as direct genotoxicants. Evidence is mounting that arsenic acts as a promoter rather than an initiator in carcinogenesis. This study reports a plausible mechanism by which arsenic could promote skin carcinogenesis with UV irradiation as an initiation step. The design of the experiment used the spontaneously immortalized keratinocyte cell line (HaCaT) which bears two UV-specific mutations on the *p53* gene as an *in vitro* model of an initiated cell. Keratinocytes were exposed to sodium arsenite (0, 1, 10, 100 nM) for 14 days. The exposure media were changed every day and cell samples were harvested every other day. We assayed p53 levels by Western blotting using chemiluminescence as the detection system. Cells viability was not affected throughout the study. p53 protein levels decreased in a dose-dependent manner after 7 to 9 days of exposure to arsenite. We conclude that arsenite treatment, after a latent period, either inhibits the activation or accelerates the degradation of the p53 protein. Decreased p53 protein levels might lead to a decrease in cell division arrest time thus increasing the probability of the cell to incur DNA damage that is left unrepaired, leading to excessive DNA mutations and ultimately carcinogenesis. (HH is supported by Department of Education Graduate Assistance in Areas of National Need Program Grant.)

1656 DISSIMILAR EFFECT OF ARSENIC ON CELL CYCLE KINETICS IN CONTROL AND RAS-TRANSFORMED C3H 10T1/2 FIBROBLASTS.

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Arsenic is a human-specific carcinogen whose mechanism of action remains undefined. Results generated in this laboratory indicate that sodium arsenite sensitizes wild-type, but not ras-transformed C3H 10T1/2 fibroblasts to LPA and EGF-stimulated DNA synthesis as measured by tritiated thymidine incorporation. To further explore the effect of arsenic on these cell types, a study was designed to test the hypothesis that transient arsenite exposure differentially alters the distribution of wild-type and ras-transformed cells in S-phase of the cell cycle following mitogen exposure. To this end, serum-starved wild-type and ras-transformed fibroblasts were treated with 300 μM sodium arsenite for 1 hour. Eight hours post-arsenite cells were treated with sub-optimal concentrations of LPA or EGF, harvested at various time points following the addition of mitogen and subjected to cell cycle analysis using a fluorescence-activated cell sorter. The data indicate that arsenic pre-treatment increases the percentage of wild-type cells in S-phase of the cell cycle; conversely, the percentage of ras-transformed cells in S-phase is decreased by arsenic pre-treatment. Viability, as determined using neutral red incorporation, indicates that the effect observed in ras-transformed cells is not due to increased cellular toxicity. Taken together, these data suggest that transient sub-lethal arsenite exposure leads to changes in the distribution of wild-type and ras-transformed cells in S-phase of the cell cycle. Because cell proliferation is a key element in the promotion stage of carcinogenesis, the effect of arsenic on wild-type cells might prove to be an important etiologic factor in the development of arsenic-related cancers.

1657 ARSENITE-MEDIATED DECREASES IN INDUCTION OF CYPs IN CULTURED CHICK HEPTOCYTES: LACK OF A ROLE OF OXIDATIVE DAMAGE.

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We have reported that arsenite decreased induction of CYP2H and CYP1A proteins in cultured chick hepatocytes by a mechanism not involving decreased availability of heme. Inductions of CYP2H1/2 and CYP1A4/5 proteins were dramatically decreased by arsenite . There was no decrease in the steady state levels of CYP2H mRNAs, however arsenite decreased the induction of CYP1A4 and CYP1A5 mRNAs. Concentrations of arsenite that decreased the induction of these CYPs (1 to 10 μM) caused no toxicity and no increased oxidative damage. Neither increasing GSH nor inclusion of antioxidants prevented the effects of arsenite to decrease induction of CYP. Treatment with inhibitors of MAP kinases themselves decreased the expression of CYP1A4/5 and, therefore, did not reverse the effects of arsenite. Inhibitors of phosphatases also did not reverse the effects of arsenite. The results suggest that the mechanism of arsenite-mediated decreases in CYP depends on the form of CYP expressed and is not solely due to oxidative damage. (This work was supported by NIEHS (ES07373) and the Dept. of Veterans Affairs.)

1658 ARSENIC-INDUCED PERTURBATION OF NEOPLASTIC RESPONSE IN MICE.

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Arsenic is a known human carcinogen but animal evidence is equivocal. For instance, one older study indicates repeated iv arsenate injections might induce lymphomas in Swiss mice (58% incidence; Osswald and Goerttler, 1971), but was considered inadequate for critical evaluation of carcinogenic potential. Thus, we studied repeated arsenic injection and neoplastic response in mice also treated with barbital (BB), a multiorgan tumor promoter. Groups (n = 24-25) of male or female Swiss mice received sodium arsenate (As; 0.5 mg/kg, iv) or saline (control) once/week for 20 weeks and were observed for 96 weeks. Starting 2 weeks after As, BB (2500 ppm) was added to the drinking water and continued until the study ended. The groups were then: control, As, BB, As + BB. Differences in survival and body weights were unremarkable. In females BB + As reduced ($p < 0.05$) spontaneous lymphomas (control 20%; BB 4%; As 12%; As + BB 0%) but in males lymphoma incidence was unchanged. BB induced increases in hepatic proliferative lesions (HPL; foci of alteration + neoplasia) which were reduced by As in males (control 4%; BB 72%; As 4%; As + BB 40%) and females (control 4%; BB 54%; As 24%; As + BB 24%). HPL were elevated in females given As (24%) compared to control (4%). As did not alter the hepatocellular hypertrophy seen with BB. Staining for the tumor suppressor protein, *P53*, and *ras* oncogene protein, *P21*, showed no expression in HPL in BB or in As + BB treated mice. BB increased testicular interstitial cell hyperplasia (84%) over control (36%) but hyperplasia was reduced by As (As + BB 60%). These data indicate that As, while perhaps showing a weak neoplastic response in the female liver, generally suppressed BB induced

increases in proliferative lesions of the liver and testes and contributed to a suppression of spontaneous lymphoma. This potential for suppression of neoplastic response by As may complicate detection of a carcinogenic response.

1659 ORGAN SUSCEPTIBILITY FOR ARSINE TOXICITY IN SPRAGUE DAWLEY RATS.

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In animals and humans exposed to AsH_3, a severe lysis of erythrocytes occurs. Other organs such as liver and kidney are also susceptible to AsH_3 toxicity *in vivo*. AsH_3 is an important environmental health hazard to employees of industries, such as the semiconductor industry. We hypothesize that AsH_3 is metabolized to other arsenic species to produce toxicity and that this process is organ dependent. The arsenic species are arsenite [As(III)], and possibly metallic arsenic or arsenic dihydride. In this investigation we determined AsH_3 and As(III) toxicity in rat blood, liver and kidney, using heme release (Vis-Spectroscopy), LDH leakage (Sigma kit 228-20) and intracellular K^+ (flame photometry) as the toxic parameters. Rat blood was very susceptible to AsH_3 (0.5 mM) toxicity that produced 70% of total hemoglobin, 68% of total LDH and 75% of intracellular K^+, released after 1 hr treatment. As(III) was nontoxic to rat blood. The primary hepatocytes were susceptible for both arsenicals with a LC_{50} and LC_{95} of 10 μM and 100 μM, respectively (measured by % of total LDH released). In this system, K^+ dropped 75-80% after dosing with both compounds. In liver precision-cut slices AsH_3 treatment caused 50-75% decrease in intracellular K^+ above 10 μM AsH_3, but LDH was not significantly affected suggesting a K^+ pump as the target. As(III) produced a dose and time-dependent increase in % of total LDH release and reached 35, 45 and 50% at 2, 4 and 6 hrs, respectively, with 1 mM As(III). Intracellular K^+ dropped 50, 60 and 75% at the same time points and same concentrations. In the renal cortical primary epithelial cells, 1 mM AsH_3 was slightly toxic with 10 and 18% of total LDH and 1 and 25% of intracellular K^+ leakage at 3 and 5 hrs, respectively. 1 mM As(III) produced 25 and 63% of total LDH and 20 and 46% of intracellular K^+ leakage at the same time points. The rat kidney has factors that allow toxicity from these arsenicals to be distinguished and was chosen to further study arsine metabolism. We concluded that arsenical toxicity is organ dependent and that As(III) is not the sole metabolite from AsH_3 biotransformation *in vitro*. (Supported by NIEHS grant ES6644, the Flinn Foundation, and Superfund Basic Research Program.)

1660 THE ANION TRANSPORTER AND THE ACCUMULATION OF ARSENIC (As) IN ARSENATE (As^V)-EXPOSED RABBIT ERYTHROCYTES.

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The characteristics of accumulation of As by rabbit erythrocytes were examined using [$^{73}As^V$] arsenic acid in an *in vitro* system containing equal volumes of washed erythrocytes and Na, K gluconate/HEPES pH 7.5 buffer that did not contain Cl^-. In this system the accumulation of As by erythrocytes was both time and temperature dependent. Over the range of 0.05 to 5 μM As^V, the fractional accumulation of As in cells was proportional to the extracellular concentration of As^V. Addition of 35 to 140 mM Cl^- to the medium reduced the accumulation of As. Similarly, the addition of 4, 4' diiisothiocyanostilbene-2, 2' sulfonic acid which binds to the band 3 component of the anion transporter, reduced accumulation of As. The accumulation of As in cells incubated in Cl—free medium at pH 6 exceeded that found in cells incubated in Cl—free medium at pH 7.5. This difference in the accumulation of As is consistent with the favored transport by the anion transporter of the monovalent oxyanion, $H_2AsO_4^-$, which is the predominant species at pH 6, over the divalent oxyanion, $HAsO_4^{-2}$, which is the predominant species at pH 7.5. The molybdate oxyanion did not antagonize the accumulation of As. In summary, As^V transport by erythrocytes involves the anion transporter and may be modulated by other anionic species in plasma. (This abstract does not necessarily reflect EPA policy.)

1661 EFFECT OF SODIUM ARSENITE OF PORPHYRIN SYTHESIS AND HEME METABOLISM IN CULTURED CHICK HEPATOCYTES.

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Arsenite has been reported to have multiple effects on heme turnover, inducing heme oxygenase, the rate-limiting enzyme in heme degradation, and increasing the urinary excretion of uroporphyrin and coproporphyrin, oxidation products of intermediates in heme synthesis. We had previously reported that 2.5 μM arsenite decreases the induction of several forms of CYP in cultured chick and rat hepatocytes. Here we report, in cultured chick hepatocytes, 1 to 25 μM arsenite did not increase uro- copro- or protoporphyrin accumulation from 5-aminolevulinic acid (ALA), indicating that the enzymes in the pathway between ALA synthase and protoporphyrinogen oxidase were unaffected by arsenite. 2.5 μM Arsenite had no effect on either the synthesis of heme from ALA or the drug-induction of ALA synthase, the rate-limiting enzyme in heme synthesis. Heme disappearance and biliverdin formation were not different in cultured chick hepatocyte treated simultaneously with arsenite and heme, or with heme alone. Our results indicate that doses of arsenite that induce heme oxygenase several-fold and cause decreases in inductions of CYP2H and CYP1A4/5 have little effect on porphyrin synthesis or heme metabolism. (Supported by NIEHS (ES07373) and the Dept. of Veterans Affairs.)

1662 A STUDY OF THE EFFECTS OF DIMETHYLARSINIC ACID ON THE LIGAND STATUS OF OXYHEMOGLOBIN IN RAT ERYTHROCYTES.

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Unlike other mammalian animal models, rats demonstrate significant accumulation of arsenic in blood over time. Studies have identified the arsenic compound to be dimethylarsinic acid (DMAsV). However, the actual binding site of DMAsV in the rat erythrocyte is not clear. Previous studies in our laboratory provide evidence that arsenic in erythrocytes is bound to hemoglobin. Possible binding sites are on the protein, porphyrin rings, or iron. This study examined the effect of DMAsV on rat hemoglobin. Studies were performed using both rat (Sprague Dawley) erythrocytes and isolated rat oxyhemoglobin (HbO_2). At concentrations ranging from 1 to 62.5 mM DMAsV, rat erythrocytes or hemoglobin solutions were dosed and incubated for up to 60 minutes at 37° C. Changes in HbO_2 in these preparations were examined from spectral changes between 450 nm and 750 nm. Spectra indicated that DMAsV was not altering the ligand state of HbO_2 since no spectral changes characteristic of other hemoglobin species were observed. Previous studies have shown that certain arsenic species cause hemolysis of erythrocytes. After spectral analysis, hemolysis of erythrocytes induced by DMAsV at concentrations up to 62.5 mM was also measured spectrophotometrically using Drabkin's reagent. No significant increase in hemolysis with respect to control preparations was observed (2-3% of total hemoglobin release in control preparations vs. 3-4% of total hemoglobin release in treatment preparations). From these results we can conclude that DMAsV does not alter the ligand state of HbO_2 or the oxidation state of iron in the heme groups of HbO_2 at these concentrations. Further studies are necessary to determine binding of DMAsV to the globin portion of hemoglobin. (Supported by NIH Grant ES6644, NIEHS Grant ES07091, and Center Grant P30 ES06694.)

1663 PHARMACOKINETIC AND PHARMACODYNAMIC STUDIES OF ARSENITE INDUCTION OF HEME OXYGENASE IN RATS.

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The identification of useful biomarkers of arsenic effects in both experimental animals and humans has been difficult. Arsenate and arsenite are good inducers of rat hepatic and renal heme oxygenase (HO); monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) are not effective HO inducers. Little oxidation of arsenite to arsenate occurs in vivo. Therefore, enzymatic induction of HO should theoretically be an excellent biomarker of biologically active arsenite concentration. Liver and kidney inorganic arsenic, MMA and DMA concentrations and HO enzyme activities were determined

2 to 144 hours after oral administration of 100 umoles/kg of sodium arsenite to adult female Sprague-Dawley rats. In rat liver the mean inorganic arsenic concentrations were 0.11, 2.15, 1.85, 1.86, 1.82, 0.68, 0.51, 0.20 and 0.10 ug/g at 0, 2, 4, 8, 16, 24, 48, 72 and 144 hours after arsenite administration, respectively. At these same time points, rat hepatic HO activities were 1.0, 10.8, 6.8, 16.3, 21.2, 5.2, 3.8, 0.8 and 0.5 times control HO values. At these time points 86, 46, 61, 66, 80, 89, 95, 97 and 98% of the total rat liver arsenic was present as DMA, an arsenite metabolite that does not induce HO. A hepatic concentration of 0.49 ug/g of additional inorganic arsenic was present before or at the time of HO enzyme induction. In rat kidney lower inorganic arsenic concentrations and less HO enzyme induction occurred after oral arsenite administration. This study links tissue dosimetry for arsenic with a biological effect (HO enzyme induction) in a pharmacokinetic and pharmacodynamic view of its action in two tissues that are targets for arsenic-induced carcinogenesis. (Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

1664 DIMETHYLARSINIC ACID EFFECTS ON SIX BIOCHEMICAL PARAMETERS IN B6C3F1 MICE.

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Historically, methylation of the inorganic forms of arsenic (arsenate or arsenite) was interpreted as a detoxification pathway; however, recent data from free radical, biochemical and carcinogenic studies of dimethylarsinic acid (DMA) suggest that methylation of arsenic may be a toxification pathway instead. DMA has tested positive as both a complete carcinogen (urinary bladder and fibrosarcomas) and as a promoter of carcinogenesis (lung, liver, kidney, urinary bladder and thyroid gland). Adult female B6C3F1 mice were given 720 mg/kg of DMA by gavage at one of three points - 2 hours before, 15 hours before, and both 21 and 4 hours before sacrifice. We determined the DNA damage, the reduced and oxidized glutathione (GSH, GSSG) content, cytochrome P-450 content, ornithine decarboxylase (ODC) activity and plasma alanine aminotransferase activity. Significant ($P < 0.05$) decreases (15 to 37%) in liver GSH and GSSG contents were observed in all exposures to DMA. This data suggests that DMA can lower cellular defenses against electrophiles and free radicals. DNA damaging and eventually carcinogenic effects may be mediated by GSH depletion and/or the enhancement of oxidative stress. A general trend toward a DMA-induced DNA damage was observed, particularly in the liver ($P < 0.1$, one tail), but no comparisons reached the $P < 0.05$ level of statistical significance. Pulmonary and hepatic ODC activities were reduced (19 to 59%) by prior DMA treatment. ODC is involved in cell proliferation. Overall, our DMA mouse results are quite different from our prior rat results. Rats and mice differ greatly in DMA pharmacokinetics, largely because of large binding of DMA to cysteine 125 on the β chain of rat hemoglobin. These biochemical studies show that mice are much less responsive to DMA than rats. (Disclaimer: This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

1665 TOUGHNESS OF SKIN IN ARSENIC EXPOSED MICE: ROLE OF GM-CSF AND TGFa.

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Hyperkeratosis on the palms of the hand and soles of the feet are characteristics of arsenic (As) poisoning. However, toughness of skin has not been described as a manifestation of As poisoning. We previously reported that As induces GM-CSF and TGFa mRNA and protein production in human keratinocyte culture. In the present studies sodium arsenite was provided at 1 and 10mg/L to C57B/6n male and female mice via drinking water for 50 days. As concentrations were determined in hair and urine. Pieces of skin were excised from shaved ventral skin by scissors for histpathological and molecular analysis. Increase skin toughness dominantly in male was apparent in exposed groups when skin sections were prepared. Skin sections were prepared and stained for Haematoxilin and Eosin. GM-CSF in serum were measured by ELISA. RNA was extracted from skin and the expression of GM-CSF and TGFa mRNA were examined by RT-PCR. Although the epidermis appeared normal, the dermis in exposed animals showed moderate thickening of the collagen bundles. Seven of 12 exposed male mice and 4 of 12 female mice showed significantly thickened collagen bundles. GM-CSF in serum were elevated in 10mg/L, but not significantly. RNA expressions for GM-CSF and TGFa were enhanced moderately in exposed groups compared to controls. These results indicate that a number of changes occur in the skin of As-exposed mice, including thickening of collagen bundles and increased growth factor expression.

1666 PARTIAL PURIFICATION OF AN ARSENATE REDUCTASE FROM HUMAN LIVER AND CHARACTERIZATION OF ITS COFACTOR REQUIREMENTS.

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An arsenate reductase from human liver has been purified 200 fold using DEAE-Sephacel, Sephacryl S-200 HR, Activated Thiol Sepharose 4B and Reactive Brown 10. The starting liver cytosol reduced 860 pmoles arsenate/30 min/mg protein. The activity is associated with a heat labile protein which is greater than 30 kDa which required both a thiol and a heat stable cofactor less than 3 kDa in size. To determine the enzyme's thiol requirement, the ability of glutathione, thioredoxin and lipoic acid were tested to see if they would stimulate reduction of arsenate in the absence of other thiols. In the presence of enzyme, either glutathione or lipoic acid stimulated reduction but were less than 20 times as active as dithiothreitol in this respect. Thioredoxin did not stimulate activity. Mg^{+2}, Ca^{+2}, Cu^{+2}, Mn^{+2}, and Zn^{+2} did not stimulate enzyme activity. In addition, enzyme activity was not inhibited by the presence of 5 mM ethylenediamine tetraacetic acid. (Supported by Superfund Program NIEHS Grant #ES-04940 and NIEHS Center Grant #P30-ES-06694.)

1667 CHRONIC ARSENITE EXPOSURE INDUCES RESISTANCE TO ACUTE TOXICITY OF ARSENIC AND ITS METABOLITES AND CROSS TOLERANCE TO CADMIUM.

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Arsenic is a known human carcinogen. A previous study in our laboratory showed that chronic (>18 weeks), low dose (500 nM) exposure to arsenite induces malignant transformation in a rat liver epithelial cell line (TRL1215). This study sought to determine if the adverse effects of metals were altered in these transformed cells. Arsenite transformed cells (ATC) and untransformed cells (UTC) were exposed to arsenite, arsenate, monomethyl arsenate (MMA), dimethylarsenic acid (DMA) and cadmium for 24 hours and cell viability was determined. The LC_{50} for arsenite was 67 μM in ATC as compared to 26 μM in UTC. ATC were very tolerant to the acute toxic effects of arsenate (LC_{50} >500 μM) compared to UTC (LC_{50} 54 μM). After metallothionein (MT) induction with zinc (80 μM) pretreatment for 24 hours, UTC showed minimal changes in sensitivity to arsenite and arsenate (15% and 20% increase in LC_{50}, respectively) compared to ATC (155% and 826% increase in LC_{50}, respectively). Prior studies have shown that MT is hyperexpressible in ATC. DMA had a LC_{50} of 32 mM in ATC as compared to a LC_{50} of 7 mM in UTC. Differences in sensitivity to MMA were modest but significantly different between ATC (LC_{50} 68 mM) and UTC (LC_{50} 50 mM). Cellular accumulation of arsenic was 2.7-fold higher in UTC (9.5 ng/10^6cells) than in ATC (3.5 ng/10^6cells). Thus, UTC accumulate more arsenic than ATC and this may, in part, account for their increased sensitivity to acute arsenic toxicity. ATC also showed a cross tolerance to the toxic effects of cadmium. The LC_{50} of cadmium in ATC was 51% greater than that seen in UTC. The results of this study show that ATC are tolerant to the acute toxic effects of arsenite, arsenate, MMA, and DMA, and cross tolerant to cadmium. This tolerance may be based, at least in part, on reduced uptake of arsenicals.

1668 METALLOTHIONEIN-I/II NULL MICE ARE MORE SENSITIVE THAN CONTROLS TO CHRONIC ARSENIC TOXICITY.

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Metallothionein (MT) is low-molecular weight, sulfhydryl-rich, metal-binding protein. MT can protect against the toxicity of cadmium, mercury, and copper, but the role of MT in arsenic (As)-induced toxicity is less certain. To better define the ability of MT to modify As toxicity, metallothionein-I/II null (MT-null) and control mice were given repeated sc injections of As(III)

(10 and 30 μmol/kg) or As(V) (100 μmol/kg), 5 days/week, for 15 weeks. Repeated As injections increased hepatic and renal MT concentrations 3-6 fold in control but not in MT-null mice. Repeated As injections produced 2-10 fold elevation of serum interleukin-1, interleukin-6, and tumor necrosis factor-α. MT-null mice were more susceptible than controls to these elevations in serum cytokines. Repeated As injections decreased hepatic glutathione (GSH) by up to 40%, but GSH-peroxidase and GSH-reductase were minimally affected. MT-null mice and controls were similarly sensitive to the effect on GSH. Histologically, repeated As injections produce damage to liver (inflammation, focal necrosis and degeneration) and kidney (interstitial nephritis, tubular cell degeneration, and increases in kidney/body weight ratios). At the same dose levels, MT-null mice appeared to have more pathological lesions when compared to control mice. Thus, MT-null mice were more susceptible than controls to the toxicity of repeated As injections, suggesting that induction of MT could be one of the cellular mechanisms decreasing chronic As toxicity.

1669 CADMIUM AND ARSENIC INTERACTIONS IN CONTROL AND METALLOTHIONEIN-I/II NULL MICE.

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Cadmium (Cd) and arsenic (As) are toxic metals in the environment. People have the potential to be exposed to Cd and As as mixtures, but their interactions are not known. Metallothionein (MT) is a cystine-rich, metal-binding protein, that plays an important role in Cd detoxication, but the role of MT in As toxicity is less certain. To examine the role of MT in Cd and As interactions, control and MT-null mice were fed Cd in their diet (100 ppm), As (III) in drinking water (22.5 ppm), or Cd plus As for up to 4 months. Subsequent nephrotoxicity and hepatotoxicity were determined by morphological and biochemical techniques. At the doses used, chronic Cd exposure produced more renal and hepatic toxicity than did As, and the combination of Cd and As produced even more toxicity. In the mice receiving Cd plus As, proximal tubule degeneration, atrophy, glomerular swelling, and interstitial fibrosis were more severe than that produced by either metal given alone. Similarly, hepatic focal necrosis and granulomatous hepatitis were more severe in the mice that received Cd plus As. Furthermore, MT-null mice were not only more susceptible than controls to chronic Cd-induced toxicity, but also to chronic As toxicity. MT-null mice were especially sensitive to the combination of Cd- and As-induced injuries, as evidenced by decreased body weight, enzymuria, glucosuria and histopathology. In conclusion, this study indicates there is an interaction between Cd and As during long-term combined exposure, and demonstrates that MT protects against Cd- and As-induced toxicity. (Supported by NIH grants ES-01142, ES-06190 and ES-07079.)

1670 METALLOTHIONEIN-I/II NULL MICE ARE MORE SUSCEPTIBLE THAN CONTROLS TO CHRONIC ORAL CADMIUM-INDUCED TOXICITY.

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Chronic exposure to cadmium (Cd) via food is becoming a major concern for human health. We have previously shown that cellular metallothionein (MT), a small metal-binding protein rich in sulfhydryl groups, plays an important role in protecting against Cd toxicity produced by repeated sc injections. To determine whether MT protects against oral Cd toxicity following long-term exposure, MT-I/II null and control mice were fed Cd via drinking water (30, 100, and 300 ppm), or food (100 ppm) for up to 6 months. Chronic oral Cd exposure produced a dose-dependent accumulation of Cd in liver and kidney of control mice, reaching 50 μg/g tissue. In contrast, tissue Cd concentrations in MT-null mice were less than 8 μg/g tissue. In spite of the lower Cd concentrations in their liver and kidney, MT-null mice were more susceptible than controls to Cd-induced nephrotoxicity and hepatotoxicity. Cd exposure produced greater urinary excretion of gamma-glutamyltransferase in MT-null mice than in control mice, while urinary excretion of protein and glucose were minimally affected. Kidney and liver/body weight ratios were increased in a dose-dependent manner following oral Cd exposure, and again larger increases were seen in MT-null mice. In conclusion, this study demonstrates that MT-null mice are more susceptible than controls to oral Cd-induced toxicity, indicating the importance of cellular MT in decreasing Cd toxicity not only after sc injection, but also after oral exposure. (Supported by NIH grants ES-01142 and ES-07079.)

1671 REDUCED UPTAKE AND INCREASED EFFLUX OF CADMIUM IN CADMIUM-RESISTANT METALLOTHIONEIN NULL CELLS.

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Metallothionein (MT) is known to play a predominant role in the protection against Cd. To investigate other factors than MT involved in the detoxification of Cd, we utilized MT I and II null mouse embryonic cells transformed with simian virus 40 large T antigen. By exposure of these immortalized MT null cells to graded concentrations of Cd up to 10 μM, we established Cd-resistant MT null cell lines. Two clones of Cd-resistant MT null cells designated as Cd-rA7 and Cd-rB5 were characterized and compared with the parental MT null cell clone, P4. IC50 values of Cd for Cd-rA7 and Cd-rB5 were approximately ten times that of P4 as judged by MTT assay. No increase in glutathione concentration or enzyme activities of glutathione S-transferase, glutathione reductase, glutathione peroxidase, superoxide dismutase, and catalase was observed in Cd-rA7 and Cd-rB5 compared to P4. Interestingly, Cd-rA7 and Cd-rB5 showed no cross-resistance to other metals (Zn, Cu, Ni, Pb, Hg, As), cisplatin, H2O2 or 1-chloro-2,4-dinitrobenzene, suggesting that these cells had acquired Cd-specific resistance factor(s). We found that Cd accumulation after 24 h exposure to Cd in Cd-rA7 and Cd-rB5 was about 15 - 25% of that in P4, which might have resulted in the decreased sensitivity to Cd. To further investigate the mechanism underlying the reduced accumulation of Cd in Cd-rA7 and Cd-rB5, the uptake of trace amount of 109Cd-labeled CdCl2 was examined. A time-dependent increase in 109Cd concentration in each cell line was observed, but the rate of uptake in Cd-rA7 and Cd-rB5 was about 10% of that of P4. To measure the efflux of Cd, each cell line was exposed to 109Cd-labeled CdCl2 for 2 h, and then the medium was changed to Cd-free one. The 109Cd remaining in Cd-rA7 and Cd-rB5 dropped to about 50% during 60 min after the medium was changed, while less than 5% of 109Cd was released from P4. Thus, both the reduced rate of Cd uptake and the increased efflux of Cd from the cells may contribute to the decreased accumulation of Cd, and thereby the decreased sensitivity to Cd in Cd-rA7 and Cd-rB5.

1672 CYPROTERONE ACETATE, THE ANTIANDROGEN, INDUCES A NOVEL FORM OF CELLULAR TOLERANCE TO CADMIUM INVOLVING REDUCED CELLULAR ACCUMULATION.

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Several reports indicate that some steroids, in particular sex steroid hormones, can modify cadmium toxicity. We recently reported that cyproterone acetate (CA), a synthetic steroidal antiandrogen that is closely related in structure to progesterone, affects cadmium toxicity in mice. In the present study, we investigated the influence of CA on cadmium toxicity in a rat liver epithelial cell line (TRL 1215) *in vitro*. TRL 1215 cells were exposed to various concentrations of CA (0, 1, 10, or 50 μM) for 24 h and subsequently exposed to cadmium (0, 50, or 100 μM; as $CdCl_2$) for an additional 24 h. CA pretreatment resulted in a decrease in the cellular sensitivity to cadmium as assessed by metabolic integrity. Additional study showed CA pretreatment provided protection against cadmium toxicity only when given more than 6 h prior to cadmium exposure. Cellular cadmium concentration was markedly reduced (60 %) in cells pretreated for 6 h or more with CA. In the presence of protein synthesis inhibitors, the protective effect of CA toward cadmium toxicity was abolished. CA alone modestly increased metallothionein (MT) levels (2.4-fold), while cadmium (50 μM) alone resulted in an 8.9-fold increase over control. However, cadmium-induced MT synthesis was markedly decreased by CA pretreatment. These data suggest that CA pretreatment provides protection against cadmium toxicity *in vitro* and indicate that this protection is due to a decreased accumulation of cadmium rather than through activation of MT synthesis. This decrease of cellular cadmium accumulation appears to be related to events that require protein synthesis and is an unusual form of tolerance in mammalian cells.

1673 DIFFERENTIAL INDUCTION OF METALLOTHIONEIN AND HEAT SHOCK PROTEIN 72 BY CADMIUM IN THE LIVERS OF THE MALE FISCHER-344 AND THE SPRAGUE DAWLEY RAT.

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Liver injury by cadmium following acute exposure is well characterized. Previous work has shown that the Fischer-344 (F-344) rat is more sensitive to cadmium (Cd) toxicity than the Sprague Dawley (SD) rat. The primary detoxification of Cd is achieved by forming a complex with the metal binding protein, metallothionein (MT). In most cases, the rate of MT induction is paramount to protecting against cadmium toxicity. The *de novo* synthesis of stress or heat shock proteins (hsp) also occurs early after Cd administration. Hsp serve to maintain the structural integrity of proteins that have been damaged or denatured by toxic insults. The objective of this study was to determine if differential expression of MT and hsp plays a role in the sensitivity of two rat strains to Cd toxicity. F-344 and SD rats were challenged with cadmium chloride (3 mg/kg, i.v.) and the induction of hepatic MT and hsp was determined from 3 to 48 hr after treatment. Liver injury was assessed by plasma alanine aminotransferase (ALT) activity. Levels of MT in the liver were quantified by atomic absorption spectrophotometry. Liver samples were subjected to one-dimensional SDS-PAGE and the inducible form of hsp70 (hsp72) was identified using a specific monoclonal antibody. Administration of $CdCl_2$ resulted in a rapid increase in serum ALT activities within 3 hr in F-344 rats compared to SD rats (1241 U/L verses 226 U/L, respectively). ALT levels reached a maximum of 4100 U/L after 24 hr in the F-344 rat compared to 229 U/L in SD rats. The induction of MT occurred earlier in the livers of the SD rat (16 μg/g tissue verses 8 μg/g after 3 hr). Interestingly, enhanced hsp72 synthesis was observed in the SD rat liver by 3 hr but not in the F-344 liver until 6 hr after treatment. From these studies it appears that earlier synthesis of MT and hsp72 may contribute, at least in part, to the decreased sensitivity of the SD rat to Cd induced hepatotoxicity. (Supported by the NIEHS (ES-06694, ES-06095) and the Yuma Friends of AHSC.)

1674 ACTIVATION OF HEPATIC CASPASE-3 ACTIVITY FOLLOWING CADMIUM CHLORIDE ($CdCl_2$) TREATMENT IN MICE.

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Acute exposure to cadmium results in liver toxicity involving both apoptosis and necrosis. Recent advances in the study of cell death suggest that the biochemical mechanisms of apoptosis and necrosis may allow for a method to discern the importance of each death pathway. Caspase-3 is a highly conserved central mediator of the apoptotic program and is a widely accepted marker for apoptosis *in vitro*. Therefore this study was designed to determine whether acute cadmium exposure causes an increase in caspase-3 activity *in vivo*. A new method for the quantification of caspase-3 enzymatic activity in liver cytosolic extracts was developed and optimized. Serum alanine aminotransferase (ALT) was measured to assess liver necrosis. Male mice were treated with saline or 0.9, 1.8, 3.7, 5.5, 7.3 and 11 mg/kg $CdCl_2$ ip for 9 hours. Endotoxin (LPS)-treated mice (2 mg/kg ip for 9 hr) were employed as a positive control for apoptosis to verify this method of caspase-3 analysis. LPS-treatment caused a maximal increase in caspase-3 activity after 9 hours exposure with no increase in ALT. $CdCl_2$-treatment caused a 9 to 13-fold increase in caspase-3 activity with doses ranging from 0.9 mg/kg up to 5.5 mg/kg. However, at higher doses (7.3 and 11 mg/kg), caspase-3 activity was decreased 75% from control. $CdCl_2$-treatment caused an 4, 30, 70, and 100-fold increase in ALT at 3.7, 5.5, 7.3, and 11 mg/kg, respectively. These data demonstrate that caspase-3 is activated by doses of $CdCl_2$ which cause little or no necrosis *in vivo*. Only slight overlap between caspase-3 activity and ALT was observed, indicating that caspase-3 activity is not involved in $CdCl_2$-induced necrosis. Decreased caspase-3 activity may be due to direct inhibition of caspase-3. Inhibition of caspase-3 activity may prevent the completion of apoptosis, resulting in necrosis. (Supported by NIH grants ES-01142 and ES-07079.)

1675 CADMIUM BLOCKS APOPTOSIS INDUCED BY CHROMIUM, HYGROMYCIN B AND ACTINOMYCIN D: THE ROLE OF CASPASE-3 INHIBITION.

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Cadmium (Cd) and chromium (Cr) compounds are both human carcinogens. Cr(VI) is taken up into cells and reduced by cellular reductants (i.e., GSH) to Cr(III), a DNA damaging agent. Reactive oxygen species are produced during Cr reduction. A previous study in our laboratory found that Cd effectively inhibits Cr-induced apoptosis (J. Toxicol. Environ. Health, 54: 159, 1998). Inhibition of Cr-induced apoptosis by Cd may allow a larger proportion of genetically damaged cells to escape from apoptosis and become transformed. The present study was undertaken to define the molecular mechanisms of suppression of Cr-induced apoptosis by Cd in CHO cells. Cells were treated with either Cd (5-20 μM), Cr(VI) (350 μM) or Cd (5-20 μM) plus Cr(VI) (350 μM) for 3 hr. The cells were cultured in metal free media for 48 hr and DNA was extracted. Cd inhibited DNA fragmentation and reduced the number of apoptotic cells caused by Cr treatment. Electron spin resonance (ESR) was used to determine if the effect of Cd was due to an alteration of the cellular reduction of Cr(VI) to Cr(III). These data showed that Cd did not alter cellular Cr(VI) reduction as assessed by the levels of the intermediate, Cr(V). There was also no difference in cellular GSH levels between Cr-treated and Cd/Cr-treated cells. Thus, Cd suppression of Cr-induced apoptosis is not through an alteration in cellular Cr metabolism. Cd also inhibited apoptotic response to the non-metal agents, hygromycin B and actinomycin D. Additional study showed that Cd was very effective at inhibiting cellular caspase-3, a central mediator of apoptosis, with 10 μM Cd, which was otherwise non-toxic, resulting in ~ 40% inhibition. Our results indicate that Cd may have a generalized inhibitory effect on apoptosis possibly through caspase-3 inhibition. This inhibition of apoptosis by Cd may allow a greater portion of genetically damaged cells to survive, and has implications as a potential, non-genotoxic mechanism of Cd carcinogenesis.

1676 COMPARISON OF THE CYTOTOXIC EFFECTS OF CADMIUM (Cd^{2+}) IN S-180 AND S-180L CELLS.

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Recent findings from our laboratory have shown that Cd^{2+} can disrupt the tight junctions between epithelial cells by interacting with E-cadherin, a calcium-dependent cell adhesion molecule. In order to further explore the role of E-cadherin in the general cytotoxic response to Cd^{2+}, we have examined the effects of Cd^{2+} in a fibroblast-like mouse sarcoma cell line that does not express E-cadherin (S-180), and a modified strain of the cell line that has been transfected to express E-cadherin and exhibit an epithelial-like phenotype (S-180L). Depending on the particular experiment, the cells were grown in 6 well plastic tissue culture flasks or on glass coverslips in DMEM supplemented with 15% calf serum, and exposed to Cd^{2+} in serum-free medium. Cell viability was evaluated by the trypan blue dye exclusion assay, and E-cadherin was visualized by an indirect immunofluorescence procedure. In the E-cadherin-expressing S-180L cells, exposure to 200 - 400 μ M Cd^{2+} for 4-6 hours caused the cells to separate from each other without causing them to detach from the growing surface or killing them. This effect coincided with the loss of E-cadherin from the cell borders. In the S-180 cells, a similar exposure to Cd^{2+} caused the cells to change from an irregular, spindle shape to a round shape. With longer periods of exposure (ie., 8-24 hours), Cd^{2+} caused both cell lines to begin to die. However, the S-180L cells were significantly more resistant to the lethal effects of Cd^{2+} than were the S-180 cells. These results are consistent with the hypothesis that E-cadherin may be an early target for the junction-perturbing effects in epithelial cells. In addition, they indicate that the level of E-cadherin expression may influence the sensitivity of the cells to more severe toxic injury by Cd^{2+}. (Supported by NIH Grant R01 ES06478.)

1677 CADMIUM ACTIVATES ONCOGENE EXPRESSION IN HUMAN PROSTATE EPITHELIAL CELLS.

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Cadmium is a toxic heavy metal classified by the International Agency for Research on Cancer as a human carcinogen. The prostate is a target site of

cadmium carcinogenesis in rats and possibly in humans. The carcinogenic mode of action for cadmium has not been elucidated, but the poor mutagenicity observed with this metal points toward an epigenetic mechanism. Thus, we are testing the hypothesis that cadmium exposure alters expression of genes involved in growth regulation of prostate epithelial cells. The present study examined the effects of cadmium exposure on gene expression in the normal human prostate epithelial cell line RWPE-1. This line is immortalized but not transformed, is androgen-responsive and represents an *in vitro* model of the suspected *in vivo* target cells of cadmium carcinogenesis. Treatment of RWPE-1 cultures with 10 μM cadmium chloride, which is not toxic to these cells, resulted in 2-fold increases in *c-myc* mRNA levels after 4 hours of exposure. By 6 hours after 10 μM cadmium, *c-myc* levels were decreasing and by 24 hours levels were back to control. In comparison, *c-jun* mRNA levels were increased 3-fold after 4 and 6 hours of cadmium exposure and 10-fold after 24 hours. Clear dose-dependent increases were observed between cadmium concentration and the levels of *c-myc* and *c-jun* mRNA. For instance, after 4 hours of cadmium treatment, *c-myc* levels were unchanged at 5 μM, 1.7-fold above control at 10 μM and 2.5-fold above control at 15 μM. A similar pattern was observed for the *c-jun* transcript. No changes in *c-fos* mRNA levels were observed after cadmium treatment. Taken together, our data show that cadmium can affect oncogene expression in a cell type analogous to potential target cells of carcinogenesis in humans.

1678 ACTIVATION OF CADMIUM UPTAKE BY CALMIDAZOLIUM DOES NOT REQUIRE CALMODULIN.

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In our investigation on cadmium (Cd) transport into Madin-Darby canine kidney (MDCK) cells, the role of calmodulin was investigated. This is because transporters, exchangers and pumps that could play a role in regulating the transport and efflux of Cd are activated by calmodulin. Whereas two calmodulin inhibitors, W-7 and trifluoroperazine, did not affect the uptake of ^{109}Cd, treatment with another calmodulin inhibitor, calmidazolium, resulted in a three-fold increase in the uptake of ^{109}Cd. These three inhibitors displayed a similar effect on the calmodulin-dependent regulation of calcium homeostasis suggesting that each interacted with calmodulin. Furthermore, treatment with an antisense oligonucleotide resulted in an almost complete loss of calmodulin but did not affect the uptake of ^{109}Cd. Calmidazolium was not a Cd ionophore since it did not stimulate the uptake of ^{109}Cd into liposomes. Calmidazolium-mediated uptake of ^{109}Cd was not affected by an activator of protein kinase C. Interestingly, copper but not several other metals including zinc, lead and nickel inhibited uptake of ^{109}Cd in cells treated with calmidazolium. Our results suggest that calmidazolium increases the uptake of Cd possibly by activating a unique transporter that does not require calmodulin.

1679 PROTEIN KINASE C REGULATES CADMIUM UPTAKE IN KIDNEY CELLS.

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Heavy metals such as lead and cadmium (Cd) accumulate in the kidney, but the mechanism by which they are transported into kidney cells is unclear. Several mechanisms including anion exchange, calcium channels, and others that are not as well defined have been proposed. Madin-Darby canine kidney (MDCK) cells were used in this study to examine Cd transport since these cells express several transport mechanisms. For example, MDCK cells express anion exchanger 2 mRNA and display the anion exchange mechanism since the uptake of sulfate at acidic pH was largely blocked by inhibitors of anion exchange. Interestingly, conditions that stimulated anion exchange did not stimulate uptake of ^{109}Cd nor was the uptake of ^{109}Cd sensitive to drugs that inhibit anion exchange. Furthermore, ^{109}Cd uptake was not inhibited by drugs that block or activate calcium channels. ^{109}Cd uptake was increased in MDCK cells incubated overnight with the protein kinase C activator phorbol 12,13 dibutyrate (PDBu). The protein kinase C inhibitor GF 109203X blocked the effect of PDBu, and the increase in ^{109}Cd uptake was inhibited by the transcription inhibitor actinomycin D. The uptake of ^{109}Cd was inhibited by several metals including lead, copper and zinc, but not calcium. Our studies suggest that a metal transporter is under the control of protein kinase C.

1680 CADMIUM-INDUCED LUNG INJURY IN MICE: EVIDENCE FOR A SPECIFIC INCREASE IN EPITHELIAL PERMEABILITY.

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Although it is well known that respiratory exposure to Cd^{2+} results in pulmonary edema, inflammation and hemorrhaging, the specific mechanisms underlying these effects have yet to be elucidated. The recent findings from our laboratory showing that Cd^{2+} can disrupt the tight junctions between several types of epithelial cells in culture have lead us to examine the possibility that some of the pneumotoxic effects of Cd^{2+} might result from specific changes in the permeability of the tight junctions in capillary endothelial and alveolar epithelial cells. Male CF-1 mice were given either saline, or $CdCl_2$ (65 nmoles in 50 μ l saline) via intratracheal instillation. After 24 hours, the animals were euthanized, and their lungs were removed and either subjected to bronchoalveolar lavage or analyzed for histopathologic changes by light and electron microscopy. The results showed that Cd^{2+} caused an increase in lung weight and an increase in the erythrocyte and protein content of the lavage fluid. The microscopic analyses showed evidence of edema and capillary congestion but little evidence of injury to the alveolar epithelial cells or the capillary endothelial cells themselves. In addition, the samples from the Cd^{2+}-treated mice showed alterations in the pattern of distribution of E-cadherin, a Ca^{2+}-dependent cell adhesion molecule that is associated with the adhering junctions of epithelial cells. These findings suggest that the Cd^{2+}-induced pulmonary edema does not result from the death of the alveolar epithelial cells, but may be a consequence of an increase in the permeability of the E-cadherin-dependent junctions between the cells. (Supported by NIH Grant #R01 ES06478.)

1681 EFFECT OF ZINC AND ALBUMIN PRETREATMENT ON CADMIUM UPTAKE IN RAT HEPATOCYTES EXPOSED UNDER PHYSIOLOGICAL CONDITIONS.

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A key factor in cadmium induced toxicity is uptake by the liver. Primary hepatocytes offer a useful tool for studying the uptake and toxicity of this heavy metal at the cellular level. However, differences in culture conditions, exposure conditions and exposure durations make it difficult to determine realistic dose-response relationships. The goal of this study was to investigate acute 1-h cadmium uptake kinetics and toxicity in healthy hepatocytes exposed under physiological conditions. Primary rat hepatocytes were exposed to cadmium concentrations ranging from 32 to 1,000 μM in buffer lacking or containing physiologic albumin levels (4%). In addition, hepatocytes pretreated with 100 μM zinc for 24 h were exposed to cadmium in buffer containing physiologic albumin. Cadmium toxicity in hepatocytes assessed 23 h following a 1 h exposure was observed to be significantly reduced by the presence of albumin in the exposure buffer and zinc pretreatment, respectively. The presence of albumin in the exposure buffer was also found to significantly decrease the rate of cadmium uptake when compared to exposure buffer lacking albumin. Pretreatment of hepatocytes with zinc did not effect the rate of cadmium uptake. It was observed that a rapid association of cadmium with the cell surface occurred over the first minute that was constant and sensitive to washing with buffer containing EGTA. A linear uptake rate was observed between 15 to 60 min of exposure that was insensitive to the presence of EGTA in the wash buffer. The results of this study indicated that hepatocyte culture and exposure conditions effect cadmium uptake kinetics and toxicity.

1682 EFFECTS OF ORAL CADMIUM ADMINISTRATION ON RENAL FUNCTION AND BONE METABOLISM IN MALE RATS.

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Male Wistar rats (6 weeks old) were given Cd (as CdCl2) at a dose of 0, 2, 5, 10, 20, 30 and 60 mgCd/kg/day by gastric tube daily for 6 consecutive days a week for 60 weeks. Animals were sacrificed at 0, 1, 3, 5, 10, 30, and 60 week after oral Cd administration. Cd concentration in the kidney, urinary excretion of enzymes (NAG,AAP,GST,ALP), the urinary excretion of Pyridinoline (Pyr) and Deoxypyridinolin (Dpyr) were determined. Bone mineral density of the femur was determined by microdensitometry (MD)

method, and the osteoid volume (%) was also determined by the Quantitative Image Analysis method. At low Cd administration groups of 2 and 5 mgCd/kg/day, the prominent renal dysfunction was not observed. On the other hand, in the high Cd dose groups of 20, 30 and 60 mgCd/kg/day, the renal proximal tubular dysfunction was observed, and the decreased bone mineral density was found at before and after the manifestation of renal dysfunction. Osteoid volume (%) in the femur was shown the lower level compared to that of control animal. The increased osteoid volume was not found in all animals except the case of 2 mgCd/kg group at the 5th week. The increased urinary excretion of Pyr, Dpyr and Ca was observed in the high Cd dose groups from the 3rd week after the Cd administration. It was suggested that the bone resorption is activated. These results suggest that the renal dysfunction, the decreased bone mineral density, and the decreased osteoid volume in the femur are resulted at different time corresponding to the Cd accumulation resulted by the different condition of Cd exposure. Dpyr and Ca was observed in the high Cd dose groups from the 3rd week after the Cd administration. It was suggested that the bone resorption is activated. These results suggest that the renal dysfunction, the decreased bone mineral density, and the decreased osteoid volume in the femur are resulted at different time corresponding to the Cd accumulation resulted by the different condition of Cd exposure. (This study is supported by the research grants from the Kitasato PRG B-10 and the Japanese EA.)

1683 ANTIOXIDANT POTENTIAL IN BLOOD OF WORKERS EXPOSED TO LEAD AND CADMIUM IN THEIR WORK-PLACE.

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The aim of the study was to examine selenium (Se), zinc (Zn), copper (Cu) levels, and activities of antioxidant enzymes such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), ceruloplasmin, total reactive antioxidant potential as well as thiobarbituric acid reactive substances (TBARS) in blood of workers exposed to lead (Pb) and cadmium (Cd). A group of 44 workers of a lead battery plant, and 21 workers of alkali battery plant (high cadmium levels) were examined. The reference group consisted of 52 persons from other industrial factories, medical staff, students with similar social background and living in the same area. It was found that both Se levels in plasma and GSH-Px activity in erythrocytes of workers exposed to high Cd concentrations (15.2± 7.7 μ g Cd/l whole blood), were significantly lower than in the reference group 50.0± 8.6 μ g/l and 63.0± 13.5 μ g/l ($p<0.0001$), and 15.3± 3.4 u/g Hb and 19.1± 4.1 u/g Hb ($p<0.0001$), respectively. No significant differences were found between other determined parameters. In the group of workers exposed to high lead concentration (504± 92 μ g Pb/l whole blood), lower activity of GSH-Px in erythrocytes and plasma was observed ($p<0.0001$) as compared to persons having no contact with lead. Concentrations of Zn and Cu in plasma of investigated workers were 0.82± 0.16 mg/l and 1.19± 0.17 mg/l and differed significantly from the reference group ($p<0.01$ and $p<0.05$, respectively). In the group of workers exposed to Pb lipid peroxidation measured as TBARS concentration in plasma was found to be increased by 43% (2.08± 1.18 μ mol/l and 1.45± 0.36 μ mol/l; respectively vs. controls; $p<0.0001$). The values of the other markers did not differ significantly from those found in the reference group. It is conclusion that antioxidative potential of the organism of people exposed to high concentrations of Pb and Cd at the work-place were lower than in workers of reference group not exposed to these heavy metals.

1684 PROTECTION AGAINST CHRONIC CADMIUM TOXICITY BY CALORIC RESTRICTION.

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Exposure to cadmium (Cd) can result in nephrotoxicity and osteotoxicity. Because Cd-induced nephrotoxicity involves oxidative stress and caloric restriction decreases oxidative stress, we examined whether reduced caloric intake will protect against nephrotoxicity. In addition, the protection against the osteotoxicity was also examined. Weanling male Sprague-Dawley rats were provided drinking water containing 100 mg Cd/l for up to 27 months. Because female rats drank more water/kg body weight than males, they were fed 100 mg Cd/l for 3 months, followed by 80 mg/l until 6.5 months, and 65 mg/l thereafter. Both males and females consumed a total of about 5 g Cd/kg body weight. After the first 3 months food was restricted to 20 g/day. The Cd-exposed females had a 23% reduction in their bone density during the 3 months of unrestricted food intake. No further reduction in bone density was noted during the dietary restriction period. Hepatic and renal Cd accumulation and corresponding MT levels were very similar in both sexes. The reported critical Cd concentration for nephrotoxicity was reached by 9 months. Renal MT levels were maximum at this time, and did not increase significantly despite a 1.5-fold increase in renal Cd concentration over the next 18 months. In spite of high renal Cd levels and lack of availability of sufficient MT, there was no sign of nephrotoxicity, as measured by urinary protein and glucose excretion. It is concluded that caloric restriction increases tolerance to both nephrotoxicity and osteotoxicity of Cd. (Supported by NIEHS grant # ES03187.)

1685 PROTECTION AGAINST CHRONIC CADMIUM TOXICITY BY GLYCINE.

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A Japan drug containing glycine, glycyrrhizin, and cysteine (Stronger Neo-Minophagen C®) has been reported to protect against chronic Cd toxicity. The present study was conducted to evaluate which of the three constituents of this drug was the main antagonist for Cd toxicity and whether the mechanism of protection involved antioxidant action. Adult female Sprague-Dawley rats were injected sc with 5 μ mol $CdCl_2$/kg/day, 5 times/week, for 15 weeks. Under this dosing schedule the animals developed hepatotoxicity and renal toxicity by week 10. Starting from week 7 four groups of Cd-injected animals received co-treatments with either 10 mg glycyrrhizin/kg, 100 mg glycine/kg, 5 mg cysteine/kg, or with a mixture of all three compounds, 5 times/week. An additional Cd-treated group was co-treated with Vitamin E (100 mg/kg) as a positive control. Only those animals that received vitamin E, mixture, or glycine were protected against Cd-induced hepatotoxicity as well as nephrotoxicity. All three co-treatments decreased Cd-induced hepatic and renal lipid peroxidation. We conclude that the reported beneficial effects of Stronger Neo-Minophagen C® are due to glycine, which appears to protect against chronic Cd toxicity by reducing oxidative stress. (Supported by NIEHS grant # ES03187.)

1686 BIDIRECTIONAL TRANSPORT OF CADMIUM ACROSS APICAL MEMBRANE OF RENAL EPITHELIAL CELL LINES VIA H^+-ANTIPORTER AND INORGANIC ANION EXCHANGER.

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The purpose of this study was to investigate whether Cd efflux across the apical membrane of renal epithelial cells is mediated via a H^+-antiportal system, and to confirm the re-uptake of Cd from the apical membrane via the inorganic anion exchanger. LLC-PK_1 and OK cell monolayers cultured on permeable membrane were incubated with 1 μM $CdCl_2$ added to the basolateral medium, and the basolateral-to-apical transport of Cd and the accumulation of Cd in the monolayers were measured. The Cd transport was increased by lowering the pH of the apical medium and was accompanied by a decrease in Cd accumulation. Coincubation with N'-methylnicotin- amide or cisplatin, typical and possible substrates of the H^+-antiportal systems, respectively, decreased the Cd transport and increased the Cd accumulation in a concentration-dependent manner. To confirm the re-uptake of Cd, LLC-PK_1 cell monolayers were pretreated with DIDS, a specific inhibitor of the inorganic anion exchanger, before incubation with $CdCl_2$. In separate experiment, $ZnCl_2$ (10 nM) or $NaHCO_3$ (2 mM) was added to the apical medium to inhibit and stimulate the re-uptake of Cd, respectively. Pretreatment with DIDS significantly increased the Cd transport and decreased the Cd accumulation in the apical medium at pH 7.4, but not at pH 5.5. The addition of $ZnCl_2$ significantly increased the Cd transport and decreased the Cd accumulation at pH 7.4, whereas the addition of $NaHCO_3$ significantly increased the Cd accumulation. These results suggest that the bidirectional transport of Cd across the apical membrane of renal epithelial cells occurs via the H^+-antiportal system (efflux) and the inorganic anion exchanger (influx), depending on the pH of the medium on the apical side.

1687 CADMIUM INDUCED BIO-ACCUMULATION IN THE SELECTED TISSUES OF A FRESHWATER TELEOST *OREOCHROMIS MOSSAMBICUS* (TILAPIA).

A U Rani. *Department of Zoology, S.V.University, Tirupati, A.P., INDIA.* Sponsor: S M Somani.

In recent years heavy metals discharged from industries are a major source of pollution which has become a threat to all forms of life. Among the myr-

iads of pollutants, cadmium(cd^{++}) is an ubiquitous, non-essential heavy metal which possesses high toxicity to both humans and aquatic organisms and it has the tendency to bio-accumulate (Ravera 1984, Usha Rani & Ramamurthy 1989). This uptake of heavy metal is mostly tissue specific and may even bio-magnify in the animals of higher trophic level. Freshwater fish *Oreochromis mossambicus* (Tilapia) were exposed to sublethal concentration (5 ppm) of Cadmium Chloride (Technical grade) for 1;15; and 30 days of long sojourn. The fish were fed daily with groundnut cake and all the physico- chemical conditions of the aquatic environment was maintained uniformly throughout the experimentation .Tissues like gill, liver, kidney, intestine and scales were isolated after each time interval and acid digested overnight in 3:2 nitric acid : perchloric acid mixture separately in air tight vials. This acid sediment mixture was ashed at 1200 °C and the left over residue was dissolved in 5ml of double distilled water. The Cadmium concentrations in all the above tissues were determined using the graphite furnace atomic absorption spectrophotometer. Results revealed accumulation of Cadmium in all the selected tissues with an exception of high tendency of accumulation in kidney (14.4 mg/l). Cadmium levels were least in intestine (0.92mg/l) and scales (0.7mg/l). Accumulation increased with increased time of exposure and kidney was found to be the specific site for Cadmium accumulation. This bio- accumulation studies can be used as indices of heavy metal pollution and are tools to identify tissue specificity of a particular heavy metal.

1688 CADMIUM INDUCED FREE RADICAL GENERATION AND LIPID PEROXIDATION IN THE KIDNEY AND GILLS OF FRESHWATER FISH,CLARIAS BATRACHUS.

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Cadmium , a non essential element is an important concern due to its accumulation in different organisms through food chains. Though cadmium induced biochemical and pathophysiological alterations are well documented, the mechanisms responsible for such changes are not clearly established. We have reported the free radical mediated lipid peroxidation during cadmium intoxication in brain and liver. The main causes for the pathophysiological manifestation is suspected to be the involvement of free radical generation. The present study is designed to study the cadium induced free radical generation leading to lipid peroxidation in the kidney and gill of a freshwater fish, Clarias batrachus, with a specific objective to find out tissue specific and time dependant variations in responses. Fishes were exposed to sublethal concentration (1/10 of LC-50 value i.e.1.2 ppm) of cadmium chloride for period of 30 days. Following cadmium exposure Xanthine Oxidase activity was measured at 1,7,15,and 30 days to assess the formation of free radicals such as superoxide anion. Further, free radicals were also measured by electron Spin Rosanance (ESR) studies. Malondialdehyde content of the tissues was also measured to quantify the amount of lipid peroxidation. The increased levels of free radical cearly reflected in the lipid peroxidation. The responses were found to be tissue specific and time dependent. The present result indicate that cadimium may cause toxicity in aquatic organisms through oxidative injury.

1689 DNA DAMAGE AND RECOVERY PRODUCED BY CD IN A HUMAN FETAL HEPATIC CELL LINE.

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Cadmium (Cd) is one of the most important heavy metal environmental toxicants. It alters a wide variety of cellular and biochemical processes. The objective of this work was to study the subcellular distribution and DNA damage and recovery after acute and chronic $CdCl_2$ treatment in a human fetal hepatic cell line (WRL-68 cells). Cd subcellular distribution was 16% nuclear fraction, 23% mitochondrial fraction, 15% in microsomal and 46% in cytosolic fraction. DNA damage was determined by an alkaline microgel electrophoresis assay that detects DNA single-strand breaks and/or alkali-labile sites in individual cells. The mean migration length in control cells was 35.37 ± 1.43 μ m (8 % damaged nucleus), whereas cells treated with 0.005 μ M $CdCl_2$ during 3 hs (acute low dose) increased 86% (88% damaged nucleus). In the 0.01 μ M $CdCl_2$ treatment during the same time (acute high dose) increased 255% (92% damaged nucleus) and 0.005 μ M $CdCl_2$ treatment during 7 days (chronic treatment) increased it by only 65% (88% damaged nucleus). Lipoperoxidative damage expressed as malondialdehyde production per mg of protein was 15.7 ± 2.6 for control cells, and the Cd treated cells values were 20.2 ± 2.4 (acute low dose), 22.9 ± 2.2 (acute high dose) and 22.6 ± 2.1 (chronic treatment). To study the DNA damage recovery, cells were washed with 0.01 μ M meso-2,3-dimercaptosuccinic acid (DMSA), and fresh DMEM was added to cells. The percentage of damaged nucleus diminished after 90 min and DNA migration presented control values after 120 min. Cd treatment produced DNA single–strand breaks and the damage was greater in acute high dose treated cells. Lipid peroxidation values did not follow a direct correlation with DNA single-strand brakes. DNA damage recovery took 120 min after the metal was partially removed with DMSA.

1690 TISSUE DISTRIBUTION OF CADMIUM IN RATS TREATED ORALLY WITH CADMIUM FOR 8 MONTHS.

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To investigate the distribution of cadmium (Cd) to various tissues after oral administration, female rats were given diets containing Cd (0.02, 0.04, 0.11, 1.1, 5, 20, and 40 ppm of Cd) for up to 8 months. At 1, 4, and 8 months after the commencement of Cd treatment, rats were administered a single dosage of ^{109}Cd, a tracer, of the matched amounts to Cd for the day (about 1.2 to 2400 μg Cd/kg BW). Then, they were necropsied 5 days later to determine the distribution of Cd to various tissues. In addition, Cd concentrations in the liver and kidneys and metallothionein (MT) in the liver, kidneys, intestinal mucosa, serum, and urine were determined. The distribution rates of Cd to the liver increased dose-dependently (40% at low doses to 60% at high doses), whereas those to the kidney decreased dose-dependently (20% at low doses to 10% at high doses). The Cd retention rates on 5 days after Cd administration (amounts of Cd in various tissues / amounts of Cd administered) ranged from 0.2 to 1.0% at any time points. The concentrations of Cd in the liver and kidneys and MT in the liver, kidney, serum, and urine at 4 and 8 months increased dose-dependently, whereas MT in the intestinal mucosa did not change markedly at any time points. These results suggested that the distribution of Cd to the liver and kidneys after oral administration were varied depending on the levels of Cd. The difference of the distribution pattern of Cd to the liver and kidney was thought to be due to difference in the form of the absorbed Cd, i.e., free ion or Cd-MT complex. However, it was not closely related to MT in the intestinal mucosa in this study.

1691 VALIDATION OF THE NAVY NEUROBEHAVIORAL TOXICITY ASSESSMENT BATTERY (NTAB).

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Since 1993, NHRC/TD has been involved in development of a comprehensive battery of animal-based tests for prediction of human performance decrements that may be a consequence of exposure to chemicals of military interest. The NTAB consists of nearly 20 tests that evaluate decrements in eight major neurobehavioral performance factors. The NTAB is currently being validated as a tool for human risk assessment through comparison of animal and human results on topographically-similar performance tests following administration of pharmacological compounds that are ethically-acceptable for human use. Pharmacologics used include: ethanol, d-amphetamine, diazepam, caffeine, and nicotine. To date, the Navy Roto-Wheel and forelimb grip strength tests have been evaluated for prediction of equilibrium, motor strength and endurance deficits; the Porsolt Forced Swim test for emotional depression; the operant Progressive Ratio/FR-5 test for motivational and motor performance deficits; the operant delayed spectral and geometric pattern delayed matching-to-sample tests for visual system and STM deficits; and the juvenile play behavior analysis test for changes in emotionality and socialization. Results of predictive validation studies are presented and compared to the existing human literature.

1692 BEHAVIORAL TOXICITY OF ETHANOL IN RATS.

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The adverse effects of ethanol on behavior and cognition are well documented but poorly understood. The present experiment sought to characterize the behavioral and cognitive effects of ethanol in 24 male Sprague-Dawley rats using tasks in the NCTR operant test battery. Tasks included: temporal response differentiation (TRD) to assess timing behavior; incremental repeated acquisition (IRA) to assess learning; conditioned position responding (CPR) to assess position discrimination; differential reinforcement of low rates (DRL) to assess timing and response inhibition; and progressive ratio (PR) to assess motivation. Task accuracy, percent task complete, and response rates were monitored for the TRD, IRA, CPR, and DRL tasks. Response rate was monitored for PR. Ethanol (0.0, 0.5, 1.0, 1.5, 2.0, and 3.0 g/kg) was suspended in water (40% v/v) and administered by orogastric gavage. Behavior testing began 15 minutes after ethanol administration. Ethanol reduced accuracy and percent task complete for the CPR, DRL, and TRD tasks. For CPR, this reduction was accompanied by a reduction in response rates. Ethanol also reduced response rates on the PR task. There were no effects of ethanol on the IRA task. Overall, the DRL and TRD tasks showed the greatest sensitivity to ethanol, followed by CPR, PR, and IRA. These data suggest that ethanol can impair performance on cognitive-behavioral tasks. Furthermore, because ethanol did not uniformly alter performance on all tasks presented, these data suggest that specific cognitive abilities are differentially sensitive to adverse effects of ethanol.

1693 NEUROBEHAVIORAL SCREENING OF CHILD AND ADULT BYSTANDERS EXPOSURE TO TOLUENE DIISOCYANATE APPLICATION.

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Rationale: Toluene diisocyanate (TDI) is a well known respiratory sensitizer. Less widely reported are its potentially permanent neurotoxic properties. TDI is incorporated into sprayed urethane foam elastomeric coatings, such as roofing materials. Application to surfaces at occupied schools puts children and others (such as the sick and elderly) at risk, who may be more vulnerable to TDI toxicity than is the average male worker, for whom occupational standards have been promulgated. Scope: Over 500 gallons of roofing products containing .6% TDI at 135 degrees F was sprayed by workers in full protective gear over 27,000 sq. ft. of low-level roof of an occupied school building. Numerous students and teachers were exposed to the products and developed respiratory sensitivity symptoms. Assessment approach: A brief neurobehavioral screen (1 hour of testing) was administered to 10 subjects six years post-exposure with troubling respiratory symptoms (asthma, chronic bronchitis) associated with roofing materials containing TDI. The assessment included tests which have an established normative base and that are known to be sensitive to neurotoxicity, including the Benton Visual Retention test, subtests of the WAIS-III battery (Digit Symbol, Digit Span, and Block Design), a logical memory test, the Neurotoxicity Screening Survey, and tests for malingering and distortion. Summary of findings: Positive symptoms on the Neurotoxicity Screening Survey were found in 7/9, with 2/9 results borderline. Neurobehavioral test results (signs) were positive in 26/39 tests (67%), borderline in 5/39 (13%) and within normal limits in 8/39 (21%). All test results of malingering were negative. Conclusion: Findings are consistent with TDI neurotoxicity. Psychogenic causes are unlikely explanations of the illness in part due to negative malingering and distortion test results, positive cognitive deficits and duration of symptoms.

1694 A 13-WEEK NEUROTOXICITY STUDY OF PHENOL ADMINISTERED IN THE DRINKING WATER TO THE RAT.

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Groups of 15 male and 15 female Sprague-Dawley (CD) rats were treated for 13 weeks, via the drinking water, with phenol at concentrations of 0, 200, 1000 or 5000 ppm. A 4-week recovery period followed the treatment phase. In-life evaluations included body weight, food consumption, water intake, clinical signs, as well as a functional observational battery (FOB) and motor activity test. At treatment termination, 5 rats/sex/group in the 0 and 5000 ppm groups underwent a neuropathological evaluation. At 5000 ppm, a lower body weight, reduced food and water consumption and abnormal clinical signs, including dehydrated appearance, were seen for many animals of both sexes. At this high dose, one female was euthanized early in the study due to poor condition. At 1000 ppm, decreased water intake and on occasion dehydrated appearance were seen. Marked improvements were noted for the 1000 and 5000 ppm groups immediately upon initiation of the recovery period. No effects were noted for any of these parameters at 200 ppm. The FOB did not reveal any findings of neurotoxicological significance at any concentration following treatment or recovery. Observations of altered motor activity (females only) were noted at 1000 and 5000 ppm, however, this finding was considered to be secondary to the reduction in water and/or food intake. No gross or histopathological lesions in nervous tissue attributed to treatment with phenol were noted. Based on these findings, the no observed effect level (NOEL) for neurotoxicity under the conditions of this study was 5000 ppm (corresponding to 308 and 360 mg phenol/kg body weight/day for males and females, respectively). (The Chemical Manufacturers Association Phenol Panel sponsored this study.)

1695 SUBCHRONIC TOXIC AND NEUROTOXIC EFFECTS OF ACPC (1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID) IN RATS.

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ACPC a partial NMDA agonist, was evaluated in this 13-week study which also included a 13-week recovery period. Four of the groups (Groups 1-4, 20/sex/group) were used for toxicological assessment; the remaining four groups (Groups 5-8, 15/sex/group) were used for neurotoxicological and pharmacokinetic assessments. Dosage levels were 200, 600 and 1800 mg/kg/day administered daily by gavage. Clinical observations, body weight, feed consumption, clinical pathology, ophthalmology, gross necropsy, organ weights and standard histologic assessments were conducted on toxicology group animals. Functional observation battery, motor activity, neuropathology and pharmacokinetic assessments were conducted on the remaining groups. ACPC was rapidly absorbed in a dose proportional fashion and was generally cleared from the system within 24 hours. ACPC at 1800 mg/kg resulted in transient changes in the clinical condition of the animals (fine tremors one hour following dosing during the first few days of treatment), slight reductions in body weight gain and widely disseminated microscopic intramyelinic vacuolation of the brain (both white and grey matter) which was less severe after the recovery period. No neuro-behavioral deficit was identified by functional observation and motor activity assessment. Neuronal degeneration and necrosis typical of NMDA antagonists was not observed in perfused rats. At 600 mg/kg, ACPC related changes were limited to vacuolation in the brain in a limited number of animals and was not present after recovery. No signs of toxicity were observed at 200 mg/kg/day.

1696 SPATIAL LEARNING AND LTP CORRELATE WITH CHANGES IN HIPPOCAMPAL NMDAR-1 mRNA AND PROTEIN IN ADULT RATS EXPOSED TO Pb^{2+} DURING DEVELOPMENT.

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N-methyl-D-aspartate receptor (NMDAR) activity is important for synaptic plasticity, in learning and memory, and during development. Lead (Pb^{2+}) neurotoxicity is known to impair both cognitive function in children, and long-term potentiation (LTP) in the rat hippocampus. Pb^{2+} is also a potent inhibitor of the NMDAR. This study examined relationships between spatial learning, LTP, and NMDAR gene and protein expression in adult, rat littermates exposed to Pb^{2+} during development. Spatial learning in the Morris water maze was impaired ($F_{2,25}$=5.77, p<0.05) in Pb^{2+}-exposed adult rats. *In vivo* LTP was induced unilaterally in the entorhinal cortex-dentate gyrus pathway of control and Pb^{2+}-exposed adult rats. Averaged over the 60 min post-conditioning period, the magnitude of LTP evoked in Pb^{2+}-exposed rats was significantly less ($F_{2,5}$=8.633, p<0.05) than in control rats. Rats exposed

to Pb^{2+} also exhibited reduced NMDAR-1 mRNA in both the granule ($F_{2,10}$=7.404, p<0.05) and pyramidal ($F_{2,10}$=6.602, p<0.05) cell layers of the hippocampus. NMDAR-1 protein was significantly reduced ($F_{2,9}$=6.826, p<0.05) in the hippocampus of Pb^{2+}-exposed adult rats. Together, these data suggest a relationship between both Pb^{2+}-induced impairment of spatial learning and Pb^{2+}-induced changes in synaptic plasticity, with molecular changes in the NMDAR-1 subunit. These findings support the hypothesis that the NMDAR is a principal target for Pb^{2+} neurotoxicity. (Supported by ES06189 to TRG and T32 ES07141 to MKN.)

1697 EFFECTS OF LEAD, MAGNESIUM AND ZINC MIXTURE ON SPATIAL MEMORY CAPABILITY AND NMDA-RECEPTOR SUBUNIT COMPOSITION IN F344 RATS.

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The N-methyl-D-aspartate (NMDA)-receptor complex (NR) is known to be involved in learning and memory processes. It undergoes changes in subunit composition early in life, affecting affinities for its ligands zinc (Zn) and magnesium (Mg). Presumably, lead (Pb) also binds to the Zn-binding site. Pb, Mg and Zn are common ground water contaminants, often occurring in combination. Goals of this study were to evaluate the effects of Pb, Mg and Zn individually and in combination: (1) on spatial learning and memory capabilities and (2) on the mRNA for the NR subunits. Male F344 rats (n=29) were gavaged once daily with Mg-, Zn- or Pb-acetate (individually or as a mixture) for 4 weeks, beginning on postnatal day 21. After 2 weeks of exposure, learning was tested daily using a water maze. Each animal was tested 3X per day for 12 days with a hidden platform, every 6th run being a probe trial. On day 13, their vision was tested using a visual cue on the platform. The behavioral data showed that all animals learned equally fast within 4 days, with no further improvement in performance thereafter. There were no significant group differences for swim speed, cumulative distance, runtime or pathlength, suggesting that short-term exposure to these metals in early post-weaning time does not result in immediate behavioral changes. Since it is possible that molecular changes indeed occurred but were not manifested behaviorally, we are currently analyzing amounts of mRNA for the different NR subunits in various brain regions using in situ hybridization. This study was supported by a number of small grants from Colorado State University.

1698 DEVELOPMENTAL LEAD (Pb) EXPOSURE FAILS TO DISRUPT SPATIAL LEARNING IN THE MORRIS WATER MAZE.

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Long-term potentiation (LTP) is a plasticity model associated with the neurobiological mechanisms of memory. Chronic exposure to environmentally relevant Pb levels has previously been shown to impair hippocampal LTP. The present study examined the effects of developmental Pb exposure (0, 0.2%, 1.0%) on a spatial learning task. From GD16 to PND21 Long Evans rats were exposed to Pb through the drinking water of the pregnant dam and directly via their own water supply at weaning. Performance on an acquisition task and a working memory task was assessed in adult male offspring using the Morris water maze. Acquisition testing consisted of 2 trials/day for 14 days. Animals located a submerged platform positioned in a constant location throughout the task. There was no difference in rate of acquisition between Pb-exposed and control rats. Probe trials in which the platform was removed were implemented throughout the acquisition period and time spent in the correct quadrant of the test apparatus during the trials was assessed. The probe trials also failed to discriminate among groups. Following the acquisition phase animals were tested on a repeated acquisition task, 4 trials/day for 5 days in which the platform position remained constant across trials, but changed daily. Performance on this working memory task revealed no differences among the groups. Finally, the effects of the NMDA antagonist, MK801, on working memory were evaluated. MK801 (0, 0.05, 0.1 mg/kg, s.c.) was administered 20 minutes prior to the start of trial 1 with a minimum of 3 drug-free days interceding between each drug dose. A dose-dependent disruption in working memory was observed following MK801 but no differential effect of Pb on task performance was detected. These data fail to reveal an impairment in spatial learning following Pb exposure that produces deficits in hippocampal LTP. The findings are consistant with reports of intact spatial learning in similarly Pb-exposed animals using the radial arm maze (Driscoll et al., 1997) and reveal a dissociation in the Pb-induced deficits in spatial learning and hippocampal LTP.

1699 CHRONIC LEAD EXPOSURE ALTERS EXPLICIT MEMORY FUNCTION.

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Despite increasing evidence that low-level lead (Pb) exposure impairs cognitive functioning, much remains to be learned about the specific processes affected and the underlying mechanism(s). Pb exposure has been shown to alter hippocampal electrophysiology (e.g., LTP) and neurochemistry. Because explicit memory depends on the hippocampus, Pb exposure would be expected to specifically impair this type of memory. However, there is no convincing evidence for this type of effect. One interpretation is that the neural changes are not of sufficient magnitude to be functionally significant. However, it is also possible that the paradigms used are not sufficiently sensitive to detect subtle impairments in explicit memory. To address this possibility, two previous studies in our lab examined the effect of chronic Pb exposure on explicit memory function, using a task that has been shown to be sensitive to manipulations that subtly alter hippocampal function. Both studies found that explicit memory function in the Pb-exposed animals was superior to controls, particularly at long retention intervals. The present study was designed to explore a wider range of exposures, to determine whether the putative memory modulatory effects of Pb manifest themselves as impairment at some BPb levels. Male Long-Evans rats were exposed to Pb chronically from birth, yielding mean BPb levels of <5, 35, & 70 μg/dl. Explicit memory was assessed in a radial arm maze with varying retention intervals (0, 4, & 8 hrs) imposed midway through the test session. Chronic Pb exposure significantly and specifically altered explicit memory function. A U-shaped dose-response curve was observed, with the low-dose group being significantly different from both controls and the higher-dose group; the latter groups did not differ. As in our previous 2 studies, the direction of the effect at the low dose was enhancement, rather than impairment, of explicit memory function. Possible underlying mechanisms will be presented as well as a regression analysis relating blood Pb level with memory function. (Supported by NIEHS ES07457, ES05950 and EPA U-915393-01-0.)

1700 ENDURING EFFECTS OF EARLY LEAD EXPOSURE: ASSESSMENT OF SUSTAINED ATTENTION, INHIBITORY CONTROL, AND INFORMATION PROCESSING SPEED.

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Despite increasing evidence that early lead (Pb) exposure can produce lasting behavioral changes, much remains to be learned about the specific cognitive processes affected. One prominent theory is that Pb exposure produces a syndrome similar to attention deficit hyperactivity disorder (ADHD). Yet this theory is based primarily on behavioral observations in the classroom; few studies have included tasks specifically designed to assess sustained attention and inhibitory control, the hallmark areas of dysfunction in ADHD. The present study was designed to test these cognitive functions, using a rodent model of early childhood Pb exposure. Long-Evans dams received Pb acetate (300 or 600 ppm) during gestation and lactation, or lactation alone. Dams receiving sodium acetate served as the control. Pb exposure stopped at weaning on PND 30. Sixty adult offspring were tested on a series of automated tasks designed to assess visual sustained attention, inhibitory control, and speed of information processing. A deficit in sustained attention was observed in rats exposed to Pb during both gestation and lactation, or lactation alone. However, impairment was only observed on the most demanding of the four tasks, in which both the onset and duration of the visual cue varied randomly across trials. Tasks in which only cue onset varied did not reveal attentional dysfunction. Speed of information processing, a measure shown to correlate with IQ in humans, was not affected by early Pb exposure. The rate of premature responding, a measure of inhibitory control, did not yield consistent evidence of Pb-induced impairment. These findings provide one of the first demonstrations of an enduring effect of early Pb exposure on sustained attention. However, the relative lack of impairment in the area of inhibitory control argues against a clear ADHD-like profile. (Supported by NIEHS grants ES07457 & ES05950.)

1701 EVIDENCE FOR THE ROLE OF DOPAMINE D2 RECEPTORS IN MEDIATING ALTERATIONS IN FIXED INTERVAL PERFORMANCE BY LOW LEVEL LEAD (Pb) EXPOSURE.

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The aim of this study was to further investigate the contention that alterations in central dopaminergic mechanisms mediate the behavioral impairments reported with low level Pb exposure. Several studies have reported region-specific modulation of D2 receptor mechanisms and measures of FI (fixed interval) performance. 21-day-old male Long Evans rats were exposed to 0 or 50 ppm Pb acetate orally for 90 days. A FI-1min paradigm was imposed after 30 days of exposure and overall response rates (ORR) assessed over 30 sessions. Subsequently, each group was subdivided into two and treated with 0.01, 0.05, 1.0, 3.0 mg/kg i.p quinpirole (Q) or eticlopride (E) respectively. ORR were monitored for 5 sessions after each treatment. After the first few sessions, the Pb-treated group exhibited higher ORR. Quinpirole resulted in significantly ($p < 0.05$) decreased responding in the Pb group at all the doses except 0.01 mg/kg. Eticlopride did not alter ORR for both groups at the two lower doses, but at 1 mg/kg, ORR were higher for the control group. At 3 mg/kg, though there were temporal differences, ORR were higher for the control group. Although there were dose-dependent differences, quinpirole reversed the higher response rates of the Pb-exposed group and these changes were associated with alterations in extracellular dopamine. On the other hand, eticlopride did not alter changes in response rates and extracellular dopamine levels in Pb-treated rats. Taking together, these results showed that D2 receptor mechanisms can modulate changes in response rates that result from low level Pb exposure. (Supported by ATSDR Cooperative Agreement #U50/ATU 398948.)

1702 Pb EXPOSURE MODIFIES DOPAMINE (DA) ANTAGONISM OF BUT NOT BEHAVIORAL SENSITIZATION TO QUINPIROLE'S LOCOMOTOR ACTIVATING EFFECTS.

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Chronic Pb exposure alters mesolimbic (MES) dopamine (DA) function, suggesting that elevated Pb burden could serve as a risk factor for MES-associated behavioral dysfunctions, including schizophrenia, drug abuse and attention deficit disorder. This study sought to further understand mechanisms by which Pb alters MES DA function. Changes in motor activity following acute administration of the D2 agonist quinpirole (QUIN; 0.05-5.0 mg/kg), its blockade by the D2 antagonist eticlopride (0.05-0.20 mg/kg) or the D1 antagonist SCH23390 (0.02-0.04 mg/kg), and sensitization to repeated 1.0 mg/kg QUIN doses were examined in rats exposed chronically to 0, 100 or 500 ppm Pb acetate drinking water from weaning. QUIN dose-dependently increased motor activity but these effects were not impacted by Pb. Nor did Pb influence either the development or reversal of sensitization following repeated QUIN treatments. DA antagonism effects, however, differed by Pb exposure level. Both eticlopride and SCH23390 decreased QUIN-enhancement of motor activity. While D2 antagonist effects were potentiated by 100 ppm Pb (blood Pb = 17 ug/dl), 500 ppm Pb (blood Pb = 47 ug/dl) potentiated SCH23390 effects and altered its time course. These findings may provide a neurochemical analogue for biphasic behavioral manifestations of Pb; they also suggest a preferential or initial impact of low-level Pb on D2-mediated behavioral functions. A question to be pursued is the relationship of these findings to reported D1-D2 interactions. (ES05017, ES05903 and ES01247.)

1703 METHYLPHENIDATE (RITALIN) DOES NOT REVERSE THE EFFECTS OF LOW-LEVEL LEAD (Pb) EXPOSURE ON A WAITING FOR REWARD BEHAVIORAL PARADIGM.

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Many of the effects of low-level lead (Pb) are similar to the symptoms of Attention Deficit Disorder (ADD). Children with ADD and those with elevated Pb levels are described as impulsive or having an inability to manage delays. Methylphenidate (ritalin) is commonly used to treat children diagnosed with ADD. This study compared acute and chronic methylphenidate effects in control and Pb exposed rats on a behavioral waiting task. Groups of 12 rats each were exposed chronically from weaning to levels of 0, 50, or 150 ppm Pb acetate in drinking water. After 40 days of exposure, rats were trained on a fixed ratio (FR) - wait behavioral baseline. A total of 50 responses (FR 50) on an operant lever produced a 45 mg food pellet. After earning an FR pellet, "free" pellets could be obtained until another lever response was emitted which reinitiated the FR component. "Free" pellets were delivered at increasing time intervals (i.e., 2 s, 4 s, 6 s, ...). Acute methylphenidate (0.0 - 16.0 mg/kg) treatment increased the number of responses per reinforcers in the 150 ppm group, suggesting that methylphenidate potentiated the effects of Pb. Chronic methylphenidate (4.0 mg/kg) increased FR resets, FR response rate, and responses per reinforcement and decreased mean longest wait times, with these effects not differentially altered by Pb exposure. These findings support previous results from this laboratory that the deficits produced by postweaning low-level Pb exposure is related to increased DA. (Supported by ES05727, ES05017, ES05903, ES01247.)

1704 EFFECTS OF CHRONIC LEAD EXPOSURE ON NEUROBEHAVIOURAL FUNCTION AND DOPAMINERGIC RECEPTORS IN RATS.

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Effects of chronic Pb exposure on radial maze performance, locomotor activity, and dopaminergic receptors in rats were investigated. Rats were exposed pre- and post-natally from day 4 ± 1 post conception with 1000 ppm lead acetate (Pb) in their drinking water. This treatment continued after weaning and until post-natal day 84. No change in working error and reference error was found in the radial arm maze following Pb exposure, indicating no influence of Pb on spatial learning. However, Pb-treated rats took less time for the first 4-arm visits and for 4 baited arm visits in the radial maze, and also showed significantly higher locomotor activity in the open-field. The results reveal that Pb may induce hyperlocomotor activity in rats. Pb treatment did not affect the motor coordination, as observed in the rotarod test. Pb produced a decrease in [^{125}I]sulpiride binding to D2-receptors in the cerebral cortex, but not in the nucleus accumbens and caudate putamen. No effect of Pb exposure on [^{125}I]SCH-23982 binding to D1-receptors was found in all brain areas examined. This research suggests that Pb-induced changes in D2-receptor in the cerebral cortex may be related to increases in locomotor activity.

1705 EFFECTS OF SUCCIMER ON REGIONAL BRAIN Pb LEVELS IN PRIMATES.

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While a number of studies have evaluated the efficacy of succimer (2,3-dimercaptosuccinic acid) for reducing brain Pb levels in rodent models, no data exist on the efficacy of this treatment for reducing brain Pb levels in primates. These data are necessary to better predict succimer's ability to ameliorate Pb-induced cognitive deficits in children, particularly since primates may metabolize succimer differently than rodents. The objectives of this study were to determine if succimer reduces brain Pb levels in a primate model, including those in brain regions associated with learning. Eleven adult male rhesus monkeys (*Macaca mulatta*) were orally exposed to Pb acetate via water for 5 weeks to achieve target blood Pb levels of 35 ug/dL. Prior to treatment, a pre-chelation biopsy of the pre-frontal cortex was collected (PRE-PFC). The next day Pb exposure ceased and the monkeys were randomly assigned to control (C, placebo, n=5) or succimer (n=6) groups. Succimer was given at 30 mg/kg BW/d for 5 days then 20 mg/kg BW/d for 14 days. The monkeys were then sacrificed and a post-chelation pre-frontal cortex biopsy (POST-PFC) collected and the frontal lobe (FL), hippocampus (H), and striatum (S) removed. Tissues were analyzed for Pb content using trace metal clean techniques and ICP-MS. Succimer treatment did not reduce brain Pb levels ($P>0.88$). However, cessation of Pb exposure reduced brain Pb in both treatment groups (PRE-PFC vs POST-PFC, $P = 0.006$). Regarding brain regions, Pb levels in the PFC were higher than the FL or H ($P<0.05$), while the S had the least Pb ($P<0.05$). Moreover, higher blood Pb levels during Pb exposure were associated with higher Pb in the H and S at sacrifice ($P<0.05$). These results indicate that 1) a standard clinical regimen of succimer is ineffective in reducing brain Pb levels, particularly when compared to the efficacy of eliminating Pb exposure, and 2) the accumulation of Pb in some brain regions is proportional to Pb exposure history. (Supported by NIEHS Grant #06918.)

1706 THE EFFECT OF CHRONIC DIETARY LEAD EXPOSURE ON GALACTOLIPID METABOLISM IN RATS.

W Deng and R D Poretz. *Rutgers University, New Brunswick, NJ, USA.* Sponsor: K R Cooper.

Lead exposure causes cognitive and behavioral deficits in children. We propose that a contributing mechanism for the neurological damage is that lead induces critically low levels of arylsulfatase A (ASA) at sensitive stages of nervous system development. Inadequate levels of ASA cause the accumulation of sulfatide and the demyelinating disease, metachromatic leukodystrophy. We hypothesize that the combined effects of a single nucleotide genetic polymorphism in human ASA which results in reduced levels of the enzyme, and lead concentrations which causes a decrease in the amount of ASA activity culminate in cellular enzymic activity below a critical threshold needed to maintain normal nervous system function. To examine the effect of lead on galactolipid metabolism, enzymes and galactolipids were studied in rat pups exposed to the metal *in utero* and subsequently through maternal milk from exposed mothers and water following weaning. Pups from control and exposed groups were sacrificed by decapitation at 1, 2, 3, 5 and 8 weeks of age. It was found that the specific enzyme levels of the relevant galactosyl and sulfotransferases and ASA were decreased by as much as 40-55% in whole brain homogenates of animals exposed to lead. Quantification of the various forms of brain galactosyl ceramide and sulfatide demonstrated the impact of lead as expected from the effect of the dietary metal on the enzymes. The results of these experiments and earlier studies with human fibroblast are consistent with the expectations of the hypothesis. (Financial support from NIH (R01-ES08373) and the March of Dimes Foundation is acknowledged.)

1707 BRAIN AND PLACENTAL LESIONS PRODUCED IN RAT FETUSES PRENATALLY EXPOSED TO LOW-LEVEL LEAD ACETATE.

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The effects of lead in the biological systems are well documented. In the central nervous system, lead toxicidad is more evident in the children than in adults and may produce either overt symptoms of acute encephalopathy such as ataxia, headache, convulsions and coma or lesser deficits including learning disorders and hiperactive behavior. During fetal development it is clear that lead crosses the placental barrier readily producing an increase in blood lead levels of fetus. The fetal blood lead levels correlate closely with that in the maternal blood. In this study the effect of prenatal lead administration was studied microscopically in the brain, placenta and umbilical cord of rat fetuses. Twenty-five pregnant Wistar rats were intoxicated with a lead solution of 32 ppm during 21 days through the drinking water. Control group rats received deionized water for the same period. Day of gestation was the day after mating and dosing began on this day. The rats were killed on gestation day 21 and fetuses were obtained for examination, the placenta and umbilical cord were collected too.The histopathological studies of the parietal-cortex, and hippocampus, showed shrunken, hiperchomatic cells and condensed nuclei with abnormal chromatin. The Purkinje cells of cerebellum were shrunken, vacuolar citoplasma and hiperchromatic nuclei, interstitial oedema in all regions studied. The placenta showed severe vascular congestion there are vacuolation citoplasmic, a lot of cells showed abnormal chromatin, Interstitial oedema . The umbilical cord showed hiperchromatic and shrunken cells with severe interstitial oedema. Results showed lead have a toxic effects in rat fetuses prenatally exposed to lead acetate.

1708 INFLUENCE OF SUBACUTE MANGANESE SULFATE ON THE NEUROBEHAVIOR OF FEMALE AND MALE RATS.

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The potential of manganese sulfate to produce neurotoxicity following two weeks of daily gavage was studied in male and female rats. The rats were gavaged with suspensions containing 0.1, 1.0, 10, 30, or 100, mg/kg manganese each day. The functional observational battery and motor activity measurements were used in an initial screen for neurotoxicity. Manganese sulfate only affected the forelimb grip strength and weight gain in male rats. The forelimb grip strength was significantly less in male rats gavaged with 1.0 mg/kg manganese, than those given 30 mg/kg. Male rats given 100 mg/kg manganese gained less weight, than the control rats. The doses of the metal salt did not alter any behavioral measurement in the female rat. The results from this initial neurotoxicity screen indicate the potential of excess manganese to elicit gender specific effects. This initial neurotoxicity screen found female rats less sensitive to any neurotoxic effect from manganese sulfate. In summary, two weeks of daily manganese gavage, at the above doses, produced a couple of toxic effects in only male rats. (Support by ATSDR grant U50/ATU398948.)

1709 THE EFFECTS OF METHYLMERCURY EXPOSURE ON VISUAL AND AUDITORY FUNCTIONS IN NONHUMAN PRIMATES.

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The present study assessed visual and auditory functions in adult monkeys (*Macaca fascicularis*) exposed *in utero* to methylmercury (MeHg), a prevalent environmental pollutant. Maternal exposure of either 0, 50, 70, or 90 μg/kg/day of MeHg resulted in blood mercury levels at birth from 1.04 to 2.45 ppm for treated infants. There were 10, 9, 2 and 2 offspring produced, respectively. At approximately 15 years of age the monkeys were tested on a spatial visual contrast sensitivity task and an auditory pure tone detection task. For the vision test, a forced choice tracking procedure was utilized. The following spatial frequencies were assessed: 1, 4, 10, and 20 cycles per degree of visual angle. For the auditory test, a signal detection procedure was used. Frequencies of 125, 500, 1,000, 4,000, 10,000, 25,000, and 31,500 Hz were assessed. The results of these studies indicate that *in utero* exposure to MeHg has long-term effects on visual contrast sensitivity thresholds. Preliminary results from the auditory task suggest that auditory thresholds were not affected. Since previous studies have shown that pre- and postnatal or postnatal exposure to MeHg alone affects auditory thresholds, the results of this study point to the postnatal period as a possible critical window for MeHg induced auditory neurotoxicity. (Funded by Grant RO1 ES03745.)

1710 ACQUISITION OF A MULTIPLE DRH EXTINCTION SCHEDULE OF REINFORCEMENT IN RATS EXPOSED DURING DEVELOPMENT TO METHYLMERCURY.

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Female rats were exposed to 0, 0.5 or 6.4 ppm Hg as methylmercury in drinking water beginning at least 4 weeks before mating and ending on postnatal day 16. Brain Hg concentrations at birth in cohorts were 0.49 and 9.8 ppm for the two exposure groups. Female offspring were trained to lever press under Multiple Differential-Reinforcement of-High-Rate Extinction (MULT DRH-N:T EXT) schedules of reinforcement. Under the extinction schedule, lever-pressing had no scheduled consequences. Under the DRH-N:T schedule, a food pellet was delivered whenever N responses occur within T seconds. Two acquisition protocols were examined. First, the DRH parameter imposed in three successive sessions was 3:1, 5:2, and 9:4, values selected so that the same average inter-response time (*i.e.*, rate) was required by the schedules. Only ##, ##, and ## % of the control, low-dose, and high-dose rats met the DRH requirement during the third-session. Second, lever-repressing was re-established and the three schedules were imposed until behavior became stable, requiring at least 10 sessions. All but ### animals acquired the response. {A marginal/no effect of mercury was detected?}. This failed to replicate a similar experiment by Bornhausen (19**) showing effects of mercury on acquisition a Multiple DRH EXT schedule of reinforcement. (Supported by ES06466 from NIH.)

1711 EFFECTS OF MERCURY VAPOR AND GENDER ON SUSTAINED ATTENTION IN RATS.

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The developmental neurotoxicity of mercury vapor (Hg°) has been of concern to the general public, due to airborne sources of Hg° (e.g., combustion of medical waste and fossil fuels) and chronic exposure to dental amalgams containing Hg. Also, dental health professionals receive episodic exposure to Hg° in the workplace. To evaluate the potential developmental neurotoxicity of Hg°, adult female Long-Evans rats were exposed during pregnancy via inhalation to air or Hg° at 4 mg/m^3 for 2 hr/day from gestational day 6 – 15. Beginning at about 120 days of age, one male and one female offspring

(16 air, 16 Hg°) were trained to press a lever for food; they then learned a signal detection task for assessments of sustained attention. Two cohorts, each with n=8/sex/group, were trained: cohort 1 was trained with visual signals and cohort 2 with auditory signals. Rats trained with auditory signals acquired the leverpress response faster than did those with visual signals; however, acquisition of this response did not differ as a function of either Hg° exposure or sex. Hg° rats trained with visual signals learned the signal discrimination more quickly and to a higher level of accuracy than did controls; males also learned faster and to higher accuracy than did females. No differences were noted in acquisition of the auditory task due to either Hg° treatment or sex. These results do not indicate a strong need for concern regarding potential cognitive dysfunction resulting from gestational exposure to Hg°. (This abstract does not necessarily reflect EPA policy.)

1712 DIFFERENTIAL EFFECTS OF TRIMETHYLTIN ON TASKS RELATED TO TIME ESTIMATION AND APPETITIVE MOTIVATION IN RATS.

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The effects of the neurotoxic agent trimethyltin (TMT) were investigated in rats using two complex operant tasks designed to model aspects of time estimation and appetitive motivation. Subjects were trained to perform a temporal response differentiation (TRD) task in which they were required to depress and hold a lever for at least ten seconds but not more than fourteen seconds in order to receive a food reinforcer. To assess aspects of motivation in the same session, subjects performed a progressive ratio (PR) task where the number of required lever presses increased for each food reinforcer. Administration of a single dose of TMT chloride (8 mg/kg, i.p.) significantly decreased the number of reinforcers obtained (accuracy) in the TRD task by the sixth day after challenge without affecting the overall rate of responding in that task. The time course of the decrease in TRD accuracy was generally not correlated with a decrease in any parameter of the PR task. In addition, the decrease in TRD accuracy that occurred on the sixth day after TMT failed to recover in the six weeks after toxicant challenge. These results suggest that TMT may selectively affect timing behavior in rats at doses that do not similarly affect performance of operant tasks related to appetitive motivation. In addition, comparison with published neuropathology data suggests that the effect of TMT on TRD performance occurs prior to the time that neuronal cell death is maximal.

1713 METALS AND DISORDERS OF CELL ACCUMULATION: MODULATION OF APOPTOSIS AND CELL PROLIFERATION.

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As a class of agents, toxic metals are one of the highest priority concerns for human exposure. The carcinogenic potential of many metals or metalloids, like chromium and arsenic, is a major issue. Other metals, like lead and mercury, are potent neurotoxins and potential immunotoxins. Metals often have essential functions within the cell, including regulation of important events in gene regulation, cell proliferation and cell death by apoptosis. Apoptotic cell death, which is an ongoing normal event in the control of cell populations, can be induced by a variety of toxicants, including many toxic metals, resulting in the loss of the affected cells. What has often received less attention is the concept that apoptosis can be impaired by environmental agents, such as metals, and that hindered apoptosis can facilitate aberrant cell accumulation, which may be a critical step in the pathogenesis of malignancy or autoimmunity. Thus for many toxic metals disorders of cell accumulation may be a crucial event in their toxicity. For instance with lead there is evidence in model systems that apoptosis is a key factor in neurotoxicity. With arsenic cell cycle dysregulation, and altered DNA repair and apoptosis may be important early events in transformation. Similarly the carcinogenic potential of chromium may be based in aberrant apoptosis while disruption of normal apoptosis appears to be a key factor in mercury-induced autoimmunity. Understanding the mechanisms by which metals induce disorders of cell accumulation will be important in defining their toxic potentials in exposed populations.

1714 MITOCHONDRIA COORDINATE THE UPSTREAM AND DOWNSTREAM EVENTS IN LEAD-INDUCED RETINAL APOPTOSIS.

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Selective retinal cell death occurs in man and animals following hypoxia-ischemia, excitotoxicity and lead exposure. This is associated with Ca2+ overload and its subsequent effects. To examine the mechanisms of lead-induced selective rod and bipolar cell apoptosis, we used in vivo (developmental) and ex vivo (isolated whole retina) lead exposure models. With both lead protocols, retinal: cGMP phosphodiesterase (PDE) - localized to only rod and bipolar cells - is decreased resulting in elevated [Ca] and [Pb]; respiration and mitochondrial ATP synthesis are decreased; mitochondrial cytochrome c is released; CPP-32 like caspase activity is increased; and high molecular weight (HMW) DNA fragmentation occurs. Chromatin condensation is seen in rods and bipolar cells during and following developmental lead exposure but only in rods following ex vivo lead exposure. The decrease in mitochondrial ATP synthesis, release of cytochrome c, activation of caspase and rod cell apoptosis (HMW fragmentation and chromatin condensation) are blocked by BAPTA, ruthenium red and/or cyclosporin A. However, only the activation of caspase and rod cell apoptosis are blocked by DEVD-FMK. These results suggest that lead-induced apoptosis in rod and bipolar cells: i) is triggered by Ca2+ and Pb2+ overload due to decreased cGMP PDE activity in these two cell types, ii) results from direct mitochondrial alterations that are upstream of DEVD-sensitive caspases and iii) is ultimately produced by the activation of downstream CPP32-like caspases. Thus, mitochondrial alterations appear to play a central and coordinating role during the effector phase of lead-induced rod and bipolar cell apoptosis. (Supported by NIH Grant ES03183.)

1715 ARSENIC-INDUCED DYSREGULATION OF CELL CYCLE AND DNA REPAIR GENE EXPRESSION.

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Arsenic is a common contaminant of drinking water worldwide. Chronic ingestion of trivalent arsenic (arsenite) causes skin, liver and bladder cancer in humans but not in rodents. Arsenite disrupts microtubules and causes an M-phase delay and aneuploidy in normal, diploid human fibroblasts. In contrast, we have observed that 5 - 10 μ M arsenite induces anaphase arrest in SV40-transformed fibroblasts. A majority of anaphase arrested SV40-transformed fibroblasts die via an apoptotic process. Flow cytometry and single cell gel electrophoresis (Comet assay) confirmed the arsenic dose dependent accumulation of cells in G_2/M, loss of DNA, and DNA fragmentation. Spontaneously immortalized human breast epithelial cells (MCF10A) and keratinocytes (HaCaT), and human premyelocytic leukemia cells (U937, HL60) were comparable to SV40-transformed fibroblasts in their sensitivity to arsenite induced cell killing. The kinetics of arsenite killing of asynchronous cultures of immortalized cells directly correlated with population doubling times, consistent with the time needed to reach mitosis. No toxicity was observed in non-dividing confluent diploid fibroblasts (G_0/G_1 arrested) treated with 100 (μ M arsenite. Levels of the DNA damage recognition protein XPA decreased in an arsenite dose dependent manner. This result is consistent with reports of the inhibition of DNA repair by arsenite. These results suggest that arsenite toxicity is manifested by an apoptotic process dependent on cells being in mitosis. Furthermore, loss of cell cycle regulation as in immortalized cells in culture predisposes cells to arsenite induced apoptosis after anaphase arrest. (Supported by ES06639, RR12242, ES06460, WSU OVPPR.)

1716 MECHANISMS AND MODULATION OF CHROMIUM-INDUCED APOPTOSIS.

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Certain chromium compounds are occupational respiratory carcinogens and are of growing environmental concern. These generally highly insoluble particulate forms of hexavalent chromium also cause respiratory mucosal irritation, ulceration and perforation. They cause tumor formation in animals and are genotoxic, mutagenic and transformagenic to cultured mammalian cells, but only at doses that evoke some cell death. We have utilized cellular, molecular, pharmacological and genetic approaches to study chromium tox-

icity and carcinogenesis using normal human respiratory fibroblasts and epithelial cells in culture, as a toxicologically relevant experimental system. We have studied the interaction of particulate chromium compounds with these cells with respect to particle internalization and micro environmental dissolution, using Cr-DNA adducts as an intracellular dosimeter of bioavailability. Structural DNA damage, especially DNA-Cr-DNA crosslinks, has been mechanistically related to functional damage in the form of replication arrest and altered transcription. Depending on the rate, magnitude and spectrum of genotoxicity and mitochondrial damage, and the nature of the affected signaling pathways, cells are fated to either terminal growth arrest or p53-dependent apoptosis. Cells which manage to resist or escape these two fates are predisposed to mutagenesis and neoplastic transformation. Agents which block end-stage apoptosis do not increase survival of replication-competent cells but agents which prevent the pre-apoptotic release of cytochrome c from the mitochondria, increase the survival of genetically damaged cells. Our goal is understanding the nexus between chromium genotoxicity, apoptosis and neoplasia. (Supported by NIEHS Grant ES05304.)

1717 MECHANISMS CONTRIBUTING TO SYSTEMIC AUTOIMMUNE DISEASE: MERCURY-INDUCED TYROSINE PHOSPHORYLATION AND DISRUPTION OF THE CD95/Fas APOPTOTIC DEATH PATHWAY.

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The CD95 apoptotic death pathway is a major regulatory mechanism for controlling the life span of activated lymphocytes. Defects in this pathway have been linked to autoimmunity. Inorganic mercury (Hg2+) induces a systemic autoimmune disease in genetically susceptible strains of rodents characterized by abnormal lymphoproliferation and accumulation of autoreactive lymphocytes. Additionally, epidemiological studies support a linkage between mercury exposure and autoimmunity. Inasmuch as many autoimmune diseases also are associated with abnormal lymphoproliferation and accumulation of autoreactive lymphocytes, we have studied the influences of Hg2+ on signaling pathways controlling cell growth and cell death. This talk will present evidence supporting the hypothesis that Hg2+modulates CD95 death pathway. Our experimental design employs human cell culture models to study the effects of Hg2+ on components of the CD95 death pathway and the epistasis of the effects of Hg2+ on this pathway. Non-toxic concentrations of Hg2+ significantly attenuate the concentration- and time-dependent response to anti-CD95 agonist. However, other apoptosis inducing stimuli such as ceramide and TNF are not attenuated by mercury, suggesting that the signaling component(s) targeted by Hg2+ is unique to the CD95 portion of the apoptotic death pathway. Inhibition of CD95-mediated apoptosis by Hg2+ was confirmed using several criteria including DNA fragmentation, nuclear condensation, phosphatidylserine externalization, and PARP degradation. Induction of caspase-3 autoproteolysis also is attenuated by Hg2+. Mercury does not interfere with the anti-CD95 agonist binding, nor does it down regulate the cell surface receptor density of CD95. Collectively, these data suggest that Hg2+, either directly or indirectly, interferes with CD95-mediated apoptosis by interacting with a signaling component downstream of the formation of the membrane proximal death inducing signaling complex (DISC) and upstream of caspase-3 activation. Additional studies are underway aimed at linking Hg-mediated activation of protein tyrosine kinases and the CD95 death pathway. The plausibility of such altered signaling mechanisms in the etiology of Hg-induced systemic autoimmune disease and the integration of these findings with the published literature concerning Hg-induced immunopathogenesis will be discussed.

1718 XENOBIOTIC EFFECTS ON CELL ADHESION MOLECULES AND EXTRACELLULAR MATRIX INTERACTIONS.

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The extracellular matrix is composed of a variety of versatile proteins and polysaccharides that are secreted locally and assembled into an organized meshwork. The extracellular matrix plays an active and complex role in regulating the behavior of cells that contact it. Cell migration, gene expression, proliferation, shape, function, and cell death are all influenced by interaction with the extracellular matrix. Cells not only secrete extracellular matrix glycoproteins, but also bind to these molecules through specific cell surface receptors. Cell surface adhesion receptors that will be highlighted in the symposium include members of the integrin and cadherin superfamilies. CYP1A1 regulation in a variety of epithelial cell types is influenced by cadherin family members. Oxidative stress and polycyclic aromatic hydrocarbons disrupt cadherin/catenin complexes leading to decreased adhesion in hepatic and uterine models, respectively. Environmental contaminants such as asbestos impact on recruitment and adherence of neutrophils and macrophages in the lung. Remodeling of the extracellular matrix occurs during normal physiological processes such as embryonic development and wound healing in the adult. However, direct activation of tyrosine kinases and the matrix remodeling enzyme, urokinase, by asbestos may contribute to fiber-induced pathology in the lung. The effects of xenobiotics on extracellular matrix organization, signaling, and adhesion receptors will be discussed in this symposium designed to highlight this emerging area in toxicology. Each speaker will provide a unique perspective on xenobiotic perturbation of cell-cell and cell-matrix interactions.

1719 EXPRESSION OF ADHESION MOLECULSES AND PROTEASE ACTIVITY IN ENDOTHELIAL CELLS AND AIRWAY EPITHELIAL CELLS EXPOSED TO ASBESTOS.

A Barchowsky, R Roussel, and M Treadwell. *Department of Pharmacology and Toxicology, Dartmouth Medical School, Hanover, NH, USA.* Sponsor: K T Shiverick.

Inhalation of asbestos and certain particles causes recruitment of neutrophils and macrophages into the lung. Chronic activation of phagocyte extravasatio plays a central role in self-perpetuation and amplification of airway inflammation and fibrosis. The cellular and molecular events that lead to this activation are unclear, but appear to involve both direct effects of fibers on airway cells and indirect effects caused by altered cytokine balance in the lung. Cell culture experiments have demonstrated that asbestos directly activates endothelial cell tyrosine kinase activity, nuclear translocation oftranscription factors, and ICAM-1-dependent adherence of neutrophils. In addition these fibers caused the endothelial cells to undergo morphological changes that may result from increased expression of urokinase and its receptor, uPAR. These effects are not limited to the endothelial cells, since chrysotile asbestos also increased NF-kB activation and urokinase expression in human bronchial epithelial cells and in intact lungs. The relative contributions of fiber-induced effects on the different cell types in the lungs that contribute to cell invasion, inflammation, and fibrosis are unresolved. (Supported by HL 52738.)

1720 OXIDATIVE STRESS DISRUPTS THE E-CAD HERIN/CATENIN CELL ADHESION COMPLEX.

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E-cadherin, the predominate mediator of cell-cell adhesion in epithelium, regulates adhesion via a Ca++-dependent, homophilic mechanism. β -catenin binds the cytoplasmic domain of E-cadherin and is in turn bound by α -catenin, which links the complex to the actin cytoskeleton. Given that E-cadherin is a morphogenic regulator and an invasion/motility suppressor, efforts have focused on the mechanism(s) which cause a loss of function. Interestingly, src family tyrosine kinases are activated by oxidative stress and stable over-expression of src is associated with enhanced phosphorylation of b-catenin and decreased adhesion. This lead to the hypothesis that chemically-induced oxidative stress disrupts the E-cadherin/catenin complex. Based on the maintenance of normal cell-cell interactions, precision-cut mouse liver slices were chosen as the in vitro system. Diamide (DA), a sulfhydryl depleting agent, and tert-butylhydroperoxide (tBHP), an initiator of radical formation, were chosen as model compounds. Initial incubations (6 hr) identified concentrations of DA (25-250 uM) and tBHP (5-50 uM) that were not associated with a significant alteration in protein expression of E-cadherin, b- or a-catenin. Oxidative stress was associated with activation of c-src (DA 200 & 250%; tBHP 340 & 400%) which correlated with tyrosine phosphorylation of b-catenin (DA 100 & 110%; tBHP 200 & 350%). This modification was associated with disruption of the protein interactions of adhesion complex, most notably a decrease in b-catenin:a-catenin interactions (DA 110 & 95%; tBHP 50 & 20%). In addition, oxidative stress induced a significant shift in the detergent solubility of b-catenin, another indication of functional disruption. Taken together, these results suggest that chemically-induced oxidative stress disrupts the E-cadherin/catenin complex and lend further support to the hypothesis that cell-cell and cell-matrix interactions are a molecular target of xenobiotics. (Supported by NIEHS Center

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1721 BENZO(A)PYRENE-MEDIATED INHIBITION OF UTERO-PLACENTAL CELL INVASION AND EXPRESSION OF CELL ADHESION MOLECULES.

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The polyaromatic compounds benzo(a)pyrene (BaP) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) have been implicated in uterine disease and infertility. Our studies with human uterine endometrial and trophoblastic cell lines have found that BaP, but not TCDD, significantly inhibited cell proliferation and invasion of matrigel-coated membranes. Because cell invasion is a multifaceted process, the effects of BaP and TCDD were further examined on the processes of cell attachment to extracellular matrix vs expression of proteases necessary for matrix degradation. Human uterine endometrial adenocarcinoma cells pretreated with BaP showed minimal attachment to matrigel, whereas TCDD-exposed cells showed a high level of attachment comparable to control. No effect of either compound was observed on the fibrinolytic activity of the serine protease urokinase plasminogen activator, nor on matrix metalloprotease MMP-2 mRNA expression. Immunohistochemical analysis demonstrated that epidermal growth factor receptor (EGF-R) was detected primarily in plasma membranes in intercellular regions of adjacent cells. BaP, but not TCDD, resulted in markedly reduced staining intensity in the intercellular membrane regions without alteration in cytoplasmic staining intensity. Expression of E-cadherin protein, the predominant mediator of cell-cell adhesion in epithelial cells, was also decreased following BaP, but not TCDD, exposure. These data provide evidence that the differential effect of polycyclic compounds on EGF-R expression is a biomarker for altered uterine cell adhesion to the extracellular matrix and cell-cell interaction which may well involve modulation of the interaction of cadherin with the actin cytoskeleton. Evidence suggests that the the BaP-mediated dysregulation of cell adhesion may involve cellular stress relating to the formation of reactive metabolites. (Supported by NIEHS Superfund Grant 07375.)

1722 CELL ADHESION AND CYP1A1 EXPRESSION: WHAT'S THE CONNECTION?

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Previous studies in our laboratory have shown that disruption of cell-cell and cell-substratum interactions in epithelial cells, but not fibroblasts, leads to a rapid induction of steady state CYP1A1 mRNA levels. In an effort to identify which adhesion receptors are involved in this process, we have employed methods which preferentially disrupt either cadherin mediated cell-cell interactions or integrin mediated cell-substratum interactions in epithelial cells. Normal human keratinocytes removed from substratum attachment as an epithelial sheet in which cell-cell contact is maintained do not show an increase in steady state CYP1A1 mRNA following a 6h treatment. In addition, reporter gene assays of Hepa 1c1c7 cells stably transfected with a luciferase gene downstream from part of the CYP1A1 promoter demonstrate that treatment of these cells with RGD-containing peptides to disrupt integrin mediated interactions does not lead to an increase in luciferase activity. Taken together, these results indicate that disruption of cell-substratum interactions may not be involved in the adhesion mediated induction of CYP1A1. To examine the role of cadherin-mediated interactions in this induction, we have disrupted cell-cell adhesion by decreasing the calcium concentration of the media to a level that is below the minimum required for cadherin function. Steady state levels of CYP1A1 mRNA increase in normal human keratinocytes following a shift from 0.66 mM Ca2+ media to 0.05 mM Ca2+ media. Concomitant with this induction, indirect imuno-fluorescent staining of the cells following a decrease in extra-cellular calcium shows that E cadherin is lost from the cell surface. These results imply that calcium-dependent cell-cell interactions may be involved in the induction of CYP1A1 following a loss of adhesion.

1723 CYTOKINES: BIOLOGY, GENE REGULATION, AND ROLE IN THE PATHOGENESIS OF LUNG DISEASE.

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Altered cytokine expression can contribute to changes in lung structure and function associated with inhalation of pneumotoxic chemical and biological agents. This symposium focuses on the role of cytokines in acute lung injury and the pathogenesis of lung diseases including: chronic airways disease; allergic asthma; and interstitial lung disease. Cytokines such as TNFα and interleukin (IL)-1 can initiate responses to inhaled agents, stimulationg a cascade of cytokine and cell:cell interactions which result in recruitment and activation of inflammatory and immunocompetent cells. Chemokines (e.g., IL-8, monocyte chemotactic protein 1) are TNFα and IL-1-inducible cytokines which play critical roles in recruitment of cells to sites of tissue injury. Recent studies suggest chemokines can influence expression of other cytokines (e.g., IL-4, IL-12) which drive a transition from Th1 to Th2 lymphocyte responses and contribute to chronicity of inflammation. In this respect, Th2 cells and their associated cytokines (e.g., IL-4, IL-5, IL-13) appear to play a role in the pathogenesis of asthma. IL-4 and IL-13 being critical in the regulation of IgE synthesis and IL-5 contributing to the recruitment and activation of eosinophils. Dysregulation of cytokine expression can be a key factor in remodeling of respiratory tract tissues after exposure to pneumotoxic agents. For example, altered expression of platelet-derived growth factor (PDGF) A and B, mitogens for mesenchymal cells, and transforming growth factor β 1 (TGFβ 1), which regulates expression of matrix proteins, plays a critical role in the pathogenesis of asbestosis and likely other interstitial lung diseases. Similarly, studies in transgenic models suggest overexpression of the IL-6 type cytokine, IL-11, can contribute to airway remodeling such as that seen in asthmatic lungs. Expression of genes coding for several inflammatory and immunoregulatory cytokines appears to be regulated, in part, by redox sensitive transcription factors such an NFkB and AP-1. Oxidant induction of cytokine gene transcription may represent a common mechanism whereby several environmental agents increase cytokine expression in the respiratory tract. Symposium presentations will discuss research at the molecular, cellular and whole animal levels relevant to the above concepts on cytokine function in normal health and disease.

1724 THE DYNAMIC INTERACTIONS BETWEEN CYTOKINES, CHEMOKINES, AND ADHESION MOLECULES DICTATES THE EVOLUTION OF CHRONIC DISEASE.

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Our current understanding of chronic inflammation would suggest that the recruitment of mononuclear cells from the lumen of a vessel into a localized area of inflammation is dependent upon the initiation and maintenance of leukocyte activation and chemotaxis. These responses are like dependent upon a cascade of events which must occur with some fidelity in order for the cells to successfully arrive at a site of inflammation. Initial phases of the activation/chemotactic responses are dependent upon a number of dynamic interactions, resulting in the expression of a variety of important inflammatory mediators. These mediators are involved in the transition from adherence to migration which allows the activated leukocytes to accumulate and pass through the endothelial cell and basement membrane barrier. Studies have now shown that chemokines are not only important in recruiting cells into an area of chronic inflammation, but they also appear to aid in dictating the evolution of particular types of chronic inflammation. For example, the monocyte chemoattractant protein-1 (MCP-1), which has been shown to be a potent chemotaxin for monocytes and lymphocytes, can also increase the expression of lymphocyte-derived interleukin-4 (IL-4) in response to antigen challenge and decrease the levels of macrophage-derived interleukin-12 (IL-12). This activity may facilitate the evolution, or switch, of a Th-2 response from a Th-1 response. In the context of chronic inflammation, the particular cytokine/chemokine phenotype that persists at an inflammatory site may play an important role in the development of long-term disease. This may be especially true in chronic inflammation where the continued cytokine expression pattern may predominate with characteristics of a Th-2 phenotype. Recent data supports the notion that this cytokine expression profile may be consistent with continued recruitment and activation of certain leukocyte subpopulations, fibroblast proliferation, and matrix deposition.

1725 THE ROLE OF TH2 CYTOKINES IN THE PATHOGENESIS OF ALLERGIC ASTHMA.

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Marked increases in the incidence, morbidity and mortality of allergic asthma have been reported over the last two decades, underscoring the need for a better understanding of the etiology of this disease. As a primary regulator of the inflammatory cascade, CD4+ T-lymphocytes have been implicated in the pathogenesis of this disease. Functional subsets of CD4+ T cells have been distinguished at both clonal and population levels by the unique profiles of cytokines that they produce. The differential presence of these cytokine phenotypes in a variety of allergic and infectious diseases of both mice and humans has provided both descriptive power and theoretical insight into disease pathogenesis. Th1 cells produce interleukin-2, TNF-β , and interferon gamma (IFN-γ), and are critical in the development of cell-mediated immunity. Th2 cells on the other hand produce IL-4, IL-5, IL-13, IL-6, and IL-10 and are important in humoral immune responses. A possible immunopathogenic role for the Th2 cells in asthma has been based on the role that these cytokines play in IgE synthesis and eosinophil regulation. In particular, IL-4 may be of critical importance in allergic inflammatory processes, as it is required for the differentiation an expansion of Th2 lymphocytes and inhibition of the development of Th1 lymphocytes, while both IL-4 and IL-13 regulate IgE syntheses. IL-5 is considered the primary regulator of eosinophil function. Experimental support for this hypothesis is provided by the fact that unique cytokine patterns (i.e. Th2) are expressed in T cells from asthmatic patients. Although considerable inferential evidence supports a role for these cytokines in allergic reactions, the exact mechanisms by which they induce the inflammatory and functional changes associated with allergic asthma are currently debated. Here we will discuss the data derived from both human and experimental animal studies (transgenic and knock-out) which have contributed to our current understanding of the mechanisms leading to the aberrant production of Th2 cytokines in allergic individuals and the mechanisms by which these cytokines induce allergic diseases.

1726 IL-6-TYPE CYTOKINES IN AIRWAYS DISEASE.

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The IL-6-type cytokine family includes IL-6, IL-11, LIF, oncostatin M and cardiotropin-1. IL-11 was initially described as an IL-6-like bioactivity and shown to regulate thrombopoiesis and hematologic recovery. Studies from our laboratory have demonstrated that: (1) IL-11 is produced by a variety of lung epithelial cells, fibroblasts and smooth muscle cells; (2) a variety of stimuli induce IL-11 elaboration by lung stromal cells including TGF-b1, respiratory syncytial virus, rhinovirus and parainfluenza virus type III and (3) TGF-b1 stimulation of stromal cell IL-11 elaboration is mediated, at least partly, via an AP-1-dependent transcriptional mechanism. To begin to address the effector functions of IL-11 in the lung, IL-11 was targeted to the murine lung using the Clara cell 10 kDa (CC10) promoter. These studies demonstrated that the prolonged overexpression of IL-11 caused: (a) enlarged alveoli; (b) peribronchiolar nodules composed of B cells, T cells and rare macrophages; (c) airway remodeling with subepithelial fibrosis and myocyte and myofibroblast hyperplasia. To determine which features of the CC10-IL-11 overexpression mice were development-dependent versus development-independent, an inducible lung-specific airway targeting system using the tetracycline transactivator was produced. When employed with IL-11, the following phenotypes were obtained: (a) short period of IL-11 expression during adulthood Ð no phenotype; (b) expression in utero-14 days of age Ð enlarged alveoli only; (c) expression in utero through 3 months of age Ð phenotype identical to CC10-IL-11 animals. These studies demonstrate that IL-11 is a stromal cell-derived cytokine which can cause development-dependent alveolar enlargement and development-independent peribronchiolar nodules and airway remodeling.

1727 EXPRESSION OF PDGF AND TFGβ1 AT SITES OF LUNG INJURY.

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Inhaled inorganic particles such as silica and asbestos damage the alveolar epithelium leading to the development of interstitial scarring. There is increasing evidence that certain peptide growth factors play a role in the fibroproliferative response that is the hallmark of the fibrogenic process. Thus, we have been studying expression of growth factors that could mediate disease development. Here, we present recent findings on two factors in rats and mice: platelet-derived growth factor (PDGF), A and B isoforms, and transforming growth factor beta1 (TGFβ1). PDGF is the most potent mecenchymal cell mitogen and TGFβ is a powerful inducer of extracellular matrix components. We have shown that during the five hrs. of a single exposure to chrysotile asbestos, both PDGF A and B as well as TFGβ 1 genes and cognate proteins were upregulated at sites of alveolar injury. In situ hybridization (ISH) and immunohistochemistry (IHC) showed that alveolar epithelial cells, interstitial fibroblasts and lung macrophages expressed the protein for at least two weeks post-exposure. Most interesting was our finding that mice with the receptors "knocked out" for tumor necrosis factor alpha (TNFα) were protected from the fibrogenic effects of inhaled asbestos. Since both PDGF and TGFβ1 expression was reduced in these mice, we postulate that TNFα acts as a key factor in controlling production of other molecules that mediate the fibroproliferative response to lung injury. (Supported by NIH Grants #ES06766 and HL60532.)

1728 CHEMICAL-INDUCED ACTIVATION OF NUCLEAR TRANSCRIPTION FACTORS AND THEIR REGULATION OF CYTOKINE SECRETION.

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Non-immune cells from a variety of organs, including the liver, lung and skin, when targeted by toxic agents, secrete pro-inflammatory cytokines and chemokines which participate in pathological as well as repair processes. In particular, tumor necrosis factor alpha (TNFα) and certain members of the neutrophil chemoattractant family of chemokines (CXC), including interleukin-8, play major roles in these responses. This presentation will describe several organ model systems for toxicity including asbestor-induced lung disease, arsenic-induced skin toxicity and heavy metal-medicated hepatotoxicity which demonstrate cytokine participation. In addition to describing the nature of these responses, the molecular events responsible will be detailed. A common pathway responsible for cytokine induction in all of these systems involves chemical-medicated, cellular oxidative stress, which, in turn, activates nuclear transcription factors, such as NF-kB, NF-IL-6 and AP-1. Unraveling the complex mechanism associated with these events may provide novel opportunities for intervention.

1729 CHARACTERIZATION OF THE METABOLIC CAPACITY OF SYRIAN HAMSTER EMBRYO (SHE) CELLS.

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Syrian hamster embryo (SHE) cells are used in the SHE cell transformation assay to predict the carcinogenic potential of chemicals. Unlike other in vitro genetic toxicity assays, the SHE assay efficiently detects carcinogens that act via non-genotoxic mechanisms. In addition, procarcinogens that require metabolic activation to their carcinogenic form are detected by the assay even in the absence of an exogenous metabolic activation system, suggesting that SHE cells possess the ability to metabolize xenobiotics. However, while many of the molecular features of SHE cells have been well characterized, very little is known about the metabolic capacity of these cells. Since cytochromes P450 are the enzyme family primarily responsible for xenobiotic metabolism and procarcinogen activation, the goal of the present work was to characterize cytochrome P450 protein expression in SHE cells. SHE cells derived from several different Syrian hamster embryo isolates were cultured, harvested, sonicated, and microsomes isolated by differential centrifugation. Western blotting with enhanced chemiluminescent detection was used to evaluate SHE cell microsomes for the presence of a battery of cytochrome P450 (CYP) isozymes. The most abundant cytochrome P450

isozyme detected constitutively was CYP4A. In addition, CYP2E isozyme and cytochrome P450 reductase, a requisite cofactor for cytochrome P450 enzyme activity, were also detected constitutively. The following cytochrome P450 isozymes were not detected constitutively in SHE cells: CYP1A, CYP1B, CYP2A, CYP2B, CYP2C, and CYP3A. In SHE cells exposed to the procarcinogen benzo[a]pyrene at levels resulting in SHE cell transformation (5 mg/ml for 24 hours), CYP1A isozymes which metabolize this compound were induced significantly to detectable levels. Importantly, these results provide direct evidence of constitutive cytochrome P450 protein expression in SHE cells and demonstrate that SHE cells are capable of regulating expression of these proteins in response to xenobiotic exposure.

1730 ROLE OF METABOLISM IN ACRYLONITRILE (ACN) CARCINOGENICITY: STUDIES IN SYRIAN HAMSTER EMBRYO (SHE) CELLS.

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Chronic oral exposure to ACN, a chemical used in the manufacture of plastics, rubber and acrylic fibers, results in brain, stomach and Zymbal gland tumors in rats. While the mode of action of ACN-induced carcinogenicity is unresolved, results from *in vivo* studies showed that ACN produced oxidative stress in the brains of ACN treated rats. Studies examining ACN metabolism have shown that one degradation pathway involves oxidative metabolism by cytochrome P450 to cyanoethylene oxide (CEO), a highly reactive epoxide metabolite. Further metabolism results in the liberation of cyanide (CN). In the present study, the SHE cell morphological transformation (MT) assay, which has been shown to be predictive of *in vivo* carcinogenicity, was utilized to determine whether ACN or ACN metabolites are involved in the transformation process. ACN treatment produced MT in a dose-dependent manner in SHE cells at concentrations of 75 μg/ml and below which was accompanied by increased 8-hydroxydeoxyguonosine (8OHdG) levels, an indicator of oxidative DNA damage. Blockage of CEO formation by 1-aminobenzotriazole, an inhibitor of cytochromes P450, prevented MT by ACN. Treatment of SHE cells with CN resulted in an increase in MT. These results indicate that ACN requires metabolism to produce SHE cell transformation and that CN formation may be contributory to the ACN-induced MT, suggesting a potential role for ACN metabolism in the induction of glial tumors in the rat.

1731 TAMOXIFEN INITIATION AND PHENOBARBITAL PROMOTION STUDIES IN LIVERS OF BIG BLUE™ TRANSGENIC F344 RATS.

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Female F344 Big Blue™ transgenic rats were given tamoxifen (tam) (20mg/kg/day p.o.) or vehicle (tricaprylin) for 6 weeks. After 6 weeks some animals were sacrificed and the remaining animals were then given phenobarbital (PB) (0.1% w/v in water) or were untreated for up to 24 weeks. Groups of 4 animals were sacrificed at 2, 12 and 24 weeks after the commencement of PB treatment and the mutation frequencies (MF) in the livers determined. MF at 2 weeks: control 5.9 ± 0.3; control + PB 6.0 ± 0.3; tam 19.5 ± 2.9; tam + PB 19.8 ± 1.8. MF at 12 weeks: control 7.6 ± 2.4 ; control + PB 10.4 ± 1.2; tam 35.6 ± 4.0; tam + PB 34.8 ± 7.8. MF at 24 weeks: control 6.8 ± 2.7; control + PB 8.3 ± 2.0: tam 30.7 ± 0.7; tam + PB 31.9 ± 1.7. Liver weights in animals given phenobarbital or tamoxifen followed by phenobarbital showed a time-related increase compared with controls or animals given tamoxifen alone. After 2 weeks promotion liver cell proliferation, measured by PCNA expression, was significantly increased in both the phenobarbital-treated groups in comparison with concurrent controls and by 12 and 24 weeks labelling in both the phenobarbital-treated groups and the tamoxifen-initiated group were significantly elevated. compared with the concurrent control. There was a progressive increase in the number of GST-P expressing foci in the livers of rats initiated with tamoxifen with or without subsequent promotion with phenobarbital. The animals given phenobarbital alone showed no increase in foci. The results demonstrate that although phenobarbital is known to promote tamoxifen-initiated liver tumours in the rat it has no influence on the frequency of *lacI* mutations induced by tamoxifen but may select for oncogenic mutations.

1732 PHENOBARBITAL IS MUTAGENIC AND ALTERS THE MUTATION SPECTRUM (MS) OF lacI IN THE LIVER OF BIG BLUE® TRANSGENIC MICE.

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Although phenobarbital (PHE) is a hepatocarcinogen in B6C3F1 mice, it is a weak mutagen in vitro and does not form DNA adducts. Thus, the mechanism by which PHE causes tumors may differ from that of mutagens which form DNA adducts. To investigate whether PHE causes mutations in vivo, B6C3F1 Big Blue® male transgenic mice harboring the lLIZ shuttle vector containing the lacI target gene were fed 2500 ppm PHE for 180 days. The mutant frequency (MF) of lacI in the liver of control mice, fed diet alone, was 5.02 ±2.4x10-5 and that in the PHE-fed mice was 6.88 ± 0.75x10-5, a significant increase ($p<0.05$). The mutation spectrum obtained from a random collection of mutants from each PHE-fed mouse was significantly different ($p<0.02$) from a spontaneously derived spectrum from control mice. In the PHE-fed mice, an increase in G:C>T:A and G:C>C:G transversions and a concomitant decrease in G:C>A:T transitions was obtained. It is postulated that the mutations found in the PHE-derived spectrum were due to oxidative damage following induction of CYP2B isozymes by chronic PHE feeding. Results from this study demonstrate that even though PHE is a weak mutagen in vitro, it is mutagenic in vivo in Big Blue® mice. The PHE-derived lacI spectrum was remarkedly similar ($p<0.8$) to that generated by oxazepam in vivo in Big Blue® mice. Oxazepam has similar pleiotropic properties to PHE, including its induction of CYP isozymes and thus, both these compounds could be tumorigenic through a similar mechanism. (Supported by CA72534-01.)

1733 SILICA-INDUCED MUTATIONS IN THE LUNGS OF BIG BLUE® RATS BUT NOT MICE.

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Crystalline silica is a cytotoxic particle which causes lung inflammation in multiple species; however, tumor formation following inhalation exposure has only been demonstrated in the rat, suggesting possible species differences in sensitivity to silica-induced lung injury or repair. It would be desirable to directly assess species differences in genotoxicity within a single assay system. The Big Blue® *in vivo* mutagenesis system, which allows such an evaluation in both rats and mice, was utilized to examine silica-induced inflammation and fixed mutations. Big Blue® female B6C3F1 mice and F344 rats were exposed to the same dose of silica based on lung mass via instillation (500 μ g for mice; 2.5 mg for rats) and groups were sacrificed 2 weeks and 2 months after exposure. Significant inflammation was found in both species and at both time points as determined by the percentage of neutrophils (PMNs) in lavage fluid. The PMN response in rats, however, was greater than in mice at both 2 weeks and 2 months. There was a significant effect of exposure on mutant frequency of DNA isolated from rats ($F_{1,16} = 5.79, p = 0.029$) but not in mice; however, no significant differences between treatments were found within the time points alone. This data suggests that rats are more sensitive than mice to the inflammatory potential of silica, but that this assay system may not be as sensitive as others (e.g. Hprt mutations) in detecting mutations caused by indirect-acting genotoxic agents such as particles.

1734 USE OF A LACZ PLASMID-BASED TRANSGENIC MOUSE MODEL FOR THE DETECTION OF MUTATIONS INDUCED BY IONIZING RADIATION.

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Transgenic animal models are generally very efficient at detecting small size changes including DNA point mutations, but are relatively insensitive to clastogens, which are thought to cause predominantly large deletions. Recently, a transgenic mouse model, based on the pUR288 plasmid containing the *lacZ* reporter gene, was developed that is sensitive to a wide spectrum of mutations (Nature 377:657-659). The plasmid is purified from the rest of the genomic DNA by a restriction enzyme digestion and immunomagnetic affinity capture. Subsequently, the plasmids are circularized and

electrotransformed into electrocompetent *E.coli C* (*lacZ⁻ galE⁻*) host cells. A small portion of the transformed bacteria is used to determine the titer while the remainder is plated onto selective media containing phenyl-beta-galactoside, allowing only cells containing a mutated *lacZ* gene to form colonies. The spontaneous mutant frequency in spleen and brain DNA from these transgenic mice was approximately 5 x 10^{-5}. X-ray exposures over 3 Gy increased the mutant frequency in brain two to five-fold and spleen three to seven-fold, in a dose-dependent manner. The *lacZ* plasmid mouse model thus enabled comparisons of mutation frequencies between tissues, demonstrating differences in mutant frequencies in spleen and brain. It also detected a wide spectrum of mutations, and was potentially sensitive enough to elucidate dose-response relationships following exposure to ionizing radiation. (Supported by U.S. DOE DE-AC09-756R000 and the American Foundation for Pharmaceutical Education.)

1735 ASSESSMENT OF THE IN VIVO MUTAGENICITY OF ETHYLENE OXIDE IN THE BONE MARROW OF B6C3F1 *lacI* TRANSGENIC MICE FOLLOWING A CHRONIC INHALATION EXPOSURE.

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Ethylene oxide (EO) is a direct acting somatic and germ cell mutagen. To assess the in vivo mutagenicity of EO in the bone marrow of B6C3F1 *lacI* transgenic mice, a chronic inhalation exposure of up to 48 weeks (wk) was conducted using exposure concentrations of 0, 25, 50, 100, or 200 ppm EO (6 h/day 5 days/wk). Animals were sacrificed at 12, 24, and 48 wk of exposure, and the *lacI* mutant frequency was determined in the bone marrow. Exposure to EO did not induce an elevated *lacI* mutant frequency at either the 12 or 24 wk time points. At 48 wk of EO exposure, an increased *lacI* mutant was not observed at either the 25, 50, or 100 ppm exposure levels. However, at 200 ppm EO for 48 wk, a significant increase (5-fold above background) in the *lacI* mutant frequency was observed. Preliminary DNA sequence analysis of *lacI* mutants isolated from the 48-wk 200-ppm exposure group indicated a specific increase in A:TmT:A transversions that was 10-fold above the background frequency of A:TmT:A transversions in age-matched controls. These data indicate that long-term exposure to EO (48 wk) is necessary for the induction of point mutations by EO detectable at *lacI* in the bone marrow of mice.

1736 TRANSGENE EXPRESSION IN LIVER AND KIDNEY OF TG.AC MICE FOLLOWING TISSUE INJURY.

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The dermal Tg.AC transgenic mouse line is currently being used as a short-term alternative *in vivo* model for carcinogenicity screening of drugs and environmental chemicals. These mice carry multiple copies of an activated v-Ha-*ras* oncogene, making them susceptible to promotionally-induced tumorigenesis caused by carcinogen exposure or deep skin wounding. Transgene expression is associated with tumor development in these animals. To evaluate the usefulness of this model in predicting the carcinogenicity of chemicals by non-dermal routes of exposure, we characterized the pattern of transgene expression in naïve animals as well as mice treated by oral gavage with cytotoxic doses of chloroform. Hepatic BrdU labeling was increased 40-fold in females (240 mg/kg/day) and 20-fold in males (140 mg/kg/day) after 4 days of dosing with chloroform. An increase in renal BrdU labeling (7-fold) was observed only in male Tg.AC mice. After 3 weeks and 13 weeks of prolonged exposure to chloroform, there was a gradual reduction in the extent of regenerative proliferation in the liver and kidneys. Although chloroform did not induce v-Ha-*ras* expression either in the liver or the kidney, a constitutive amount of transgene message was evident in the kidneys of Tg.AC mice (n=30). This expression was not dependent on age, zygosity or the colony from which the mice were bred. V-Ha-*ras* transgene expression also correlated with the expression of GATA-3, a transcription factor that binds the zeta-globin promoter of the Tg.AC transgene. These studies suggest that tissue injury and regenerative cell proliferation *per se* are not sufficient for the induction of transgene expression in the liver and kidney of Tg.AC mice.

1737 DERMAL CARCINOGENICITY STUDIES IN HOMO- AND HEMIZYGOUS TRANSGENIC Tg.AC MICE WITH TPA: COMPARISON OF TRANSGENE EXPRESSION, GENOTYPE, AND PHENOTYPE.

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The Tg.AC transgenic mouse is gaining support as a short-term alternative *in vivo* model for carcinogenicity screening of chemicals. These mice carry multiple copies of an activated v-Ha-ras oncogene making them susceptible to promotionally-induced skin tumorigenesis. It has been suggested that the responsiveness of these mice to papillomagenesis was dependent upon the zygosity of the transgene as well as other genetic rearrangements in the transgene. The objectives of the present investigation were (1) characterize the tumor responsiveness of homo- and hemizygous female Tg.AC mice (15 animals/group) to dermal treatment of TPA, a phorbol ester and tumor promoter (2.5 μ g/mouse, 3 days/week, 26 weeks), (2) correlate the tumor responsiveness with the genotype by presence of 2 kb band (Thompson *et al.*, Toxicol Pathol Vol.4, 1998), (3) examine transgene expression by RT-PCR, in various tissues of homo- and hemizygous mice, and (4) compare the pattern of transgene expression in individual animals responding to TPA treatments with that of the non-responders. No differences were observed either in the onset or magnitude of papilloma development between homo- and hemizygous mice following treatment with TPA; approximately a 50% response rate was observed for both genotypes. All mice developing papillomas were confirmed to have the responder genotype by Southern analysis. Expression of v-Ha-ras transgene was observed in several tissues of the Tg.AC mice. These tissues included kidney, spinal cord and hindbrain with the highest expression observed in the spinal cord for both responder and non-responder phenotypes. These data suggest that zygosity does not influence responsiveness to treatment with a tumor promoting agent in the Tg.AC mouse model. Furthermore, expression of the transgene is not altered in the non-responder phenotype despite the absence of the 2 kb band. Further characterization of the Tg.AC model currently underway in several laboratories will help establish it's future role in carcinogen identification.

1738 TRANSPONDER-INDUCED SARCOMA IN THE HETEROZYGOUS $p53^{+/-}$ MOUSE.

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Heterozygous $p53^{+/-}$ transgenic mice are currently being studied as a potential short-term alternative model to the 2-year rodent carcinogenicity bioassay. During a 26-week study undertaken to assess the carcinogenicity of oxymetholone and *p*-cresidine in this mouse strain, masses were observed arising from the transponder site. Male and female $p53^{+/-}$ mice were individually housed and uniquely identified with subcutaneously implanted IMI™ radio signal transponders (BioMedic Data Systems, Inc., Seaford, DE). Upon study termination, all animals were subjected to a complete necropsy and histopathological analysis. Eighteen of the 177 animals on the study (10%) were microscopically diagnosed with sarcomas arising at the transponder site. Of these 18 animals, a total of 5 died prior to study termination between the 18th and 25th week of the study. Palpable masses were late developing but rapidly increased in size. The first detectable transponder site mass was identified in the 15th week and measured 12 mm x 8 mm x 6 mm. By the 25th week, this mass had attained a size of 55 mm x 39 mm x 35 mm. Transponder-associated neoplasms were characterized by undifferentiated sarcomas present at transponder sites in a number of animals in all study groups including controls. In addition, mesenchymal dysplasia was present in these study groups and was considered to be a prodromal change to the sarcomas. Light and scanning electron microscopic evaluation suggested that tumors arose from the mesenchymal tissue capsule surrounding the polypropylene component of the transponder. The mesenchymal changes appeared to begin at the barbed anchor, glass-polypropylene interface of the transponder and then later extended to completely surround the entire transponder in some instances. Capsule membrane endothelialization, inflammation, mesenchymal basophilia, dysplasia and sarcoma were all considered unequivocal responses to the transponder and were not obviously related to treatment with either oxymetholone or *p*-cresidine test substances.

1739 USE OF LASER CAPTURE MICRODISSECTION (LCM) TO DETECT HETEROGENEOUSLY DISTRIBUTED *p53* MUTATIONS WITHIN AFLATOXIN B_1 (AFB_1)-INDUCED MOUSE LUNG TUMORS.

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Inactivation of the *p53* tumor suppressor gene is one of the most frequent genetic alterations observed in human lung cancers. However, *p53* mutations are more rarely detected in chemically-induced mouse lung tumors. Seventy-one lung tumors from AFB_1-treated AC3F1 mice were examined for over-expression of p53 protein by immunohistochemical staining (IHS). Positive nuclear p53 staining was observed in 79% of the AFB_1-induced tumors, but the pattern was highly heterogeneous. Single strand conformation polymorphism (SSCP) analysis of the evolutionarily conserved regions of the *p53* gene (exons 5-8) from AFB_1-induced whole lung tumor DNA samples revealed banding patterns consistent with point mutations in 20 of 76 (26%) samples, with 85% of the mutations in exon 7 and 15% in exon 6. Direct sequence analysis identified apparent point mutations; however, bands representing the putative mutations appeared only weakly on autoradiograms. LCM of different regions of paraffin embedded AFB_1-induced tumor tissue sections, and subsequent SSCP analysis of exons 5-8, revealed banding patterns consistent with point mutations in 15 of 30 (50%) DNA samples. Direct sequencing of microdissected samples revealed clearly identifiable mutations at several different codons in exons 5-8. The results indicate that molecular analysis of laser capture microdissected lung tumor DNA can reveal the presence of *p53* mutations that are not detected by analysis of whole lung tumor DNA. (Supported by Medical Research Council of Canada Grant No. MT-10382.)

1740 RESPONSE OF THE TRANSGENIC P53+/- MOUSE 26-WEEK CARCINOGENICITY ASSAY TO CHLOROFORM.

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The transgenic $p53^{+/-}$ mouse model has been proposed as an alternative to the conventional 2-year bioassay for the detection of genotoxic carcinogens. However, the response of this model to well documented, MTD-induced epigenetic events (e.g., cytotoxicity and cell proliferation) has not been fully documented. In this study, the known non-genotoxic carcinogen chloroform, was given to $p53^{+/-}$ males at 0, 28, 90, or 140 mg/kg/day and females at 0, 24, 90, or 240 mg/kg/day via gavage in corn oil for 5 days/week for 13 (5/sex/dose) or 26 (15/sex/dose) weeks. $p53^{+/+}$ wild-type mice were also given 0, 140 (male) or 240 (female) mg chloroform/kg/day as above, to compare their sensitivity with that of $p53^{+/-}$mice. Standard toxicologic parameters were measured at 13 and 26 weeks; additionally, cell proliferation in the liver and kidneys was determined at 13 weeks using BrdU immunohistochemical methods. Administration of cytotoxic/MTD doses was confirmed by the observations of increased relative liver weights (30-40% at 26 weeks), degenerative hepatic lesions, and hepatocellular proliferation (10-fold in females at 13 weeks) at the high dose. Males also had decreased body weights (8%), renal tubular degeneration, and proliferation of renal tubular epithelial cells (approx. 2-fold at 13 weeks). There were no treatment-related increases in tumors of any tissue either in the transgenic or wild-type mice. Under the conditions of the study, cytotoxic/proliferative tissue injury *per se* was not sufficient to induce tumors in the $p53^{+/-}$ model following 26 weeks of dosing.

1741 CHARACTERIZATION OF THE *p53*-HETEROZYGOUS MOUSE: SPONTANEOUS PROLIFERATIVE LESIONS, ORGAN WEIGHTS, BODY WEIGHTS.

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The aim of this cross-sectional study was to assess lead exposure in children aged 6-9 years attending three primary schools. One of the schools is located ~500 m distant from a smelter complex which includes a lead refinery (high exposure group); the second is located ~ 900 m away from the complex and at the side of a heavy traffic road (medium exposure) in Torreón, Coahuila. The third school is located in Gómez Palacio, Durango, 5,000 m away from the smelter complex and distant from heavy vehicular traffic or industrial areas (low exposure group). Lead was measured in air, soil, dust and well water. Lead in blood (PbB) was determined in 394 children attending the above mentioned schools. Determinations were performed by atomic absorption spectrophotometry. Diet, socioeconomic status, hygienic habits and other variables were assessed by questionnaire. Average PbB (± SD) values were 8.7 ± 3.8 μ g/dL in the low exposure group, 22.4 ± 7.8 μ g/dL in the medium exposure and 28.8 ± 11.0 μ g/dL in children attending the school nearest to the lead refinery. The percentage of children having PbB ≥ 15 μ g/dL was 6.8%, 84.9% and 92.1%, respectively. In this order, the average Pb concentrations in air were 3.25 ± 2.59 μ g/m^3, 6.03 ± 2.07 μ g/m^3 and 8.16 ± 5.65 μ g/m^3. The range of Pb concentrations in soil and dust in playground areas in the medium and high exposure settings was 1,000 to 4,000 mg/kg. Pb values in drinking water were less than 6 μ g/L. Soil and dust ingestion and inhalation appear to be the main routes of exposure. Our results indicate that environmental contamination has resulted in an increased body burden of Pb, suggesting that children living in the vicinity of the smelter complex are at high risk of suffering the well known adverse effects of lead if exposure continues. Further studies are needed to evaluate the specific contributions of lead sources in the city and the presence of other metals in the environment.

1742 EVALUATION OF THE GENOTOXICITY OF CHEMICALS CURRENTLY UNDERGOING IN VIVO CARCINOGENICITY TESTING USING THE p53 INDUCTION ASSAY.

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In vivo carcinogenicity testing is an expensive and time–consuming process, and as a result, only a relatively small fraction of new and existing chemicals can be tested in this manner. Therefore, the development and validation of alternative approaches is desirable. The Predictive Toxicology Evaluation 2 (PTE2) project, initiated in 1990, listed thirty chemicals currently undergoing in vivo toxicity testing. A number of laboratories have subjected this set of chemicals to analysis using a variety of alternative methods and on the basis of these results, predicted their carcinogenicity in the rodent models. We have previously developed a mammalian, in vitro assay for DNA damage based on the ability of cells to increase their level of p53 in response to DNA damage. Cultured cells are treated with several concentrations of the test substance, and at defined times following treatment, are harvested and lysed. The lysates are analyzed for p53 by Western blot and/or ELISA. An increase in cellular p53 following treatment is interpreted as evidence for DNA damage, and a lack of such an increase is interpreted as evidence of no significant DNA damage. To determine the ability of this p53–induction assay to predict carcinogenicity in rodents and to compare such results with those obtained using other alternate approaches, we subjected chemicals from the PTE2 list to analysis with this method. The results obtained from the p53 induction assay corresponded to those obtained with the Salmonella assay for most chemicals; however, there were a few differences.

1743 THE USE OF IN- SITU HYBRIDISATION FOR THE OPTIMISATION OF POLYMERASE CHAIN REACTION-IN SITU METHODOLOGIES IN FROZEN TISSUE.

A Lawrence , N Bullough, L McLoughlin and P R Ryle. *Huntingdon Life Sciences, Huntingdon, Cambs, England.* Sponsor: R J Harling.

In-situ polymerase chain reaction (IS-PCR) technique can be invaluable for localising specific DNA sequences in the safety evaluation of gene therapy products and xenotransplantation. Our initial attempt to validate IS-PCR using a low copy number gene was of limited success as appropriate controls for each stage of the complex PCR procedure were unavailable and the cause(s) of inconsistent results were difficult to identify. However the first major problem to be overcome was poor tissue morphology following PCR thermal cycling . In this presentation we describe the use of a high copy number porcine retro virus env gene as a model to optimise tissue sectioning and fixation in in-situ methods. The effect of thermal cycling on tissue morphology was also investigated. The initial investigation focused on tissue fixation, where twelve micron thick frozen sections of pig and human liver were fixed in acetone or buffered formalin. Formalin fixed sections gave superior in situ-hybridisation (ISH) staining (using FITC labelled oligonucleotide probe) and improved morphology compared to acetone fixation where high levels of background staining and poor morphology were seen. The effects of thermal cycling on tissue morphology and the ISH reaction were examined. The methodology involved thermal cycling with PCR buffer, porcine liver and testis sections, human liver was used as the nega-

tive control, followed by ISH technique using the oligonucleotide probe. The results showed that satisfactory morphology was achieved between zero and forty cycles. However, background staining increased significantly with those samples that had been subjected to thirty and forty cycles. In summary optimisation of the methodology for ISH depends on twelve micron thick sections and buffered formalin fixation of frozen tissues. Satisfactory morphology for ISH following PCR was observed up to thirty thermal cycles. This ISH approach can now act as a model system for the optimisation of PCR conditions in frozen tissues.

1744 SCREENING BY QXL-PCR AS A TOOL TO ASSESS GENOTOXICITY FROM AIRBORNE PARTICULATES.

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This study assesses the genotoxic potential of airborne pollutants in the San Juan port area of Puerto Rico. Total suspended particles and PM-10 airborne extracts were obtained from filters placed at monitoring stations, and bioactivated using a chemically-induced rat liver microsomal cocktail. Known genotoxic agents were employed as positive controls for the two regions analyzed, a 17.7 kb fragment of the ß-globin gene and a 16.2 kb region of the mitochondrial DNA. Benzo[a]pyrene was employed as control agent for nuclear DNA damage, whereas H2O2 was used to evaluate the mitochondrial DNA region. Cultured human fibroblasts were exposed to different agents to assess DNA damage and repair. DNA was isolated, quantitated by fluorometry, and amplified by 32P-radioactively labeled QXL-PCR (Yakes, 1997). QXL-PCR is based on the inability of rTth DNA polymerase to elongate DNA fragments in presence of DNA lesions, leading to a reduction in amplification signal, which is proportional to the extent of damage suffered by the template. When performing a direct bioactivation of B[a]P using 0.5 mg/ml of hepatic microsomes, we observed a dose-dependent effect of DNA damage. The highest concentration of B[a]P induced the highest DNA repair response, both in the mitochondrial and the nuclear DNA. HPLC analysis of our B[a]P bioactivation cocktails demonstrated the presence of a polar B[a]P metabolite. H2O2 experiments also demonstrated dose-dependent DNA damage and repair, both in the mitochondrial and nuclear DNA. This damage was more persistent in the mitochondrial DNA. Genotoxicity of airborne extracts were more prominent in the PM-10 fractions. This technique is being optimized to monitor human exposure to genotoxic agents by analyzing human lymphocytes DNA from blood of both smokers and non-smokers as a preliminary test of this methodology.

1745 EVALUATION OF CYTOTOXICITY ASSAYS OF HUMAN EPIDERMAL KERATINOCYTES EXPOSED *IN VITRO* TO SULFUR MUSTARD.

C L Gross, S A Kelly, J Guzman, and W J Smith. *Drug Assessment and Pharmacology Divisions, United States Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD.* Sponsor: T M Shih.

Sulfur mustard (HD) is a potent cytotoxic chemical agent. Reproducible analysis of HD cytotoxicity is a useful tool in screening candidate prophylactic and therapeutic (P/T) compounds as medical countermeasures against vesicant agents. Viability assessment of human lymphocytes (PBL) by flow cytometry of propidium iodide uptake has been a staple of the compound screening assays for years. Our laboratory has reported on problems associated with simple vital dye uptake as a measure of cytotoxicity in human keratinocytes (HEK) following *in vitro* exposure to HD. We have evaluated 6 different measures of cell viability for HEK exposed to HD *in vitro*. These are propidium iodide uptake (membrane integrity), viable cell number, calcein-AM (membrane integrity), alamar blue (mitochondrial metabolism), neutral red (lysosomal activity), MTS (mitochondrial dehydrogenase activity). The sensitivity of each assay to HD toxicity appears to be: viable cell number > calcein = alamar blue = PI uptake > MTS = neutral red. All of these assays can be performed by moderately trained laboratory personnel without the need for expensive flow cytometry instrumentation.

1746 AMNIOTIC FLUID AND MATERNAL/FETAL BLOOD COLLECTION IN LABORATORY MICE.

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Researchers have become increasingly interested in quantifying the amount of test material crossing the placenta. The analysis of prenatal amniotic fluid and blood samples can provide this information. The method developed in this laboratory allows for determination of test substance levels in the amniotic fluid and in fetal/maternal blood in mice. The pregnant mouse, gestation day 17, is placed in a clear chamber for induction of anesthesia with isoflurane. The anesthetic (isoflurane) is driven through a vaporizer at a concentration of 4%-4.5% via the pressure from an oxygen tank set at 0.4 liters/min. Once the dam has reached a surgical plane of anesthesia, it is placed on a heating pad in a supine position. Anesthesia is maintained with isoflurane via a nose cone at an approximate concentration of 1%-1.5% and is adjusted as needed. The abdominal cavity is opened and the uterus is exposed. The fetuses are maintained in situ until the amniotic fluid is collected. Approximately 0.5 ml amniotic fluid per mouse litter can be obtained. The fetuses are removed sequentially from the uterus. Each fetus is placed on a heating pad, the thoracic cavity is opened and the great vessels anterior to the heart are exposed. The vessels are severed and a heparinized capillary tube is placed near the incision. After collection, the samples from each litter may be combined for analysis. Approximately 0.20 mL of pooled mouse fetal blood can be obtained. After all fetal samples are collected, a maternal blood sample is obtained from the vena cava. The dam may then be sacrificed by exsanguination while under anesthesia. This method allows for the maintenance of the maternal/fetal vascular supply and fetal viability during the procedure. We believe that the technique developed in the laboratory is advantageous and reliable based on the consistent results.

1747 ELISA-BASED OLIGONUCLEOTIDE LIGATION ASSAYS FOR THE DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISMS (SNPs) IN BIOTRANSFORMATION ENZYMES.

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High throughput genotyping methods are becoming more important for the timely completion of large-scale molecular epidemiologic studies. An ideal genotyping assay should be efficient, economical, automatable, and should yield consistent single-base pair discrimination. We have developed ELISA-based oligonucleotide ligation assays (OLAs) to detect specific SNPs in the cytochrome P450 (CYP) 1A1, CYP 2E1, CYP 2D6, microsomal epoxide hydrolase, NAD(P)H quinone oxidoreductase, glutathione S-transferase P1, and N-acetyl transferase 2 genes. These genotyping assays employ 96-well microtiter plates in a diallelic, single-well format. The OLA protocol is as follows: a specific region surrounding the SNP is PCR amplified from genomic DNA or a cell lysate and is incorporated as a template for an allele-specific ligation reaction using two adjacent oligonucleotides: a wild-type or variant specific reporter probe (labeled with digoxigenin or fluorescein) and a biotinylated anchor probe. The two allele-specific ligation oligomers are complementary to sequences nested within the PCR amplicon. Only probes that are perfectly complementary are ligated by a high-stringency thermal-cycled ligation step. Capture of the biotinylated anchor probe on immobilized streptavidin is followed by sequential analysis for the covalently-linked reporters using an ELISA. This procedure allows automated detection for the presence or absence of allelic variation in the PCR product thereby enhancing genotyping analytical capacity in a low cost format. (Supported by NIEHS Center ES-07033.)

1748 HORMONE DETERMINATIONS IN MACAQUES: VALIDATION OF A TESTOSTERONE ASSAY.

W Müller, F Vogel, and R Korte. *Covance Laboratories GmbH, Münster, Germany.* Sponsor: J C Norris.

Testosterone is a core parameter in reproduction toxicology and generally in reproduction research. Non-human primates are often the most appropriate model for this research due to their close relationship to man. As the number of assays for hormone determinations in animal species including primates is very limited, there is often no alternative other than using assays which have been developed for determinations in human samples. The implementation of such assays may be affected in an adverse manner by interferences of matrix differences between man and animal species and, in certain cases (but not regarding testosterone), differences in the molecular structure of the target hormone. The proper performance of an assay must therefore be demonstrated with respect to the target species. This validation procedure should follow national and international standards including the Good Laboratory Practice Regulations if acceptance by regulatory authori-

ties is aimed for. This poster outlines the validation procedures and the acceptance criteria for the routine use of the testosterone radioimmunoassay DSL-4100 from Diagnostic Systems Laboratories Deutschland GmbH for determinations in serum samples from macaques. Data are presented on intra- and inter-run accuracy (with one exception between 86.3% to 109.8%) and precision (between 2.2% and 15.9%), on stability (samples stable under the tested short- and long-term conditions), cross reactivity (less than indicated for human samples), parallelism (good parallelism within the examined range), sensitivity (not examined below 0.4 nmol/L) and robustness (no influences by staff or laboratory changes or by examination dates). The data confirm suitability of the assay for determinations in serum samples from macaques in the range of 0.4 to 86.7 nmol/L.

1749 ASSAYS FOR DNA DELETIONS DETECT CARCINOGENS.

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Genetic instability and deletions are involved in carcinogenesis. We have constructed and/or used assays (DEL assays) that select for DNA deletion events in yeast, human cells and in vivo in the mouse. DEL events in all three formats are inducible by a wide variety of carcinogens including carcinogens, such as benzene and PCBs, that are negative in many other short-term tests. Furthermore, the yeast DEL assay correctly differentiates between carcinogen-non carcinogen structural analogs. Of 62 chemicals of known carcinogenicity status the yeast DEL assay detects 85% correctly compared to the Salmonella assay that detects only 33% correctly. The DEL assays are currently used to determine dose response relations for carcinogens and to investigate the biological activity of these carcinogens. Mouse DEL assays may detect tissue specific effects and allow in vitro - in vivo comparisons. The yeast DEL assay is currently being further developed into a high throughput screening format and is currently used in governmental and industrial applications in attempts to identify the potential carcinogenic activity of chemicals. Further validation with 500 chemicals is ongoing. 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 biologic activity of carcinogens than current assays.

1750 ARE RIGOROUS STUDY METHODS REPORTED IN RESEARCH USING ANIMAL MODELS?

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In 1996 the CONSORT statement was adopted by many medical journals to standardize the reporting of rigorous study methods, including randomization of subjects and blinding of investigators to the treatment of subjects. No such methodological requirements have been adopted for animal studies. We evaluated the reporting of study design in animal studies from four toxicology-oriented journals. Issues of the Journal of Toxicology-Clinical Toxicology (JTOX) from 1994 to 1997, Annals of Emergency Medicine (AEM) from 1994 to 1997, Veterinary and Human Toxicology (VHT) from 1996, and one month of Fundamental and Applied Toxicology (FAAT) from 1996 were reviewed. Using a standardized data collection form, three non-blinded investigators reviewed articles that studied a poison or drug effect using an animal model. Investigators assessed for reporting of the following data elements: use of a control group; randomization of animals to treatment groups; blinding of the investigator responsible for assessing study outcome; and age, weight, sex and species of the model. The model was considered adequately described if sex, species and age or weight were provided. Of the 83 articles reviewed, 58 (73%) reported adequate model descriptions, 76 (95%) use of control groups, 36 (45%) randomization of animals and 12 (15%) blinding of study outcome assessments. The use of controls and model descriptions were common in these journals, though blinding and randomization, considered crucial in human studies to distribute bias evenly, were not reported in a majority of studies. We advocate the adoption of randomization and blinding as standard practice in animal research until data demonstrates that these safeguards are not required.

1751 DEVELOPMENT AND UTILIZATION OF A PHARMACO/TOXICOKINETIC MODEL FOR EXTRA CELLULAR MATRIX PROTEINS USED IN THE TREATMENT OF PERIODONTAL DISEASE.

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Disease of the periodontium continues to be one of man's most widespread afflictions. Approximately 15% of the population in the United States suffers from this disease. Fibronectin was used as a surrogate ECM protein to develop a pharmaco/toxicokinetic model capable of assessing acute toxicity and bioavailability of I-125 radiolabeled ECM proteins administered I.V. or through a clinically relevant gingival flap procedure. The flap procedure consisted of resecting the gingival tissue from the mesial portion of the maxillary first molar, debriding the exposed root surface, administering fibronectin, and returning the flap to its original position. Chronically cannulated male rats were administered 5 μL of a 100 μg/mL (0.5 μg) into a gingival flap (n = 4) representative of the clinical route and dose received in human periodontal flap surgery therapy. The systemic bioavailability, urine clearance, through a bladder catheter, and tissue accumulation of the gingivally applied protein was compared to increasingly high systemic doses of fibronectin. Doses of 1, 10 and 100 μg (n = 4, 3 and 4 respectively) were administered I.V. Results showed no signs of acute toxicity, organ bioaccumulation or altering kinetics for the I.V. treated animals. A therapeutic ratio of greater than 2000 was demonstrated by comparing peak systemic doses of clinical dose to the highest systemic dose. Preferential binding to site of action, e.g.: tooth, gingiva and maxilla was also demonstrated in this model. The model was designed to evaluate the acute safety and true clinical exposure of ECM proteins in the periodontium. The model can be further utilized to address the pharmacokinetics of chemical entities where exposure in the periodontium occurs.

1752 ACUTE TOXIC CLASS METHODS: USE, PROPERTIES AND INTERNATIONAL HARMONIZATION.

W Diener and E Schlede. *Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV), Berlin, Germany.* Sponsor: J Pauluhn.

The expected numbers of animals in acute toxicity testing is significantly reduced by the use of the Acute Toxic Class (ATC) Methods, on average 70% in comparison to the classical LD/LC50 tests. In March 1996 the oral ATC Method was adopted as an official OECD Test Guideline (No. 423). Thus, a worldwide use is possible. The oral ATC Method is as reliable as the oral LD50 Method with respect to classification and observation of clinical signs. Since 1996 in Germany the oral ATC Method has been used in 21% of all oral acute tests conducted for the notification procedure of new chemicals in the European Union. This Method is considered as an excellent alternative to the oral LD50 test which may be deleted in the near future by the OECD. For the inhalation and dermal routes of administration new guidelines based on the oral ATC Method were developed, which are also appropriate as alternatives to the respective LD/LC50 test methods. The ATC Methods (oral, inhalation, dermal) are also applicable in case that an international harmonization based on LD/LC50 as cut-off values is achieved. Nevertheless, all tests carried out before the completion of harmonization process can be used due to the specific procedure of the ATC Methods. In addition, modified test procedures can be easily applied with the harmonized cut-off values. Examples for the modified ATC Methods together with classification probabilities and expected numbers of used and dead animals are given according to the agreement on acute toxicity systems for all routes of administration at the 8th high level meeting of the OECD in September 1998.

1753 HUMANE END-POINTS IN ACUTE TOXICITY TESTING.

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The reduction of severe pain and distress of test animals and avoidance of death as an end-point in acute toxicity testing should be feasible within the framework of the regulation of chemicals, drugs, pesticides etc. Examples for humane end-points with respect to acute toxicity testing are: -Theoretical considerations and in vitro tests prior to animal testing: Within a tiered testing strategy structure-activity (SAR) considerations and specific in vitro

tests can be used in order to predict severe acute toxic effects like skin corrosivity or eye damage. If severe effects can be predicted, animal testing should be avoided. - Prediction of local irritation/corrosion after a single contact: When no severe local irritant/corrosive properties of a substance can be predicted, Draize irritation tests using one single rabbit should be conducted. If corrosivity, severe corneal opacity or pannus are observed, the test can be terminated because these lesions do not resolve within an adequate observation time and thus, a reliable classification of local effects is possible. If a one-animal test does not demonstrate severe local effects, two further rabbits are used in order to rank local irritation according to international guidelines. - Clinical signs preceding death of test animals in oral toxicity testing: Neither a single nor a combination of clinical signs can be reliably associated with death. However, there are specific clinical signs that should be considered as „alert signs" for an impending death. These signs include all forms of convulsions followed by lateral position and tremor. Convulsions are almost non-existent in surviving animals (< 1%) in comparison to moribund animals (44%) and animals that die (28%). For example, only 10 out of 210 animals suffering from clonic convulsion survived. These data are based on the evaluations of clinical signs of 3942 rats that were used in the ring studies of the oral Acute Toxic Class Method.

1754 IN VITRO PERCUTANEOUS ABSORPTION STUDIES OF CONTAMINATED SOILS: HPLC DETECTION OF DERMALLY BIOAVAILABLE BENZO[A]PYRENE.

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A high performance liquid chromatography (HPLC) method has been developed to determine benzo[a]pyrene (BaP) in an aqueous (6%/Wt.) polyethylene glycol 20 oleyl ether surfactant (Volpo 20) receptor fluid used for *in vitro* percutaneous absorption studies. Previously, *in vitro* studies have estimated the dermal bioavailability of BaP and other carcinogenic polynuclear aromatic compounds (PAC) from contaminated soils by determining levels of (surrogate) ^{3}H-BaP in surfactant-modified receptor fluid. However, this method is disadvantageous as it may not accurately reflect the true bioavailability of endogenous PAC, which can be more tightly bound to soil than ^{3}H-BaP added to soil pre-(dermal) dosing. The present method is designed to detect endogenous BaP in surfactant-modified receptor fluid at the termination of *in vitro* percutaneous absorption experiments and thus allow a better estimate of the effect of soil aging on dermal bioavailability. This is a simple and fast method involving direct analysis of BaP without the need for radiotracers or tedious extraction procedures. The *in vitro* dermal studies are conducted in the usual manner and the receptor fluid analyzed by reverse-phase HPLC using fluorescence detection. The method shows good linearity in the range studied (10-400 picograms BaP in receptor fluid). The enhanced detection method for *in vitro* dermal bioavailability studies should improve the accuracy of experimentally determined environmentally acceptable endpoints for (contaminated) site risk assessment and remediation.

1755 EVALUATION OF BENZENE BIOMARKER S-PHENYLMER CAPTURIC ACID, A SENSITIVE BIOMARKER OF EXPOSURE.

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The American Conference of Governmental Industrial Hygienists (ACGIH) proposed S-phenylmercapturic acid (SPMA) as the biological exposure index (BEI) for benzene. We conducted an inhalation study in rats to evaluate the SPMA assay in a rodent model. This assay was developed by Van Sittert *et al.* (1993, 1995) in humans. Four Wistar rats per dose group were exposed for 8 hrs to 0, 0.5, 1.0 and 10 ppm benzene in individual metabolism cages. These cages served as the exposure chambers and facilitated the collection of urine. Chamber airflow and benzene concentrations were monitored continuously throughout the exposure and recorded every 60 minutes. Urine was collected at 8, 24 and 48 hrs after initial exposure. SPMA was analyzed using capillary gas chromatography with mass selective detection with a quantitation limit of 50 nM (24 ng/mL). S-benzylmercapturic acid (s-BMA; n-acetyl-s-benzyl-DL-cysteine) was used as the internal standard instead of deuterated SPMA. In addition to SPMA, phenol and creatinine were quantified in each sample. Exposure to 0.5, 1.0 and 10 ppm benzene resulted in mean SPMA concentrations (standard deviation) of 19.0 (2.3), 63.3 (10.1) and 1024.2 (380) μ g/g creatinine, respectively, at 8 hr time interval and 6.4 (2.6), 11.6 (5.3) and 79.2 (32.7) μ g/g creatinine, respectively, at the 24 hr interval. By 48 hrs after exposure, SPMA was detected only in the 10 ppm dose group with a mean concentration of 0.4 (0.2) μ g/g creatinine. SPMA was not detected in any of the control animals. Phenol concentrations in the urine were elevated only in the 10 ppm exposure group and only at the 8 hr time interval. These data confirm the sensitivity of the SPMA assay in the rat and validate SPMA as a suitable biomarker for low level benzene exposure.

1756 PRODUCTION OF ANTIBODIES TO METHADONE FOR TOXICOLOGICAL STUDY.

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A specific antibody to methadone (Mtd) was produced in rabbits by immunization with a conjugate of methadol - hemisuccinyl - bovine serum albumin. A hapten was first prepared by reduction of methadone with sodium borohydrure, then followed by esterification with succinic anhydride. The conjugation of hapten with albumin was achieved by the mixt anhydride method. Rabbits were immunized, and titres and specificity of antibodies were determined by ELISA using a conjugate of methadone - hemocyanin (KLH) as coating antigen. The antibodies obtained were tested with methadone, its major metabolite : 2-ethylidene, 1,5-dimethyl 3,3 diphénylpyrrolidine (EDDP) and other drugs and drugs of abuse. The antibody titers were 1:640,000. The detection sensitivity of methadone was10 ng/ml. The cross-reactivity with its EDDP metabolite was about 1.0% and with other drugs and drugs of abuse less than 0.01%. This specific antibody to methadone will be used for the development of an enzymatic method for the determination of methadone in biological fluids for maintenance treatment of opiate addicts and of an immunoaffinity chromatography method for the extraction of this compound especially in polypoisoning, intended to physico-chemical methods (HPLC, GC, MS).

1757 NEST BOX FOR EXCRETA COLLECTION FROM MOUSE DAMS THROUGH PARTURITION.

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Standard metabolism cages cannot be used to collect excreta from mouse dams through parturition and early lactation because newborn pups fall to the excreta collection area where the dam cannot reach them. A nest box that solves this problem was constructed from a rectangular polyethylene box with a removable lid and designed for placement inside a standard metabolism cage (patent filed). The nest box was tested in a metabolic study with pregnant mice, strain 129/SvJ (metallothionein-normal and metallothionein-knockout); nonpregnant mice without a nest box and pregnant mice in conventional plastic cages served as controls. Each mouse received two gavage doses of $^{109}CdCl_2$, one in late gestation and one in midlactation for dams, and at analogous time points for nonpregnant mice. Recovery of ^{109}Cd in excreta was determined for each dose. Dams urinated only outside the nest box and deposited <4% of their feces inside the box; all urine and the balance of feces were collected from the metabolism cage collection tray. In contrast, a nonpregnant test mouse with a nest box routinely deposited feces and urine both inside and outside the box. For dams, the percentage ^{109}Cd recovered after the first gavage — with nest box, collection through parturition (95±6%, mean+SE, n=5) was the same as after the second gavage — no nest box, collection during midlactation (95±6%, n=5). Results demonstrated that values for litter size, dam weight, pup weight, and pup survival were not significantly different from those obtained for mice in conventional plastic cages. The nest box provided a safe haven for mouse pups in the metabolism cage environment while allowing for quantitative excreta collection from mouse dams (and pups) through pregnancy, parturition, and the early postnatal period. With this box, drugs or toxic compounds that need testing in females can undergo continuous metabolic evaluation through parturition and early lactation in a metabolism cage setting. (Work supported by NIH grant ES07398.)

1758 EVALUATING THE CYTOTOXICITY OF 181 CHEMICALS TO HELA CELLS USING SAR EXPERT SYSTEM.

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Cellular toxicity (CelTox) is often used as a surrogate for other toxicological endpoints ranging from ocular irritation to systemic toxicity. In order to explore the validity of this assumption, we developed an SAR model of toxicity of chemicals towards cultured Hela cells. Based upon a database of 181 chemicals assayed for their growth-inhibiting activity after 24 hours of exposure, an SAR model based upon CASE/MultiCASE was developed. For chemicals external to the learning set, the model displayed a concordance between experimental and predicted results of 74% (Sensitivity: 0.75; Specificity: 0.72). This suggests that CelTox is a complex phenomenon reflecting a series of independent and/or sequential actions. Yet the SAR model is robust and the chemicals used to build the models are a good representation of the universe of chemicals. Moreover, it is to be expected that the predictivity of the model will improve as the number of chemicals included is increased. The structure overlap between SAR models can be taken as a measure of mechanistic similarity. There was significant, but not complete, structural overlap between CelTox and maximum tolerate dose (mice and rats), LD_{50} in rats, toxicity to fish, eye irritation and phenomena associated with electrophilicity (mutagenicity in *Salmonella;* allergic contact dermatitis). There was only minimal structural overlap between CelTox and α 2μ - induced nephrotoxicity or binding to the Ah receptor. These findings indicate that while CelTox can be used as a surrogate for other toxicological endpoints, this should be done as part of a battery of assays.

1759 A METHOD FOR QUANTITATION OF TISSUE VINCLOZOLIN AND METABOLITES USING A BENCHMATE® AUTOMATED SPE WITH GC-MS.

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Vinclozolin (*Vin*, CAS 50471-44-8), a dicarboximide fungicide, has been reported be an antagonist of androgen receptor binding in rats, effects that could result in decreased fertility, defects in reproductive organs or cancer. It is thought that *Vin* activation to the metabolites 2-[[(3,5-dichloro-phenyl)carbamoyl]oxy]-2-methyl-3-butanoic acid (*M1*, CAS 119209-27-7), 3',5'-dichloro-phenyl-2-hydroxy-2-methylbut-3-enanilide (*M2*, CAS 83792-61-4), and 3,5-dichlorobenzenamine (*M3*, CAS 626-43-7) could induce similar toxicity in workers. A potential biomonitoring method was developed to measure tissue levels and evaluated using Dutch Belted rabbits after exposure to *Vin*, either as a single dermal dose or multiple dermal doses. *Vin* (100 mg/kg) was applied to the skin in 100 µL DMSO. Plasma samples were taken at 0, 6, 8, 12, 24, and 48 hr after a single dose or weekly during multiple dose exposure. Skin from dermal application sites and testes were excised at termination, homogenized and filtered. A BenchMate® robotic workstation was used for automation of sample preparation. Bond Elute® 500 mg C8 SPE columns were conditioned with acetone, MeOH and H_2O pH 7. Samples were loaded onto columns, rinsed twice with H_2O. A fraction containing >96% of *Vin* and *Vin* metabolites was collected in 1.5-mL acetone, and reduced to 1 mL for GC-MS SIM analysis (Hewlett-Packard quadruple using a 12 m X 0.2 mm Ultra 1 column). Chromatographic standards *M1* and *M2* were synthesized by alkaline hydrolysis of *Vin* and isolated by HPLC. *Vin*, *M1*, *M2*, and *M3* were detected in both plasma and tissues, but animal variation was problematic. After a single application *Vin* plasma levels were highest at 6 hr whereas levels of *M1*, *M2*, and *M3* were highest at 12 hr. After 4 weeks of dosing *Vin* levels in plasma were 162.2 ng/mL — consistent with testes levels. *M2* was detected in the testes and skin levels of *Vin* were ~10 fold that of plasma. Thus, *Vin* and *Vin* metabolite internal exposure levels can be rapidly quantified in a single assay using an automated system.

1760 A FLUORESCENCE PLATE READER ASSAY FOR MONITORING THE SUSCEPTIBILITY OF BIOLOGICAL SAMPLES TO LIPID PEROXIDATION.

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The susceptibility of biological samples to lipid peroxidation can be determined by exposing samples to a lipid peroxidation initiator and measuring the length of time prior to the onset of lipid peroxidation. Previous studies have shown that aldehydes generated by lipid peroxidation can react with amines to produce fluorescent products. We have utilized this principle to develop a fluorescence plate reader assay for measuring susceptibility to lipid peroxidation. In this assay, samples are placed in glycine/phosphate buffer and loaded into a 96 well plate. Lipid peroxidation initiators are added, and fluorescence is monitored over time at excitation and emission wavelengths of 360/430 nm. Samples were assayed for susceptibility to lipid peroxidation by both the thiobarbituric acid reactive substances assay and the fluorescence plate reader assay. We found good agreement between these two methods in assessing relative susceptibility to lipid peroxidation in liver microsomes and mitochondria. The fluorescence assay was also used to monitor lipid peroxidation in liposomes and rat liver homogenates. The addition of 4-hydroxy-2-nonenal but not malondialdehyde to glycine/phosphate buffer produced a fluorophore with similar excitation/emission maximums as liposomes peroxidized in glycine/phosphate buffer. Fluorescence was stable over an extended time period and could be induced by a variety of lipid peroxidation initiators. The fluorescence plate reader assay offers a rapid method for monitoring lipid peroxidation in a large number of samples. (This work was supported by NIEHS/NIH grant # R01ES 05452.)

1761 A RETROSPECTIVE ANALYSIS OF CLINICAL PATHOLOGY PARAMETERS IN DOMESTIC SWINE: A COMPARISON OF VALUES BEFORE AND AFTER JUGULAR VEIN CATHETERIZATION.

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Domestic swine are often utilized in preclinical safety and efficacy studies. We decided several years ago to surgically implant jugular catheters in domestic pigs to minimize stress and pain when multiple serial blood collections are required within a study. Animals arriving at our Test Facility are placed in quarantine for a minimum of seven days for observations (clinical and physical) and clinical pathology testing, including fecal samples for analysis of intestinal parasites and blood collection for analysis of hematology (red cells, white cells and platelets) and serum chemistry parameters. This blood collection requires tranquilization and/or physical restraint, and generally occurs 4-5 days after receipt of the animals into their new habitat. We compared the hematology and serum chemistry values obtained from this analysis with the same parameters from a sample collected in conscious, unrestrained pigs via a jugular catheter at least five days post-surgery. This analysis includes collation of clinical pathology data obtained from more than 200 castrated male and female Yorkshire pigs (*Suis sus*) approximately 2-3 months old. The pigs weighed between 25 and 40 kg at the post-surgical blood collection time point. Total white blood cell counts, moderately elevated at the quarantine blood collection, were decreased when collected via a jugular vein catheter. This was primarily due to a decrease in polymorphonuclear cells. In addition, there was often a small decrease in red blood cell values (total red cells, hematocrit, hemoglobin, MCV and MCH) post-surgically compared to values obtained from pigs without catheters. This retrospective analysis provides a set of baseline values for evaluation of treatments in future studies using domestic swine. (All animal studies at Primedica Corporation are conducted in compliance with USDA and NIH regulations, and reviewed by the Primedica Animal Care and Use Committee.)

1762 THE EFFECTS OF SODIUM NITRITE ON METHEMOGLOBIN FORMATION IN NONHUMAN PRIMATES.

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Methemoglobin (MHb) formers are protective against cyanide (CN) toxicity in multiple species. Sodium nitrite-produced MHb was studied in nonhuman primates as part of an anti-CN pre/treatment development program. The time-course of sodium nitrite ($NaNO_2$)-produced MHb formation and changes in related hematological parameters were measured in six rhesus monkeys (*Macaca mullata*) (two females and four males with mean weights of 5.3 and 9.7 kg, respectively). A surgical plane of anesthesia was produced and maintained with tiletamine HCl/zolazepam HCl (3 mg/kg, IM) and inhalation of 0.5 - 2.0% isoflurane in oxygen, respectively. Arterial blood was collected serially from a centrally placed catheter and analyzed to determine the animals capacity to form MHb following increasing concentrations of intravenous administration of $NaNO_2$ (total cumulative dose per animal ranged from 16.0 - 20.0 mg/kg). Total hemoglobin, oxyhemoglobin, reduced

hemoglobin, saturated oxygen, oxygen content, sulfhemoglobin and MHb were measured with a OSM3 hemoximeter. Continuous body temperature was monitored by rectal probe. A dose-response relationship was developed for $NaNO_2$-produced MHb in the nonhuman primate, with maximum levels ranging from 8.6 - 15.5%. The data suggest that the relationship between the dose of $NaNO_2$ correlates well with the concentration of MHb formed, although differences across subjects were observed. This relationship lays the foundations for further study of MHb-forming anti-CN therapeutics in nonhuman primates.

1763 RAT ERYTHROCYTE ACETYLCHOLINESTERASE (AChE) MEASUREMENT USING A RADIOMETRIC METHOD.

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The objective of this work was to evaluate the sensitivity and the reproducibility of a simple radiometric method for the measurement of rat erythrocyte acetylcholinesterase. The radiometric method is based on the extraction of ^{3}H-acetate generated by the action of AChE on ^{3}H- acetylcholine. The method published by Johnson and Russell was modified to include the following: a) pH of reactive medium set at 8.0; b) addition of a biological and a commercial control; c) preparation of an hemolysate from packed red blood cells. The following results were obtained: Intra-assay precision: 1.2% C.V. (n=10), Inter-assay precision 4.1% C.V. (5 runs), Linearity of dilution: coefficient of correlation = 0.9999 for a range of 152-787 nmoles/min/mL hemolysate, slope = 0.9896. Sensitivty was found to be 84.9 nmoles/min/mL. Physiological Range for 12-15 weeks old Sprague-Dawley rats was calculated as 673-940 nmoles/min/mL hemolysate. In conclusion, this method is considered to be very sensitive and reproducible for the measurement of rat erythrocyte acetylcholinesterase.

1764 THE DEVELOPMENT OF AN IN VITRO MODEL FOR URINARY TRACT CELLS FROM C57BL/6N MICE.

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) produces hydronephrosis by altering the proliferation of ureteric epithelial cells in the embryonic C57BL/6N mouse urinary tract. The mechanism appears to involve effects on growth factor regulation and in this study an *in vitro* model was developed to facilitate study of the role of growth factors in this response. Ureters were removed from the urinary tracts of gestational day (GD)18 fetuses. The ureters were minced and trypsinized and the digest was centrifuged to produce a cellular pellet. Single cells and small clusters were resuspended in medium and plated onto transwells (Millicell-CM inserts, Millipore Corporation, Marlborough, MA) coated with purified and defined extracellular matrix components. The basal medium with low calcium (Keratinocyte Growth Medium-2, Clonetics , San Diego, CA) was supplemented with trace elements, a complex mixture of lipids, a defined mixture of purified hormones and growth factors tailored for the epithelial cells. Fetal bovine serum was added at 2% for the first 48 hours and at 1% thereafter. The cultures were incubated at 37°C in a humidified atmosphere containing 5% CO_2 in air. These conditions permitted selective expansion of the ureteric epithelia relative to stromal cells by inhibiting differentiation and inducing growth of epithelial progenitor cells. These epithelial cultures were viable and functional for 21 days. This culture model will be important in further study of the TCDD-induced disruption of coordinated expression of epithelial growth factors that coordinates with excessive epithelial cell proliferation. Disclaimer: (This abstract does not necessarily reflect EPA policy.)

1765 A METHOD FOR SPECIFICALLY ASSAYING ACETYLCHOLIN-ESTERASE ACTIVITY IN PERIPHERAL TISSUES.

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The total cholinesterase activity present in a given tissue consists of butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE) activity. The Ellman *et al.* (1961) assay, routinely used to assay total cholinesterase activity, does not distinguish between BuChE and AChE activity. Furthermore, assay of a large number of samples often depends on automated instrumentation. The present group of experiments was designed to assess whether an automated instrument could be used to assay AChE in peripheral tissues. Plasma, lung, retina, brain, diaphragm, heart, and muscle were collected from adult, female Long-Evans rats. All subjects were perfused transcardially with saline. Following homogenization in 0.1 M Na phosphate buffer (pH 8) with 1% Triton X-100, the relatively specific BuChE inhibitor iso-OMPA (0.1 mM final concentration) was added to an aliquot of each sample. The samples with and without iso-OMPA were then assayed on an Hitachi 911 Automatic Analyzer and also a ThermoMax Microtiter Plate Reader. Using the plate reader, acetylthiocholine-hydrolyzing-activity and butyrylthiocholine-hydrolyzing-activity of all samples were assayed approx-imately 15 minutes after the addition of iso-OMPA. Acetylthiocholine hydrolysis was assayed using the 911 at three time points: 15, 60, and 120 minutes after the addition of iso-OMPA. The amount of acetylthiocholine hydrolysis assayed by the 911 at 15 minutes after addition of iso-OMPA was the same as the activity measured by the plate reader. One hour after addition of iso-OMPA, the AChE activity had not increased nor decreased more than 10% for any tissue. By 2 hours after the addition of iso-OMPA, however, AChE activity was decreased in most of the tissues. Taken in concert, the data indicate that addition of iso-OMPA to brain and peripheral tissues is a valid method for inhibiting BuChE activity (and therefore measuring only AChE activity)if assay is completed within one hour after the addition of iso-OMPA.

1766 COMPARISON OF DOG THYROID PROFILES AND MECHANISM OF ACTION IN AMITROLE, FOE 5043, KTU 3616 AND YRC 2388 REGULATORY STUDIES.

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Primary hypothyroidism due to chronic amitrole (0, 10, 500 and 1500 ppm) reflected inhibition of thyroid peroxidase. There was decreased T4, T3, TSH, increased cholesterol, globulin, AST; nonregenerative, normochromic anemia with reduction in muscle mass, and reduced R- and P-amplitudes. Thyroid weights were increased and heart weights were decreased. Micropathology revealed moderate/ severe diffuse thyroid follicular cell hyperplasia with capsular fibrosis, vascular dilatation, inflammation and thrombosis, with ectopic thyroid tissue near the aortic arch, minimal/slight hypertrophy with vacuolization in the pituitary. *Extrathyroidal clearance with metabolic abiotrophism* characterized chronic FOE 5043 treatment (0, 40, 800; 1600 ppm). There was decreased T3, T4 as ALP increased (0-180 days), then adaptation of T3, T4; regenerative anemia, hypercholestrolemia and ECG arrhythmias. Central neurologic deficits and QEEG became pronounced as T4 & T3 rebounded. Micropathology revealed a minimal/slight hepatocytomegaly and hepatocyte vacuolization; vacuolization of ciliary body epithelium and peripheral optic retina; minimal/moderate axonopathy in brain, spinal cord and sciatic nerve, with no thyroid microscopic findings. The *"Euthyroid Sick Syndrome"* characterized subchronic KTU 3616 (0, 100, 500, 5,000; 20,000 ppm). There was decreased T4 & T3, decreased T-Prot & Gluc, with cachexia, body weight loss (>30%), and decreased food consumption with mild anemia and decreased P-wave amplitudes. Micropathology revealed parenchymal atrophy of heart, muscle and/or kidney; depletion of thymic and bone marrow tissue; hepatopathy with protoporphyrin pigmentation and individual hepatocyte necrosis. The chronic YRC 2388 (0, 20, 40, 200 and 750 ppm) increased *extrathyroidal clearance via xenobiotic induction of T4-UDP-GT.* T3 and T4 were little changed, but Phase I, UDP-GT and ALP were increased. Cholesterol was decreased, but with no anemia or ECG changes. Micropathology revealed hepatocytomegaly and hyperplasia of gall bladder mucosa related to bile secretion/flow, but no thyroid abnormalities. We conclude that mechanistic work during chronic or subchronic studies benefits from concurrent measurement and time course data, which adds to data package integrity per FQPA.

1767 EVALUATION OF THE REPRODUCTIVE PARAMETERS IN RATS AFFECTED BY THE INTERACTIONS OF POLYCHLORINATED BIPHENYLS AND METHOXYCHLOR RESULTING FROM EXPOSURE DURING DEVELOPMENT.

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Reproductive toxicity has been linked to the disruption of the endocrine system. Polychlorinated biphenyls (PCBs) bioaccumulate in the food chain and are passed on to offspring, inducing their cytochromes P450. Methoxychlor

(MXC), a pesticide used as a substitute for DDT, has estrogenic properties. The objective of this study was to investigate whether basic reproductive parameters are altered by the gestational and lactational exposure to PCBs in combination with pre-weanling exposure to MXC. Female Sprague-Dawley rats were fed 4 mg/kg/day Aroclor 1254 from gestation day 6 until postpartum day 22. Pups (n= 745 total) were treated from postnatal days (PND) 10-14 with 0, 0.3, 3, or 300 mg/kg/day (IP) laboratory grade MXC in the vehicle safflower oil. Pups were sacrificed on PND 15, 23, 31, and 70. Day of vaginal opening, weight at vaginal opening, sacrifice weight (used as covariable where appropriate), and reproductive organ weights were determined. There was an interaction of PCBs by MXC on PND 15 ($P<0.003$) such that at 300 mg/kg/day MXC, control rats had greater ovarian weight (22 mg vs 11 mg) and greater uterine weight (38 mg vs 28 mg) than PCBs-treated rats. At PND 23, uterine weight was consistently suppressed by PCBs ($P<0.0004$). Testis weight was decreased at the highest dose of MXC at PND 15 and 31 ($P<0.0001$), but was increased by PCBs only on PND 23 ($P<0.015$). Day of vaginal opening ($P<0.0001$) was accelerated by 4 days and weight at vaginal opening ($P<0.0006$) was lowered by 25 g by the highest dose of MXC. PCBs appear to mitigate negative effects of the highest dose of MXC on ovarian and uterine weight, while positively affecting testis weight. (Supported by EPA R 825296.)

1768 DETECTION OF THE ANTIANDROGEN *p,p'*-DDE IN SPRAGUE-DAWLEY AND LONG-EVANS RATS USING A TIER I SCREENING BATTERY.

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p,p'-DDE has been examined in a Tier I screening battery for detecting endocrine-active compounds (EACs). The Tier I battery that was used to examine *p,p'*-DDE consisted of a 15-day intact male *in vivo* battery and an *in vitro* yeast transactivation system (YTS). In addition, because other investigators have reported strain sensitivity differences with *p,p'*-DDE, Sprague-Dawley and Long-Evans rats were evaluated in Tier I. In the *in vivo* male battery using Sprague-Dawley rats, responses to *p,p'*-DDE included organ weight changes [increased relative liver and decreased absolute epididymis] and hormonal alterations [increased serum estradiol (E2) and decreased serum FSH and T_4]. Responses to *p,p'*-DDE in Long-Evans rats included organ weight changes [increased relative liver, absolute epididymis, relative accessory sex gland (ASG) unit, as well as the individual component weights of the ASG (prostate and seminal vesicles)], and hormonal alterations [increased serum testosterone (T), E2, dihydrotestosterone (DHT), thyroid stimulating hormone (TSH), and decreased T_4]. These data demonstrate that there are considerable strain sensitivity differences to *p,p'*-DDE exposure. The described *in vivo* male battery using Sprague-Dawley rats did not identify *p,p'*-DDE as an EAC. In contrast, the *in vivo* male battery using Long-Evans rats identified *p,p'*-DDE as an EAC. The pattern of responses suggest that *p,p'*-DDE is acting as an AR antagonist centrally, but not in the periphery, and that the increased ASG weights are a result of the increased serum T. In the YTS for the AR, *p,p'*-DDE had an EC_{50} value of 3.5×10^{-4} M; however, in the AR YTS competition assay, *p,p'*-DDE did not inhibit DHT binding to the AR. In summary, these data suggest that strain selection will affect the ability to detect certain weak EACs. However, a Tier I screening battery consisting of both *in vivo* and *in vitro* endpoints would reduce the chance that weak-acting compounds such as *p,p'*-DDE would not be identified as potential EACs. This example illustrates that a the proposed Tier I screening battery consisting of both *in vitro* and *in vivo* endpoints would detect *p,p'*-DDE as an EAC.

1769 EVALUATION OF A TIER I SCREENING BATTERY FOR DETECTING THE THYROID TOXICANTS PHENOBARBITAL AND PROPYLTHIOURACIL.

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Phenobarbital (PB; thyroid hormone excretion enhancer) and propylthiouracil (PTU; thyroid hormone synthesis inhibitor) have been examined in a Tier I screening battery for detecting endocrine-active compounds (EACs). In addition, several additional thyroid endpoints [serum rT_3 levels, thyroid weights, thyroid follicular cell proliferation, and hepatic UDP-glucuronyltransferase (UDP-GT) activity] have been evaluated for their ability to detect thyroid modulating effects at 7, 14, and 28 days of treatment using PB and PTU. The Tier I battery incorporates a 5-day ovariectomized female battery, a 15-day intact male battery, and an *in vitro* yeast transactivation system. In the female battery, PB increased relative liver weight and uterine epithelial cell height, and caused hormonal alterations (increased serum FSH , and decreased serum T_3 and T_4). In the male battery, PB administration increased relative liver weight and caused hormonal alteration (increased serum estradiol and TSH, and decreased serum prolactin, FSH, LH, T_3, and T_4). In the female battery, PTU caused hormonal alterations (increased serum TSH, and decreased T_3 and T_4). In the male battery, PTU administration decreased relative liver weight, caused thyroid gland hyperplasia, and caused hormonal alteration (increased serum TSH, and decreased serum T_3 and T_4). At all three timepoints in the thyroid timecourse experiment, PB induced UDP-GT activity and caused hormonal alterations [increased serum TSH (4-week timepoint only), and decreased serum rT_3, T_3, and T_4]. At all three timepoints, PTU increased absolute thyroid gland weight, caused hormonal alterations (increased serum TSH, and decreased serum T_3, and T_4), and increased thyroid follicular cell proliferation. Thyroid gland hyperplasia was evident in the PTU- but not in the PB-treated rats. It has been proposed that thyroid gland histopathology coupled with decreased serum T_4 levels be used as the criteria for identifying thyroid toxicants. However, using that criteria, weak thyroid toxicants like PB would not be detected using a study design of 28 days or less.

1770 EFFECTS OF DIETARY GENISTEIN ON REPRODUCTIVE DEVELOPMENT OF SPRAGUE-DAWLEY RATS.

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Phytoestrogens such as genistein (GE) can affect sexual differ-entiation in rat pups when administered by gavage to their dams, but whether normal dietary levels of these compounds are suffi-cient to alter perinatal reproductive development is unknown. A modified NIH-07 open formula rodent ration was obtained in which casein and corn oil were substituted for soybean and alfal-fa meal and soy oil, respectively. In contrast to standard NIH-07, which contains about 0.016% GE, soy- and alfalfa-free diet (SAFD) had no detectable isoflavones. To determine whether dietary GE can affect sexual developmental end points, groups of six timed-pregnant SD rats were fed SAFD, SAFD + 0.02% GE (GE.02), SAFD + 0.1% GE (GE.1), or NIH-07 starting on gestation day (gd) 0. Body weights (BW) of dams or pups were monitored until postnatal day (pnd) 56. Birth weights of pups and BW gain of dams and pups were similar in animals consum-ing SAFD or NIH-07 but were slightly reduced in those fed GE.02 or GE.1. Anogenital distance/BW on pnd 1 was signifi-cantly higher in animals of both sexes ingesting GE.1 than in those fed SAFD. On pnd 21 a 2.5-fold higher uterus/BW was observed in animals ingesting GE.1 relative to the other three groups, all of which had similar weights. Vaginal opening was accelerated in animals ingesting GE.1 relative to the other three groups, all of which had similar values. The results suggest that dietary concentrations of GE = 0.02% do not induce marked effects on sexual development relative to a GE-free diet, but that effects can occur at a 5-fold higher level. Based on the above end points, it appears unlikely that the concentrations of genistein present in NIH-07 are sufficient to alter sexual development of rats in the perinatal period.

1771 A COMPREHENSIVE PROTOCOL FOR THE EVALUATION OF POTENTIAL ENDOCRINE DISRUPTING CHEMICALS.

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Exposure to endocrine modulating substances may occur from ambient environmental exposures, foods, food packaging materials, pharmaceuticals, and medical devices. An Interagency Agreement has been established between the NIEHS/NTP and FDA/NCTR to evaluate five compounds in a comprehensive protocol capable of assessing effects on multiple structural and functional endpoints expected to be sensitive to modulation of estrogenic or androgenic pathways. The compounds selected for study (genistein, methoxychlor, nonylphenol, ethinyl estradiol, and vinclozolin) include chemicals that have been demonstrated to have widely varying potencies as endocrine disrupting agents. The compounds are being administered to CD (Sprague-Dawley) rats in a natural diet modified to eliminate phytoestrogens contributed by soy and alfalfa. For short term dose range finding studies, exposure to 5 or 6 doses of test chemicals is conducted from gestation day 7 through postnatal day 50 to 77, depending on the specific endpoints to be evaluated. Developmental, neuroanatomical and neurochemical endpoints, organ weights and histopathology, sperm counts, blood chemistry, hematology, and evaluation of immune function and sexually dimorphic behaviors are included. The main study is a multigeneration design in which the F_0-F_4

generations are evaluated. A comprehensive assessment of reproductive effects, including determinations of cyclicity, ovarian follicle counts and staging, sperm motility and staging, will be included. Recovery from effects observed in earlier generations by withdrawal of exposure will be determined. Immunological, neurological, and behavioral effects of the test compounds will be evaluated in the main study if warranted. Evaluations of chronic effects resulting from multiple exposure windows are included for 2 compounds. This comprehensive protocol will thus address many of the central issues of concern related to exposure to endocrine disrupting chemicals.

1772 ASSESSMENT OF ESTROGEN AGONIST AND ANTAGONIST ACTIVITY USING A MODIFIED RAT UTEROTROPHIC ASSAY.

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An increasing awareness of environmental chemicals and natural products with estrogen agonist activity has ignited research efforts to determine mechanistic links between xenoestrogen exposure and observed positive (therapeutic) and adverse health effects in animals and humans. As an increase in uterine weight in ovariectomized adult female rats following a 72 hour treatment period is generally considered to be the "gold standard" in vivo bioassay for estrogenicity, this assay was used to assess potential estrogen agonist effects of a chloroacetamide. We have also extended this assay to assess inhibition of 17β -estradiol induced increases in uterine weight (i.e., estrogen antagonism) using ovariectomized adult female rats injected with 17β -estradiol (40 μ g/kg/day). Here, adult ovariectomized female Crl:CD® (SD)BR rats (10 rats per group; 10 days post-surgery) received vehicle (corn oil) or 200 or 800 mg of the chloroacetamide/kg BW/day, with or without 17β -estradiol, daily by subscapular injection for 3 days. This chloroacetamide did not induce significant increases in uterine wet weight compared to vehicle controls and also did not inhibit the 7.7-fold increase in uterine wet weight induced by 17β -estradiol. The trophic effects of 17β -estradiol on uterine wet weight were not influenced by changes in body weight as body weights at necropsy were similar among all groups. These data suggest that this chloroacetamide does not exhibit estrogen agonist or antagonist activity using the above protocol. Future dose-response studies with lower dosage levels of 17β -estradiol and a positive anti-estrogen control will be completed to further examine the potential of this chloroacetamide to act as an anti-estrogen.

1773 HISTOLOGIC ANALYSIS OF THE TISSUE SPECIFIC ACTION OF THE XENO-ESTROGENS BISPHENOL A, p-tert-OCTYLPHENOL, o,p'-DDT AND THE PHYTOESTROGENS DAIDZEIN AND GENISTEIN.

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Epidemiologic data suggest that phytoestrogens and xeno-estrogens may affect the endocrine system and may cause beneficial but also developmental, reproductive and oncological effects. For this reason we have analyzed the endocrine profile of xeno- and phytoestrogens in ovariectomized female DA/HAN rats. The animals were treated for 3 days p.o. with the xeno-estrogens bisphenol A (5, 50 and 200 mg/kg/day), p-tert-octylphenol (5, 50 and 200 mg/kg/day), o,p'-DDT (20, 100 and 500 mg/kg/day), daidzein (1, 10, 100 and 500 mg/kg/day), genistein (10, 50 and 100 mg/kg/day) and ethinylestradiol (100 μg/kg/day). A morphometric analysis of the uterus epithelia, mammary glands and the vaginal epithelia was performed. The uterus wet weight was dose-dependently stimulated after administration of DDT (100 and 500 mg/kg/day), genistein (50 and 100 mg/kg/day) and after administration of 200 mg/kg/day p-tert-octylphenol. A significant stimulation was detectable after administration of 200 mg/kg/day bisphenol A and 500 mg/kg/day daidzein. The thickness of the uterine epithelium was stimulated after administration of DDT and genistein. The stimulation of uterus weight after octylphenol administration correlates with a stimulation of the myometrium. Analysis of the vaginal epithelium revealed clear evidence for an estrogenic action of DDT and genistein. After administration of daidzein, octylphenol and bisphenol A a weak but significant effect of these compounds on the vaginal epithelium was visible. In the mammary glands estrogenic effects were detectable after administration of DDT and genistein. In summary, uterotrophic response and histologic analysis revealed clear evidence for an estrogenic action of DDT and genistein. The data also demonstrate an estrogenic action of octylphenol. Its mechanism of action, especially possible androgen like effects, is under further investigation. Only a weak estrogenic action of daidzein could be demonstrated. The potential estrogenic activity of bisphenol A at high doses will e investigated in further studies.

1774 EVALUATION OF THE EDSTAC FEMALE PUBERTAL ASSAY IN CD RATS USING 17β -ESTRADIOL, STEROID BIOSYNTHESIS INHIBITORS AND A THYROID INHIBITOR.

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The Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) has designated the 20-d female pubertal assay as a Tier I screening test to detect potential endocrine disrupting (ED) chemicals. In two separate studies (S1 and S2), we began evaluating the ability of this assay to detect EDs acting through a variety of mechanisms. Weanling female rat pups were dosed for 20 d by gavage with vehicle (0.5% methocel) or the following test compounds (mg/kg/d): 17β -estradiol (E_2, 0.1, 2 or 4), ketoconazole (Keto, 24, 50 or 100), finasteride (Fin, 20), testolactone (TL, 220), fadrozole (Fad, 0.6, 1.2, or 6.0) or 6-propylthiouracil (PTU, 240). In vehicle-treated females, ages at pubertal onset, as evidenced by vaginal opening (VO), were 32.3 ± 1.6 d (S1) and 33.5 ± 1.8 d (S2). At 0.1 mg/kg E_2, age at VO was reduced slightly to 31.0 ± 1.6 d (S1), a reduction which was not statistically significant (α =0.05). However, at 2 or 4 mg/kg E_2, age at VO was maximally reduced to 28 d (S2). Keto, a broad spectrum steroid biosynthesis inhibitor, delayed VO, but this delay was statistically significant only at 100 mg/kg (39.7 ± 2.4 d). Fin, a 5α -reductase inhibitor, and TL, an aromatase inhibitor, had no effect on age at pubertal onset; however, Fad, a more specific aromatase inhibitor, significantly delayed VO. PTU, a thyroid inhibitor, significantly delayed VO to 34.2 ± 1.1 d (S1) and produced pronounced effects on thyroid weight, histology, and hormone levels. Thus, this assay failed to detect statistically significant differences in pubertal onset in rats receiving 0.1 mg/kg E_2, 24 or 50 mg/kg Keto, or 220 mg/kg TL, suggesting that VO is a relatively insensitive endpoint for assessing endocrine function; however, at higher doses, EDs operating through a variety of mechanisms can be detected. Considerable inter-animal variability suggests that even in the presence of EDs, intrinsic factors exert strong control over this endpoint.

1775 EVALUATION FOR ACTIVITY OF UTEROTROPHIC ASSAY USING OVRIECTOMIZED RATS TO DETECT ESTROGEN-LIKE COMPOUNDS.

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In order to develop a short-term in vivo screening method for the detection of estrogen-like compounds, we evaluated the activity of a uterotrophic assay using ovariectomized (OVX) rats, based on the method of O'Connor et al. (Fundam.Appl.Toxicol. 33,182, 1996). The ability to identify 17_$B&B_(B-Estradiol (E2), a pure estrogen receptor agonist, was evaluated by comparing the responses to E2 between OVX and sham-operated rats. Female Crj:CD(SD)IGS rats (6 weeks old) were OVX or sham-operated 1 week before treatment, and then treated with E2 (0, 0.001, 0.05 mg/kg/day, ip) for 4 days. One day after the final treatment, endpoints including body weights, food consumption, organ weights, gross pathology, uterine histopathology, uterine epithelial cell height, and uterine stromal cell proliferation using BrdU labeling were determined. In OVX rats, responses to E2 included decreases in body weight gain and food consumption, as well as uterine enlargement in gross pathology, and increases in uterine weight, uterine epithelial cell height, and uterine stromal cell proliferation. The changes in uterotropic endpoints were observed even with the lowest E2 dose. On the other hand, examination of sham-operated rats did not reveal any of these responses, demonstrating that OVX rats are extremely sensitive for detecting E2-induced alterations. For further validation of this assay, methoxychor, a proestrogen, was tested. As previously described, methoxychor (95% purity) demonstrated relatively weak but apparent estrogenic activity in the current assay. These findings demonstrate that the current design of the uterotrophic assay is a useful short-term screening method for the detection of estrogen-like compounds.

1776 UTEROTROPHIC EFFECTS OF ADMINISTRATION OF COUMESTROL AND 17β -ESTRADIOL IN OVARIECTOMIZED SPRAGUE DAWLEY RATS.

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Under the Food Quality Protection Act of 1996, the US EPA has been directed to develop and implement a testing program to screen chemicals for the potential to interfere with the function of estrogen and other hormones deemed appropriate by the agency. The uterotrophic assay utilizing rats has been proposed by the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) for inclusion in Tier 1 screening of chemicals for endocrine disruption. This particular study was conducted to assess the sensitivity of this assay in this laboratory. The uterotrophic responses of groups of 10 ovariectomized Charles River Sprague-Dawley rats to coumestrol and 17β -estradiol were assessed. The parameters evaluated included estrous cycling, uterine weight (wet and blotted), vaginal cornification, serum prolactin levels and serum estrogen levels. The administration of coumestrol in the diet at a concentration of 0.05% for 4 days resulted in the restoration of estrous cycling and an increase in uterine weights. The mean prolactin levels were increased in comparison to control animals (58 ng/mL vs 28 ng/mL) whereas estrogen levels were similar among the treated and control animals. Various dose levels of 17β -estradiol (1 to 50 μ g/kg/day) were administered by once daily, subcutaneous injection for 4 days to separate groups of animals. Doses up to 10 μ g/kg/day did not result in measurable estrogenic activity. The administration of 50 μ g/kg/day produced increases in uterine weight and restoration of estrous cycling. In the uterus, estrogenic activity was characterized by hypertrophy of the superficial epithelium covering the endometrium and an increase in the thickness and cornification of the stratified squamous epithelium covering the vaginal os of the cervix. Based on these results, coumestrol (0.05% in diet) and 17β -estradiol (50 μ g/kg/day, subcutaneously) can be used as positive control materials for this assay.

1777 UTEROTROPHIC ASSAY OF LOW AND HIGH ORAL GAVAGE DOSES OF 23 POLYSTYRENE MIGRATES USING IMMATURE WISTAR FEMALE RATS.

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This study was performed to address any concerns regarding styrene dimers and trimers potentially migrating from polystyrene containers into human food. Representative polystyrenes [9 general purpose (GPPS), 8 high impact (HIPS) and 6 expandable (EPS)] were eluted (50% ethanol) to obtain levels of dimers and trimers simulating human consumption of 0.5 or 5 kg of food (GPPS and HIPS samples) and of 0.5 or 3.15 kg of food (EPS samples), respectively. These two doses of polystyrene migrates were administered (oral gavage) to Wistar (Chbb:THOM-SPF) female rats (10 rats/ group) for 4 days, beginning at 22 ± 1 days of age (doses adjusted daily for body weights); absolute and relative (% body weight) uterine weights were determined at 26 ± 1 days of age. Untreated and vehicle (25% ethanol) controls, a positive control (diethylstilbestrol-dipropionate, DES-DP, 5 μg/kg/day), and low and high doses of 7 or 8 polystyrene oligomer migrates were tested in 3 blocks. DES-DP significantly increased absolute and relative uterine weights. No polystyrene oligomer migrate significantly increased absolute or relative uterine weights; thus, low and high doses of 23 polystyrene oligomer migrates were not estrogenic in the immature rat uterotrophic assay.

1778 PCBs EXHIBIT DIFFERENTIAL BINDING TO RECOMBINANT HUMAN α AND RAINBOW TROUT ESTROGEN RECEPTORS.

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Although the function and activities of estrogen and the ER are conserved between species, the amino acid sequence of the regions involved in ligand binding (D, E and F domains) are poorly conserved. This suggests that species may exhibit different responses and sensitivities to non-traditional ligands and that one species may not be an appropriate surrogate to identify and predict responses in other species. In order to investigate potential species-specific sensitivities to non-traditional ER ligands, a comparative study was undertaken in which we examined the ability of 20 structurally distinct PCBs (IUPAC: 41, 51, 58, 60, 68, 78, 91, 99, 104, 112, 115, 126, 143, 153, 169, 173, 184, 188, 190, 193) at concentrations ranging from 10^{-9} to 10^{-5} M to compete with [^{3}H]-estradiol (E_2) for binding to bacterially expressed GST-human ERα DEF (GSThERαdef; amino acids 264-595) and GST-rainbow trout ER DEF (GSTrtERdef; amino acids 224-576) fusion proteins. Both fusion proteins were purified to greater than 90% and yields provided sufficient GSThERαdef and GSTrtERdef fusion protein for approximately 300 and 100 binding assays, respectively. The GSThERαdef and GSTrtERdef both exhibited high affinity for E_2 with dissociation constants (K_d) of 1.28 ± 0.10 nM and 2.14 ± 0.30 nM, respectively. Of the 20 PCBs tested, 184 (2,2',3,4,4',6,6'-hpCB; IC_{50}=0.51 μM), 104 (2,2',4,6,6'-pnCB; IC_{50}=0.83 μM) and 188 (2',3,4',5,6,6'-hpCB; IC_{50} = 1.3 μM) effectively competed with [^{3}H]E_2 for binding to the GSThERαdef fusion protein. Conversely, six PCBs competed with [^{3}H]E_2 for binding to the GSTrtERdef fusion protein: PCBs 184 (IC_{50} =0.92 μM), 104 (IC_{50}=1.8 μM), 188 (IC_{50}=3.8 μM), 51 (2,2',4,6'-tCB; IC_{50}=5.5 μM), 91 (2,2',4,4'6-pnCB IC_{50}=6.7 μM), 41 (2,2',3,4-tCB IC_{50}=9.5 μM). This data demonstrates that ERs from different species exhibit differential ligand preferences and affinities, and that the use of a single surrogate model may be unsuitable to predict estrogenic responses in all species.

1779 BIOCHEMICAL INDICES OF ENDOCRINE EFFECTS: AN *IN SITU* APPROACH TO ASSESS THE ESTROGENIC ACTIVITY OF MUNICIPAL WASTEWATER.

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A number of environmental chemicals possess estrogenic activity including chlorinated hydrocarbon pesticides and degradation products of high-use industrial surfactants. Included in this list are potent natural and synthetic estrogens which may find their way to the aquatic environment through municipal sewage effluent. In fish and wildlife, reproductive disorders resulting from exposure to environmental estrogens may include reductions in fertility and decreased viability, as well as alterations in hormone levels or adult sexual behaviors. The estrogenic activity associated with effluent from two municipal wastewater treatment plants (WWTP) was assessed in Fall 1996-97, Spring 1997, and Summer 1998. Fish were maintained at two sites within each WWTP; sites were located prior to pre-chlorination and within the post-chlorination stream. Male channel catfish (n=10; 200 to 300 g) were placed in cages at each experimental and reference site and exposed *in situ* for 21 days. Serum vitellogenin levels ranged seasonally from no detection to 4.8 fold above reference values at the WWTP. Hepatic ethoxyresorufin O deethylase (EROD) activity was elevated 2.2 to 4.1 fold above the reference and CYP1A content also varied from 1.4 to 10.1 fold above reference values. Tissue indices showed little to no change when compared to reference values. These data demonstrate estrogenic activity in WWTP effluent at levels sufficient to elicit a biological response that appears to be dependent on the WWTP design and seasonal changes in microbial populations within the WWTP.

1780 IN VITRO VITELLOGENIN PRODUCTION BY CARP (*CYPRINUS CARPIO*) HEPATOCYTES AS A TOOL FOR DETERMINING THE (anti-)ESTROGENIC ACTIVITY OF XENOBIOTICS.

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The yolk-protein vitellogenin (Vtg) is excreted by the liver of female, as well as male fish, in response to 17β -estradiol (E2) or other estrogenic compounds. Elevated levels of Vtg have been observed in the field, in male fish exposed to sewage effluents. In this study an in vitro system was developed, using isolated hepatocytes from a genetically defined strain of carp (Cyprinus carpio), in which Vtg-production could be measured by means of a competitive ELISA, after exposure to estrogenic compounds. A number of xenobiotics was tested for (anti-)estrogenic activity, both alone and in combination with E2. The estrogenic potencies of methoxychlor, op-DDT, bisphenol-A, 4-t-pentylphenol and chlordecone, were $1*10^{-3}$ to $1*10^{-4}$ when compared to E2. Dieldrin, β -endosulfan, op-DDE and toxaphene did not cause vitellogenesis at the concentrations tested. The synthetic estrogen DES had a relative potency of: 0.5. Because Vtg-production is an integrated result of (anti-)estrogenic activity, this assay system can detect estrogens and anti-estrogens working through different modes of action. Anti-estrogens tested include Tamoxifen and dioxin-like compounds. Carp hepatocytes were also shown to be very sensitive for the Ah-receptor mediated EROD induction. This assay is the

first of its kind in a fish-species other than rainbow trout. It can be performed in a 96-well plate format from the culturing of the cells to the ELISA-determination of Vtg, making the assay both rapid and convenient.

1781 USE OF A RAINBOW TROUT HEPATOMA CELL LINE (RTH-149) FOR SCREENING ESTROGENIC CHEMICALS: COMPARISON OF TRANSFECTION METHODS AND OPTIMIZATION OF REPORTER ACTIVATION.

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A rainbow trout hepatoma cell line (RTH-149) was used to develop a screening protocol for potential estrogen disrupting chemicals in the environment. Transfection of a plasmid containing an estrogen-responsive luciferase reporter into the cells was used to determine the activation potential of these chemicals within the physiologically relevant temperature range for rainbow trout from 11 to 18°C. Multiple reagents for transfection including calcium phosphate, DEAE-dextran, and FuGENE™ 6 transfection reagent (Boehringer Mannheim) were used to optimize transfection efficiencies in this cell line. The FuGENE™ 6 reagent consistently produced higher responses compared to the other methods. To reduce the amount of background activity due to endogenous estrogen present in fetal calf serum and to avoid the growth inhibition, morphological changes and cell death produced in these cells when incubated in media with dextran-coated charcoal stripped serum, trout cells were weaned onto media containing 4% fetal bovine serum with serum components added. The estradiol concentration in this media was 0.015 - 0.06 nM as determined by RIA. This procedure provides a means of screening xenobiotic chemicals for estrogenic or anti-estrogenic activity in a model fish cell system under conditions in which cell viability is optimized.

1782 VITELLOGENIN INDUCTION IN TROUT LIVER SLICES EXPOSED TO 17-β-ESTRADIOL: A COMPARISON OF TWO INCUBATION METHODS.

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The egg-yolk precursor protein vitellogenin (Vtg) is normally only produced in the liver of female rainbow trout (RBT). However, it's production can be induced in male RBT livers, upon exposure to estrogenic chemicals. An *in vitro* male RBT liver slice assay has been developed to detect Vtg protein induction in response to xenobiotics. Two methods of slice incubation are compared: 1) individual slices in media (11°C), in culture plate wells with continuous shaking (PL); or 2) individual slices on a titanium screen, in and out of media (11°C), in a scintillation vial, termed dynamic organ culture (DOC). Slices (200 μm X 8mm) were incubated in 1.7 mL modified L-15 media and exposed to 1.0 μM 17-β-estradiol for 72 h. Protein from media and slices were sampled at 24, 48, and 72 h. Western blot analysis, using a polyclonal antibody to Vtg, was used to detect appearance of Vtg protein. Maximum Vtg was seen at 72 h, with detectable protein at 24 and 48 h in slices and media following PL incubation. In contrast, slices incubated in DOC showed little detectable Vtg above background levels after 72 h. This difference was not attributable to protein adsorption onto vial or plate surfaces. Viability assays and histopathology did not reveal any striking differences between slices incubated in PL or DOC, yet production of Vtg was significantly impacted in DOC. An optimized method of RBT liver slice incubation, for detection of Vtg induction upon exposure to estrogenic chemicals, is presented.

1783 INDUCTION AND INHIBITION OF VITELLOGENIN PROTEIN SYNTHESIS IN RAINBOW TROUT LIVER SLICES UPON EXPOSURE TO ESTROGENS AND ANTI-ESTROGENS.

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The induction of vitellogenin (Vtg) in a male rainbow trout liver slice model was used to determine estrogenic effects of diethylstilbestrol (DES), 17β-estradiol (E2) and ICI-182,780. Liver slices, 200 μm x 8 mm, prepared from immature male rainbow trout (400-600 g) were incubated at 11°C, in L-15 media, using a culture plate incubation method. Slices were exposed for 72 h to 0.01, 0.1, 1.0, 10, 100, or 1000 nM E2 or DES in slice media. Vtg protein concentration in media samples was determined by Western blot analysis and ELISA. Relative concentration of Vtg mRNA in slices was determined by ribonuclease protection assay (RPA). Vtg mRNA was detectable 8 h after exposure to as little as 1 nM E2, with peak levels recorded at 24 h. Vtg protein was detectable 24 h after exposure to E2 or DES with protein production increasing through 72 h. DES appeared slightly more potent than E2 at inducing Vtg protein in male trout livers. Vtg protein was detected in the slice incubation media after exposure to as little as 0.1 nM DES, or 1 nM E2, with maximum Vtg induction reported for the highest concentration tested, 1000 nM. The ER antagonist ICI-182,780 only partially inhibited the ability of 1000 nM E2 to induce Vtg protein, but in a dose-dependent manner, up to a maximum inhibitory concentration of 1000 nM. These results indicate the estrogenic detection sensitivity of the mRNA (RPA) and protein (ELISA and Western) assays were similar. Detectable Vtg mRNA was seen at least 16 h earlier than measurable Vtg protein. This could potentially allow assay time to be shortened from 72 h to 24 h, and theoretically increase number of chemicals that could be tested for potential estrogenicity in this system. However, advantages gained in decreased assay length with RPA are countered by the disadvantage of relatively longer sample processing times with reduced sample capacity, when compared with protein endpoints.

1784 EFFECTS OF ENDOCRINE-DISRUPTING CHEMICALS (EDCs) ON LABORATORY-EXPOSED MALE JUVENILE SUMMER FLOUNDER.

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Effects of 17β -estradiol (+ control), *o,p'*-DDT, octylphenol and *p,p'*-DDE on hepatosomatic and gonadosomatic indices, plasma steroid hormone levels, vitellogenin induction, and gonadal development were evaluated in sexually immature, laboratory-raised summer flounder, *Paralichthys dentatus.* This study was conducted to determine which of the above indicators best reflected exposure to EDCs. Test chemical, dissolved in acetone and mixed with coconut oil, was injected into fish in a body sinus twice, two weeks apart. Estrogenic (*o,p'*-DDT; octylphenol) and anti-androgenic (*p,p'*-DDE) chemicals were evaluated alone and in combination (octylphenol plus *o,p'*-DDT or *p,p'*-DDE). Blood and tissues were sampled four, six and eight weeks after the first injection. Fish exposed to a combination of *o,p'*-DDT plus octylphenol were also sampled after 15 weeks. Chemical analysis of livers from fish treated with *o,p'*-DDT or *p,p'*-DDE confirmed significant concentrations of chemical eight weeks after the first injection; octylphenol was never detectable. In our experiments, reduced GSI and plasma testosterone levels were consistent indicators of treatment with estrogenic chemicals (*o,p'*-DDT; octylphenol). However, low GSI did not necessarily reflect changes in developmental stage of the gonads. Vitellogenin synthesis was not dependable as a diagnostic indicator of exposure to estrogenic chemicals. No indicators were found that would identify exposure to the anti-androgen *p,p'*-DDE.

1785 INHIBITION OF ECDYSONE-DEPENDENT GENE EXPRESSION BY PHYTOCHEMICALS.

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Many plant compounds are able to modulate growth and reproduction of herbivores by directly interacting with steroid hormone systems. In insects, several classes of chemicals, including the phytoestrogens, interfere with molting. We investigated whether the anti-ecdysone activity may be due to interaction with ecdysone-dependent gene regulation at the level of the Ecdysone Receptor (EcR) using a reporter-gene assay. Chinese Hamster Ovary cells, which stably express the *Drosophila* ecdysone receptor (VgEcR) and the heterodimeric partner, retinoic acid receptor (RXR), were transiently transfected with response element linked to β -galactosidase. Cells were dosed with one of 8 phytochemicals, either in the absence or presence of the potent EcR agonist, muristerone A. We tested the flavone-glycoside, rutin (delays molt in insects); the four flavones luteolin and quercetin (metabolites of rutin), and apigenin and chrysin; and three non-flavones, coumestrol and genistein (both estrogenic) and tomatine (alters molt in insects). None of the phytochemicals tested were ecdysone agonists. The flavones were able to significantly inhibit EcR-dependent gene transcription at 10 and 25 μ M concentrations. We suggest that the rutin effects on molting are most likely due

to the metabolites, luteolin or quercetin, while tomatine acts via a non-EcR pathway. Neither coumestrol nor genistein inhibited EcR-dependent gene transcription. In plants, flavones are signaling molecules and cause nitrogen-fixing bacteria to form root nodules. The bacterial NodD protein which regulates this symbiosis has two ligand-binding domains which are similar to that of human estrogen receptor (hERα). NodD, EcR and ER all interact with flavones, which may have evolutionary significance.

1786 APPLICATION OF THE CALUX BIOASSAY FOR DETECTION OF PHARMACOLOGICAL AGENTS WHICH ACTIVATE THE AH RECEPTOR SIGNALING SYSTEM.

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The Ah receptor (AhR) is a ligand dependent transcription factor that mediates the majority of the biochemical and toxicological effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin) and related chemicals. Utilizing two recently developed AhR-based screening systems, the CALUX (Chemically Activated LUciferase eXpression) cell bioassay (employing cell lines stably transfected with a TCDD/AhR-responsive firefly luciferase reporter gene) and an inducible AhR-dependent DNA binding (gel shift) assay we have begun large scale screening of chemicals and chemical mixtures for their ability to activate the AhR signaling pathway. We have identified several pharmacologically-relevant chemicals including imidazoline receptor ligands (idazoxan, guanabenz and epinephrine) and benzimidazole drugs (omeprazole, albendazole and thiabendazole) which induced luciferase activity in several different CALUX cell lines. Although some of the imidazoline receptor ligands were also potent activators of AhR transformation and DNA binding in vitro, others were essentially inactive; all of the benzimidazoles were significantly less potent in the in vitro bioassay. These results, combined with those obtained using other chemicals reveal significant discrepancies between the ability of a chemical to activate the AhR in vitro and in intact cells. This clearly demonstrates the serious limitation of only using in vitro AhR-based bioassays in chemical screening approaches. (Supported by NIEHS (ES08372, ES07685, ES04699).)

1787 EFFECTS OF PHYTOCHEMICALS ON MCF-7 CELL GROWTH AND ESTROGEN RECEPTOR BINDING.

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Recent attention has focused on the isoflavanoids in soya and the flavanoids in tea as possible mediators of carcinogenesis. In this study, we measured the binding properties of three isoflavanoid compounds found in soya- genistein, daidzein and coumestrol-, and two flavanoid compounds found in tea- catechin and epicatechin. The effects of these phytochemicals on the cell proliferation of estrogen-responsive MCF-7 breast cancer cells was also studied. Receptor binding to human estrogen receptor (ER) was measured with a Beacon 2000 fluorescence polarization system (PanVera, Inc, Madison, WI). Expressed, purified ER (PanVera, Inc, Madison, WI) was preincubated for 30 min with an optimal concentration of a fluorescent estrogen analog (EF, PanVera, Inc, Madison, WI) and then concentrations of the test compound (10^{-9} to 10^{-4} M) were added to the ER-EF complex. competitive binding of the phytochemicals was measured as a decrease in polarized fluorescence after 2 hrs. MCF-7 cell proliferation was determined by seeding 10^4 cells into 24 well plates and exposing the cells to varying concentrations of the test compounds in the presence and absence of estradiol for 5 days. On the sixth day, the cells were fixed and the effects on cell growth determined by measuring total protein in the cells as compared to the protein in cells incubated with an optimum concentration of estradiol or with vehicle alone. Genistein and coumestrol displaced EF from ER in the range of 10 μM while catechin and epicatechin displaced EF from ER in the range of 100 μM. Coumestrol stimulated MCF-7 cell growth at concentration ≥10 nM and genistein stimulated MCF-7 cell growth at concentration ≥100 nM, but both catechin and epicatechin strongly inhibited the growth of MCF-7 cells at all concentrations tested (10^{-10} M to 10^{-5} M). The effects of these phytochemicals on the growth of estrogen-responsive MCF-7 breast cancer cell, therefore, do not correlate with their competitive binding to the human ER.

1788 OPTIMAL pH AND SOLVENT CONCENTRATION FOR AN *IN VITRO*, HUMAN ESTROGEN RECEPTOR GENE TRANSACTIVATION ASSAY.

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The estrogen receptor (ER) gene transactivation assay is currently being considered as a tool for large scale screening of materials from a wide range chemical classes (e.g., strong acids/bases). Therefore, the goal of this study was to determine optimal ranges for pH and solvent (dimethylsulfoxide; DMSO) concentration in this assay. MCF-7 cells were transfected with a chimeric Gal4-ER construct and a Gal4-regulated lucerifase reporter gene, then were exposed overnight to a range of 17β-estradiol (E2) concentrations (10^{-12} to 10^{-7} M), each tested at various pH levels (pH 6.8, 7.3, 7.5, 7.8, 8.0, 8.2, 8.8) or DMSO concentrations (0.1, 0.5, 1.0% v/v). At the end of incubation, the cells were lysed and the lysate assayed for luciferase activity. Maximal E2-induced reporter activity (25-40 fold relative to vehicle controls) occurred at pH 7.8, with a marked decrease in E2-induced activity at pH $\leq$ 7.5 or $\geq$ 8.0 (pH effect significant at $p<0.001$). E2-induced reporter activity tended be highest at a DMSO concentration of 0.1%, and lowest at 1.0% DMSO, while 0.5% DMSO produced somewhat variable responses across studies. These results show that pH is a critical variable in the conduct and interpretation of this ER reporter gene assay, and also suggest that DMSO concentration should not exceed 0.1% for optimal assay performance. (*Funded by The Chemical Manufacturers Association (Arlington, VA).*)

1789 USE OF A RECOMBINANT HUMAN BREAST CANCER CELL LINE, MVLN, TO SCREEN FOR ESTROGENIC ACTIVITY AMONG STRUCTURALLY-RELATED ALKYLPHENOLIC COMPOUNDS.

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Alkylphenolic compounds are used in great volumes, primarily in the manufacture of surfactants. Several of these compounds have been observed to possess weakly estrogenic activity. The purpose of the current study was to utilize the cell line, MVLN, which is a MCF-7 human breast cancer cell line that is stably transfected with a luciferase reporter under the transcriptional control of the estrogen receptor (ER) to determine relative estrogenic potencies for several alkylphenolic compounds compared to 17-β-estradiol (E_2) as a standard reference compound. Individual test compounds were tested for both their ability to produce a statistically significant response relative to the solvent control and their ability to elicit a full dose-response curve. Each test agent was tested in the absence of E_2 and in the presence of an EC_{50} concentration of E_2 (10 pM). The most potent test agent was tecnical nonylphenol (a mixture of ring and chain isomers) with a relative potency of 0.0001 compared to E_2. As expected, significant structure-activity relationships were observed. The number and position of substituents on the phenolic ring, length of carbon chain, and branched versus straight carbon chains were found to be important determinants of activity. A test agent with a straight carbon chain, 4-*n*-nonylphenol, was found to be inactive as an estrogen agonist up to 5 μM and was cytotoxic at greater concentrations. All of the alkylphenolic compounds that were tested, except technical nonylphenol, were either inactive as estrogen agonists or were weakly estrogenic. The MVLN cell line was found to be a useful screening assay that is sensitive and specific for estrogenic chemicals.

1790 SIMILARITIES BETWEEN ACETAMINOPHEN- AND ESTRADIOL-INDUCED PROLIFERATION OF CULTURED, ESTROGEN-RESPONSIVE BREAST CANCER CELLS.

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Studies in this laboratory have shown that acetaminophen, a widely used analgesic/antipyretic, induces DNA synthesis and proliferation in estrogen-responsive (ER+) but not estrogen-nonresponsive (ER-) cultured, human breast cancer cells. Like estradiol, acetaminophen contains a *p*-phenol moiety; this moiety appears to influence estradiol- and xenoestrogen-induced ER+ cell proliferation. Thus, the first goal of this research was to determine

the importance of the *p*-phenol moiety in acetaminophen-induced proliferation. To this end, effects of *p*-, *m*-, and *o*-acetamidophenol on ER+ (MCF7 and T47D) cell proliferation and on % cells in S (DNA synthesis) phase of the cell cycle were determined. Therapeutic concentrations of *p*-acetamidophenol (~ 0.1 mM) significantly increased proliferation, and the relative order of potency was *p*- > *m*- > *o*-acetamidophenol. These data suggest the *p*-phenol moiety is important in both estradiol- and acetaminophen-induced ER+ cell proliferation. The second goal of this research was to establish if estrogen receptor pathways are involved in mediating the acetaminophen-induced proliferation of ER+ cells. Two antiestrogens (ICI 182,780 and 4-hydroxytamoxifen) were tested in 2 ER+ (MCF7, T47D) and 1 estrogen-nonresponsive, ER- (MDA-MB-231) cell lines. Effects of antiestrogens on acetaminophen-induced proliferation were assessed by flow cytometry (% S phase cells) and by cell counting. In MCF7 and T47D cells (but not in MDA-MB-231 cells), both antiestrogens inhibited proliferation induced by acetaminophen and by estradiol. These data also suggest that there are similarities between estradiol- and acetaminophen-induced proliferation. Furthermore, estrogen receptors may directly or indirectly mediate the acetaminophen-induced proliferation of ER+ cells.

1791 ROLE OF THE RAT SPLICE VARIANT OF ESTROGEN RECEPTOR BETA IN ESTROGEN SIGNALLING; IMPLICATIONS FOR ENDOCRINE DISRUPTION TESTING.

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We have analysed the functional activity of an isoform of the estrogen receptor beta (ERβ) (found in rat and mouse) which has an 18 amino-acid insert in the ligand binding domain (ERβ2; ^{320}SSEDPHWHVAQMKSAAPR^{338}). The ERβ2 is capable of binding to the vitellogenin estrogen response element as demonstrated by electrophoretic mobility shift assays. The ability of ERβ2 to activate transcriptionally a reporter plasmid (consisting of a consensus vitellogenin ERE cloned upstream of a minimal SV40 promoter driving luciferase) when transiently transfected into Cos-1 cells was examined. The reporter in cells transfected with ERβ alone was activated in a dose-dependent manner by stimulation with estradiol-17β (10^{-12} - 10^{-6} M). In contrast, estradiol failed to activate the reporter in cells transfected with ERβ2 alone. Of all ligands tested to date, only the synthetic estrogen diethyl-stilbestrol (DES) was able consistently to activate ERβ2 in this assay. In addition, when cells were co-transfected with a constant amount of ERβ and increasing amounts of ERβ2 there was a significant decline in reporter activation by estradiol. Using *in situ* hybridization and RTPCR analyses, we have demonstrated expression of ERβ and ERβ2 in the rat prostate, ovary and pituitary while ERβ2 mRNA is not detectable in the αT3-1 rat pituitary gonadotrope specific cell line. These data provide evidence for an isoform of ERβ which may act as a negative regulator of ERβ function. The differences in relative abundance of these two receptors in different tissues, and the species specific nature of ERβ2 may play a role in determining species and tissue-specific responses to estrogenic substances.

1792 NEW DEVELOPMENTS IN A HAZARD IDENTIFICATION ALGORITHM FOR HORMONE RECEPTOR LIGANDS.

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Recently we described the Common REactivity PAttern (COREPA) technique to screen data sets of diverse structures for their ability to serve as ligands for steroid hormone receptors (Environ. Sci. Technol. 31:3702-3711). The approach identifies and quantifies similar global and local stereoelectronic characteristics associated with active ligands through a comparison of energetically-reasonable conformer distributions for selected descriptors. For each stereoelectronic descriptor selected, discrete conformer distributions from a training set of ligands are evaluated and parameter ranges common for conformers from all the chemicals in the training set identified. The use of discrete partitions of parameter ranges to define common reactivity patterns can, however, influence the outcome of the algorithm. To address this limitation, the method has been extended by approximating continuous conformer distributions as probability distributions. The COREPA-Continuous (COREPA-C) algorithm assesses the common reactivity pattern of biologically similar molecules in terms of a product of probability distributions, rather than a collection of common population ranges determined by examination of discrete partitions of a distribution. To illustrate the algorithm, common reactivity patterns based on interatomic distance and charge on heteroatoms were developed and evaluated using a set of 28 androgen receptor ligands. Using receptor binding affinity as the measure of biological activity, the algorithm successfully discriminated 'active' from 'non-active' ligands. Notable attributes of the COREPA-C algorithm include flexibility in establishing stereoelectronic descriptor criteria for identifying active and non-active compounds and the ability to quantify three-dimensional chemical similarity without the need to predetermine a toxicophore or align compounds(s) to a lead ligand.

1793 MOLECULAR MODELING SIMULATIONS OF RECEPTOR-LIGAND INTERACTIONS FOR ENVIRONMENTAL ESTROGENS.

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Molecular modeling and molecular dynamics have been used to study the interaction of various environmental estrogens (EEs) with the human estrogen receptor α (hERα). EEs are endocrine disrupting chemicals (EDCs) that interfere with the normal function of ER in the effected tissues of exposed species. The modeling was based on the 3-D structure of the ligand binding domain of the hERα as recently determined by X-ray crystallographic studies from two different laboratories. The available structures include two of hERα with estradiol-17ß and one of hERα with the antiestrogen raloxifene. The purpose of this study was to develop an understanding of the possible ER binding modes of nonsteroidal environmental chemicals that are known to have the capacity to displace estradiol-17ß binding *in vitro*. Ligand-receptor docking studies were performed with the classic xenoestrogens DES, DDT isomers, methoxychlor, HPTE, chlordecone (kepone), t-octylphenol and bisphenol A. Each ligand-receptor model was subjected to a molecular dynamics annealing protocol and energy minimization to achieve a final binding conformation and orientation. The relative contribution of the van der Waals, electrostatic and hydrogen bonding energies to the total potential energy of each complex was determined and compared to reported *in vitro* binding affinities. An effort was also made to characterize ligand-receptor interactions that may be responsible for the relative differences in ER affinity among these xenoestrogens. These studies have resulted in the formulation of a ligand-receptor model which addresses the disparate binding capacities of various EDC's. Furthermore, these "virtual" receptor binding studies may also be of general utility as a component of endocrine disruptor screening and testing protocols.

1794 ENANTIOMER SELECTIVE ESTROGEN ACTIVITY OF o,p'-DDT AND o,p-DDD OPTICAL ISOMERS.

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Racemic mixtures of the chiral pesticides o,p'-DDT and o,p'-DDD have previously been described as endocrine disrtuping chemicals with estrogen activity. In this report, we account the estrogen activity of the individual enantiomers of o,p'-DDT and o,p'-DDD. Optically pure samples as well as racemic mixtures were evaluated for estrogen receptor (ER) binding and reporter gene activity in mammalian cells. Binding assays utilized recombinant ER and were performed at 20ûC using the PanVera fluorescence polarization method. Reporter gene assays employed MVLN cells which are MCF-7 human breast cancer cells containing ER that have been stably transfected with an estrogen responsive vit-Luc construct. All racemic mixtures and optically pure samples tested were found to have some capacity to bind the ER as well as induce MVLN reporter gene activity at concentrations up to 10 μM. It was also shown that the MVLN activity of each test compound was inhibited by the antiestrogen ICI-182,780. However, while both enantiomers and the racemic mixture of o,p'-DDD maintained minimal estrogen activity (EC_{50} from 3000nM to 10000 nM), the R or (-) enantiomer of o,p'-DDT displayed enhanced ER binding activity and was found be 25 times more potent in the MVLN assay than the S or (+) enantiomer (EC_{50} of 250 nM vs. 6500 nM respectively). This study highlights the importance of resolving the optical isomers of chiral compounds for both endocrine disruptor characterization studies as well as environmental monitoring. Furthermore, this study suggests that the enantiomer specificity of certain chiral compounds my be utilized to develop optically pure pesticides and other industrial chemicals with decreased endocrine activity.

1795 DOES FUNCTIONAL DISRUPTION OF P53 AND THE FAS SYSTEM HAVE EFFECTS ON MALE GERM CELL APOPTOSIS?

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Mice with a targeted disruption of the p53 tumor suppressor gene were crossed with *gld* mice (deficient in Fas ligand) to produce mice deficient in both p53 and the Fas ligand. Several lines of evidence suggest that both p53 and the Fas system are important in regulating germ cell apoptosis in the rodent testis; the goal of developing a mouse deficient in both of these systems is to ascertain what the effects are on spermatogenesis and response to injury. The first aim of this study is to examine the effects of the double deficiency on the physiology of the testis, including spermatid counts, apoptotic germ cell assessment, gene expression and examination of the histology at timepoints between birth and 8 weeks. In the second aim, male mice will be exposed to injuries known to induce Fas system or p53-dependent responses such as mono(2-ethylhexyl)phthalate and γ-irradiation. The effects of these toxicants on the double deficient mice will be compared to similarly exposed p53 knockout mice, *gld* mutant mice and wild type controls. To obtain the double deficient mice, F1 heterozygote mice resulting from the initial p53 knockout and gld mouse cross were mated to produce 266 viable F2 offspring. The 8 possible genotypes were determined using a modified multiplex PCR technique, and double deficient male mice were produced at the expected frequency (9/144). Preliminary examination of testes of paired male siblings - wild type and double deficient - indicates an overall decrease in spermatogenesis: decreased testis weights, decreased seminiferous tubule diameters, and occasional retained elongate spermatids in stages IX and X. Given the viablility of the double deficient males and the preliminary data on the physiology of the testis of these mice, further characterization of spermatogenesis and response to injury in double deficient males will give more insight into the importance of the Fas system and p53 in male germ cell survival.

1796 ASSESSMENT OF TOXICANT-INDUCED MORPHOLGIC ALTERATIONS IN SERTOLI CELL-GERM CELL INTERACTIONS *IN VIVO*.

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Sertli cells are key in promoting and regulating spermatogenesis. Sertoli cells both structurally organize the seminiferous epithelium and secrete a number of factors which are essential to germ cell survival. Chronic exposure of rats to 2,5-hexanedione (2,5-HD), a metabolite of *n*-hexane, results in a testicular atrophy in which only Sertoli cells and immature germ cells remain. While *in vitro* data obtained in our laboratory have demonstrated that 2,5-HD stabilizes microtubules and interferes with kinesin-based transport, little is known about the mechanism by which 2,5-HD exposure elicits this injury, *in vivo*. This enhanced polymerization and stability of microtubules, and impaired microtubule-based transport has been proposed to underlie the progressive loss of germ cells following exposure to 2,5-HD. Intimate contact with germ cells is required for Sertoli cells to deliver nutrients and paracrine signals to germ cells, functions which are believed to be essential to their differentiation and survival. Because spermatogenesis is a highly dynamic process requiring extensive reorganization of Sertoli cell-germ cell junctions, compounds which stabilize microtubules may also impair the ability of Sertoli cells to remodel the cytoplasmic extentions surrounding germ cells, thereby impairing the ability to maintain spermatogenesis. To assess alterations in Sertoli cell-germ cell interactions following toxicant exposure, we have infected Sertoli cells *in vivo* with a replication-deficient adenovirus which expresses cytoplasmic green fluorescent protein (GFP). By confocal microscopy, we have obtained an exquisitely detailed, three-dimensional images of Sertoli cell-germ cell interactions, and can observe morphological alterations in Sertoli cells within intact seminiferous tubules. Using this tool, we plan to assess alterations in Sertoli cell-germ cell interactions following exposure to microtubule disrupting compounds colchicine, taxol and 2,5-HD.

1797 DELAYED LEUPROLIDE ADMINISTRATION RESCUES SPERMATOGENESIS IN 2,5-HEXANEDIONE-INDUCED TESTICULAR ATROPHY.

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2,5-Hexanedione (2,5-HD), a metabolite of the environmental toxicant n-hexane, produces irreversible testicular atrophy in rats, and therefore provides a useful model for studies of male infertility. Leuprolide, a gonatropin-releasing hormone agonist, reestablishes spermatogenesis after 2,5-hexadione-induced testicular atrophy when administered immediately after cessation of 2,5-hexanedione treatment. The current studies were undertaken to examine whether a delay in leuprolide administration may also affect recovery. 2,5-HD (1% v/v) was administered to Fisher rats in drinking water for 3.2 weeks. At week twelve, animals were divided into two groups, with one group receiving 3 s.c. injections of leuprolide (1.5 mg/animal; 24 days apart) and the other saline. Both groups were then allowed to recover for 12 weeks. Leuprolide treatment resulted in a significant recovery of 2,5-HD induced testicular atrophy_compared with their 2,5-HD treated controls, as determined by a significant 20% increase in testis weight, as well as histopathological analysis. Seminiferous tubules of rats exposed to 2,5-HD alone showed nearly complete (>95%) atrophy, however rats exposed to 2,5-HD and leuprolide showed a significant 20% recovery. This study indicates that administration of leuprolide can reestablish spermatogenesis when it is administered 3 months after the onset of atrophy. These findings may be of relevance to the clinical setting of male infertility, where intervention occurs long after exposure and the onset of atrophy .

1798 SEMEN QUALITY AND HUMAN MALE FERTILITY: A PROSPECTIVE STUDY WITH NORMAL COUPLES.

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A prospective study of 210 reproductive age couples was conducted to quantify relationships between semen parameters and human male fertility. Eligible couples without known risk factors for infertility, other than subsequently identified poor semen quality, were recruited. Each couple was followed for up to 12 complete menstrual cycles while they attempted to conceive. Semen quality measures were evaluated from multiple ejaculates per man with known abstinence intervals. For each cycle, the day of ovulation was predicted based on the preceding cycle, and the couple was advised to have intercourse on and bracketing that day (± 2 days). Pregnancy rate for the 12 cycle interval was 82%. Pregnancy rate was influenced significantly by parity and contraception status at study entry. Relationships between the semen quality measures and 12 cycle time to pregnancy were analyzed using Cox time to event regression analysis. Treating the variables as log-linear and adjusting for parity and contraception status, significant relationships with 12-cycle time to pregnancy (probability that relationship was not real) were found for sperm concentration (0.005), number of sperm (0.002), number of motile sperm (0.002), % normal sperm by strict criteria (0.0001) and number of normal sperm by strict criteria (<0.0001). In multivariate regression analyses, percent normal sperm by strict criteria and number of motile sperm were the most effective predictors after adjustment. These results demonstrate that several semen quality measures are predictive of human male fecundity. In addition, they provide information to allow prediction of the risk to men's fecundity from decrements in semen quality.

1799 DIFFERENTIAL SENSITIVITY OF RAT AND MOUSE TESTIS TO THE MICROTUBULE DISRUPTORS CARBENDAZIM AND COLCHICINE.

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The alkaloid, colchicine (CLC) and the benzimidazole, carbendazim (CBZ) are well known testicular toxicants in the laboratory rat. The toxicity associated with these compounds is characterized by vacuolization, detachment, and sloughing of seminiferous epithelium via a mechanism involving microtubule disruption. The present study investigated the susceptibility of mouse testis to the effect of these cytoskeletal disruptors. Adult mice received CBZ (1000 mg/kg, po) or corn oil vehicle and were killed by CO2 inhalation 15, 30, 60, 120 min, and 6 and 24 hrs later. One testis from each animal was processed for histopathology and the other was snap frozen for HPLC determination of testicular CBZ levels. Adult rats receiving CBZ (250 mg/kg, po) and sacrificed 6 hrs later, served as positive controls and these animals exhibited histopathology characteristic of CBZ toxicity. However, mice administered CBZ at a dose level four times that required to cause a lesion in the rat, showed no indication of testicular damage. Immunocytochemical staining of alpha and beta tubulin in testis was carried out 6 hr after treatment, and no differences were observed between control and treated mice. The sensitivity of rat vs. mouse to damage after intra-testicular administra-

tion of CLC was investigated at dose levels of 117.6 ug/g testis and 5.3 ug/g testis for the mouse and 5.6 ug/g testis for the rat. Sloughing of the seminiferous epithelium was extensive for the high dose CLC in the mouse but did not occur at the lower (5.3 ug/g testis) dose. In contrast, 5.6 ug/g testis administered into rat testis caused severe testicular damage. These data indicate that mouse testis is more resistant than rat testis to the effect of microtubule disrupting agents. (Supported by NIH grant #ESO7832.)

1800 EPIDIDYMAL TOXICITY OF α-CHLOROHYDRIN: EFFECTS ON GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE ACTIVITY.

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α-Chlorohydrin (ACH) directly inhibits glyceraldehyde-3-phosphate dehydrogenase (G3PDH) in caudal sperm and is thought to impair sperm motility and thus fertilizing ability. Epididymal damage seen at high ACH doses can lead to permanent infertility. The present studies have investigated ACH epididymal toxicity in the rat and the effects of ACH on G3PDH activity. Onset of epididymal changes occurred rapidly within 6 hours of ACH administration (50 mg/kg, po). Early changes included epithelial vacuolization in the initial segment and increased tubular diameter with debris and few sperm cells in the initial segment lumen. The severity of the lesion increased with time and by 24 hours, no cellular structures lined the basement membrane of the initial segment. The possibility that ACH inhibited G3PDH in cell types other than spermatozoa was investigated by measuring G3PDH activity in multiple tissues 3, 6 and 24 hours following a single dose of ACH (50 mg/kg, po). G3PDH activity was decreased by 85% in sperm, 31% in kidney, 49% in liver and 35% in epididymis 3 hours after ACH. These remained low at 6 and 24 hours. G3PDH was immunolocalized within the initial segment and found associated with the microvilli and adluminal area. Smooth muscle surrounding the tubule throughout the epididymis also showed immunopositive stain. Overall, the effect of ACH on the initial segment of the epididymis occurs rapidly, within 6 hours. These data raise the possibility that G3PDH in initial segment cells may serve a specific function which when disrupted by ACH leads to toxicity.

1801 ESTERASE INHIBTION BY MOLINATE: ROLE IN TOXICITY.

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Molinate, a thiocarbamate fungicide widely used on rice, has been shown to be a testicular toxicant in male rats. The ability of molinate to inhibit ester hydrolysis has been implicated in its toxicity. This study was designed to determine whether there is a dose and time dependent recovery of ester hydrolytic capacity in liver and testes microsomes following molinate exposure. In addition the ability of molinate to limit mobilization of cholesterol was examined. Testes and livers were collected from adult male rats previously exposed to single i.p. injections of molinate at 5, 50, 100 and 200 mg/kg in corn oil, 2 hours, 1, 2 and 7 days prior to sacrifice. Microsomes were prepared and assayed for nonspecific esterase (NSE) activity using alpha-naphthyl acetate as substrate. NSE activity in both liver and testes microsomes was markedly inhibited at all time points by the higher dose levels of molinate. The lower doses of 5 and 50 mg/kg also inhibited NSE activity in both tissues but recovery was complete by 2 days. NSE activity after 5 mg/kg was not reduced by more than 20% of control. The ability of the molinate-inhibited esterase to hydrolyze cholesterol esters was investigated using cholesteryl oleate as substrate. Addition of molinate to microsomal preparations markedly inhibited hydrolysis of the ester. These data indicate that molinate-induce inhibition of esterase activity is readily reversible especially at the lower dose levels. Moreover the esterase inhibited by molinate is capable of hydrolyzing cholesterol esters. The testicular toxicity of molinate may result from reduced availability of cholesterol required for testosterone biosynthesis. (Supported by the California Rice Growers Association.)

1802 PERCHLOROETHYLENE REDUCES RAT SPERM FERTILIZING POTENTIAL.

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Perchloroethylene exposure appears to reduce semen quality in dry cleaning workers. The objective of this study was to develop an animal model for perchloroethylene exposure. Mature male rats (100+ days of age) were exposed to perchloroethylene by inhalation (1 or 2 hours/day at 1700 ppm). Sperm were recovered after 2 or 3 weeks of exposure and evaluated for fertilizing potential in in vitro fertilization trials and subjected to motility assessment (Hobson Tracker). In an additional trial, male rats were exposed to perchlorethylene in drinking water (0.8% in 3% emulphor) or control (3% emulphor). In a third trial, young female rats (25 to 32 day) were exposed by inhalation to perchloroethylene for one week prior to oocyte recovery for in vitro fertilization. Sperm from male rats breathing perchloroethylene for two 1-hour periods/day for 3 weeks had reduced fertilizing potential (1.8 penetrated sperm/zona-free oocyte compared with 2.9 penetrated sperm/zona-free oocyte for sperm from control rats breathing room air in the same exposure chambers; $P < 0.05$). Testosterone levels were similar in exposed and control rats. Exposure to perchloroethylene in the drinking water also reduced sperm fertilizing potential (36.7% vs 51% fertilization of cumulus-intact oocytes, $P < 0.05$ and 4.4 vs 2.5 penetrated sperm/ zona-free oocyte, $P < 0.005$.) The kinematic parameter ALH (amplitude of lateral head displacement) was consistently affected by perchloroethylene exposure. Preliminary experiments suggest subtle histopathological changes in the efferent ducts consistent with the timing of initial exposure and gamete recovery. Oocytes from females inhaling perchloroethylene had reduced fertilizability (1.6 vs 2.5 penetrated sperm/zona-free oocyte). High exposures to perchloroethylene reduced gamete fertility and caused changes in rat sperm motility similar to those observed in man. Metabolic activation by cytochrome P4502E1 enzymes, particularly within the efferent ducts may be responsible for the observed changes. (Supported in part by EPA R825351 to TB and MGM.)

1803 DETECTION OF TOXICANT INDUCED CHANGES OF RAT SPERM MITOCHONDRIAL MEMBRANE POTENTIAL BY FLOW CYTOMETRY.

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The mitochondria of the sperm midpiece provides energy for motility, hence any compound affecting this organelle may ultimately affect the motility of spermatozoa. The JC-1 fluorescent stain gives a green fluorescence when mitochondrial membrane potential is low and red-orange when the membrane potential is high. The present study has used flow cytometric analysis of JC-1 staining patterns of large numbers of sperm to detect toxicant induced alterations of sperm mitochondrial function. Spermatozoa were recovered from the cauda epididymides of rats by tubule puncture and allowed to swim out into Toyoda's Medium (TM). Sperm samples were diluted to 25×10^6 sperm/ml, and 200μ L aliquots were prepared. Samples were incubated for 10 min in the presence and absence of pentachlorophenol (PCP; 0.1μ M and 1.0μ M), a known uncoupler of mitochondrial oxidative phosphorylation, then labeled with JC-1 for mitochondrial function and SYTOX-Green for viability. Microscopic evaluation showed that the mitochondria of live, highly motile sperm stained bright orange, while the mitochondria of the live, non-motile sperm stained green. Mitochondria of an intermediate class of slightly and non-progressively motile sperm stained a faint orange-green. The heads of dead spermatozoa stained bright green. Flow cytometric analysis showed populations of sperm staining patterns similar to those viewed microscopically. The percentage of spermatozoa stained bright orange, faint orange and total orange (bright orange + faint orange) in the control samples of spermatozoa were significantly higher ($P<0.01$) than in the 1.0μ M and 0.1μ M PCP treated samples. Total orange staining was nearly 4-fold higher in control samples (92-100%) than in the 0.1μ M PCP samples (8-42%) and 10-fold higher than in 1.0μ M PCP samples (4-18%). A significant ($P< 0.05$) dose response effect was found between PCP treatments. These data indicate that sperm mitochondrial membrane potential is highly sensitive to the uncoupling effects of PCP and that JC-1 staining and flow cytometric analysis may be a sensitive assay for detecting the effects of toxicants on rat sperm function.

1804 ADHESION AND SIGNALING PROTEINS ASSOCIATED WITH INHIBITED SPERMIATION.

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Spermiation, the process by which late spermatids separate from the Sertoli cell, is disrupted by many / most testicular toxicants. In this study we used immunohistochemistry to identify some of the proteins associated with the

late spermatid-Sertoli junction. We confirmed the presence of tubulin, actin, and vinculin at the luminal edge, and report that paxillin is also present here (in both round spermatids and Sertoli cells). In other cell types, these proteins have been reported to colocalize with β -integrins. While numerous attempts to find β -integrins in the testis using immunohistochemistry and Western blots were unsuccessful, clear evidence was found for the presence in Sertoli cells of N-cadherin and it's associated intracellular proteins: β -catenin, pp120, pp60^{src}, and Csk. These proteins have been shown, in other cell types, to control cadherin adhesion. Cadherins bind homotypically; we found desmoglein (another cadherin) on late spermatids in a distribution pattern complementary to that of N-cadherin. In addition, N-cadherin and desmoglein were found around late spermatids retained by the epithelium after treatment with boric acid. From these data and previous literature reports, we propose a model wherein N-cadherin and desmoglein function in adhesion, and the Sertoli cell controls this adhesion through the intracellular proteins associated with N-cadherin. This model provides a framework for hypotheses of the steps involved in the complex process of spermiation in the rat testis, and its disruption by toxicants.

1805 A NEW METHOD FOR CHARACTERIZING THE MOTION OF SPERMATOZOA.

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Proper characterization of the motion of spermatozoa is an important prerequisite for interpreting differences in sperm motility that might arise from exposure to toxicants. We set out to develop an alternative method for describing sperm motion, since existing methods do not adequately summarize the complexity of the movement pattern. Based on an exponential model that relates the curvilinear velocity to the tracking rate, we develop a statistic that indexes the predictability of the path for individual sperm. We summarize the path of each sperm using this and 2 other statistics: (1) the path displacement velocity; (2) linearity of movement. The proposed method is shown to outperform standard CASA measures in discriminating between normal and cooled sperm.

1806 RAT SPERM ACROSOMAL STAINING USING PEANUT AGGLUTININ LECTIN (FITC-PNA) LABELLING.

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The ability of spermatozoa to capacitate and undergo an acrosome reaction in vitro can be used as a test for sperm function in toxicology studies. Detection of the sperm acrosome in the rat, the animal of choice in toxicology studies, has been difficult because the acrosome is not visible by phase contrast microscopy. Several staining techniques, including labelling with fluoresceinated lectins which bind to specific carbohydrate groups in glycoproteins and glycolipids, have been used to differentiate between acrosome-intact and acrosome-reacted sperm. Fluorescein isothiocyanate-conjugated Arachis hypogea (peanut) agglutinin (FITC-PNA) has been used previously to detect the acrosome in several species. The aim of this work was to evaluate the use of FITC-PNA to detect the acrosome in rat sperm and to develop a fast, simple and reliable procedure to quantitate in vitro rat sperm capacitation and acrosome reaction. Spermatozoa were stained with FITC-PNA (50 μg/ml) using two different methods. In the first method, spermatozoa were washed in DPBS, air dried on a slide, followed by a 30 min fixation and permeabilization with cold- 95% ethanol, and staining with FITC-PNA for 30 min at 37 °C. Alternatively, sperm were fixed in 2% formaldehyde (2 h, 4 °C) followed by staining with FITC-PNA. Sperm nuclei were counterstained with the DNA fluorochrome Hoescht 33258. Confocal Laser Scanning Microscopy confirmed that FITC PNA stained the acrosomal region, with the fluorescence brightest along the dorsal crest and absent from the postacrosomal region. In contrast, when the acrosome was disrupted by sonication, the sperm did not stain with FITC-PNA. While the first method is better suited for long term storage, the alternate method provided a better image quality. Both methods offer a practical approach for detecting the rat acrosome reaction under in vitro capacitation conditions. (Disclaimer: This abstract does not necessarily reflect EPA policy).

1807 RELATIONSHIP BETWEEN DOSE AND EXPOSURE TIME ON INHIBITION OF SEA URCHIN SPERM MOTILITY BY $HgCl_2$ AND $CdCl_2$.

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Sperm of sea urchins (*Lytechinus pictus*) were collected after intracoelomic injection of 0.5M KCl in artificial sea water at pH=6.0. Under these conditions sperm are immotile and can be stored for several days at 4-5°C. Motility was restored by dilution with activation buffer (pH=8.0) for controls or with activation buffer containing the appropriate concentration of metal salts for toxicity studies yielding approximately 20-40 sperm per field in a motility chamber. Video-microscopy was carried out at room temperature using an inverted microscope connected to a video recorder. Motility was recorded on multiple viewing fields for 3 minutes. Quantitation of sperm motility was performed by collecting data from a minimum of 400 cells. Experiments were carried out in triplicate. Dose-response curves were established for $HgCl_2$ (0.1-2.5 μM) and $CdCl_2$ (1.0-10.0 μM) using 20 min exposure time. $HgCl_2$ and $CdCl_2$ inhibited motility of sea urchin sperm in a dose-dependent manner, with EC_{50}s of 0.91 μM and 2.2 μM, respectively. Dose-time-response relationships were determined under conditions of steady-state at 5, 10, 15 and 20 min with concentrations of 1.0, 1.5 and 2.0 μM for $HgCl_2$ and 2.5, 3.5 and 4.5 μM for $CdCl_2$. There was a linear relationship between exposure time and inhibition of motility at each concentration with $r^2>0.97$. Multiplying the dose (c) with exposure time (t) at EC_{50} yielded constants, 14.97 ± 1.03 μMA min (mean ± S.E., n=3) for $HgCl_2$ and 34.05 ± 2.30 μMA min (mean ± S.E., n=3) for $CdCl_2$. These findings support the notion of $c \times t = constant$ under near ideal conditions.

1808 SMOKING IS ASSOCIATED WITH SPERM ANEUPLOIDY AND LOWERED SEMINAL PLASMA ANTI-OXIDANT ACTIVITY.

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Sponsor: E S Harpur.

Studies have shown that tobacco smoking is associated with reduced semen quality and oxidative damage to sperm DNA. Our objective was to determine if smoking increased the frequency of aneuploidy in human sperm and produced oxidative stress in seminal fluid. Sperm aneuploidy was assessed using multicolor fluorescence *in situ* hybridization (FISH) from semen samples donated by 20 non-smokers and 20 smokers (>15 cigarettes per day). Only fertile samples, as defined by World Health Organization criteria, from donors aged between 20-30 years, were used in the study. Aneuploidy was assessed on 10,000 cells per sample, using DNA probes specific for chromosomes Y and 1. Malondialdehyde (MDA), a product of lipid peroxidation that reacts with thiobarbituric acid to give an absorbing compound at 535 nm, was measured to indicate oxidative stress. Cells were separated from seminal plasma (SP) by centrifugation, allowing MDA concentrations to be determined in both spermatozoal and SP fractions. Total anti-oxidant activity was measured according to the ability of SP anti-oxidants to scavenge the radical cation $ABTS^+$, (2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate)), inhibiting its absorption at 734 nm. Smokers possessed significantly higher levels of sperm aneuploid for YY1 compared to non-smokers (0.13% and 0.08% respectively; $P<0.05$), although no difference was seen in the frequency of sperm aneuploid for Y1-1 (0.09% and 0.11% respectively; $P>0.05$). Total anti-oxidant activity was significantly lower in smokers' SP compared to that of non-smokers' (1232.22± 273.17 and 1514.49± 224.82 μmol/l respectively; $P<0.05$). MDA concentration was similar in both smokers' and non-smokers' SP (4.56± 0.34 and 5.24± 0.41 nmol/ml respectively; $P>0.05$) and spermatozoal fraction (0.53± 0.04 and 0.51± 0.08 nmol/ml respectively; $P>0.05$). The results indicate that smoking is associated with elevated levels of aneuploid sperm and reduced SP anti-oxidants.

1809 A PEROXYNITRITE GENERATOR MODULATES CALCIUM-MEDIATED RAT UTERINE CONTRACTILITY.

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Nitric oxide is important in maintaining vascular tone and uterine quiescence during pregnancy. Oxidative stress in the pregnant uterus may promote the

generation of superoxide anion and, subsequently, peroxynitrite. Using standard muscle baths, this study evaluated the possible role of Ca^{2+} in peroxynitrite-induced modulation of spontaneous oscillatory contractions of uterine segments isolated from gestation day 10 rats. Exposure to 3-morpholinosydnonimine (SIN-1), a compound that generates peroxynitrite, reduced contractile frequency in a concentration-dependent manner. After 40 min of exposure, frequencies were 77, 44 and 43% of controls for 0.5, 1 and 2 mM SIN-1, respectively. The voltage-dependent Ca^{2+} channel blocker nifedipine (20 nM) reduced contraction amplitude compared to controls, followed by complete abolishment of contractions within 10 min. Co-treatment of the uterine segments with 0.5 mM SIN-1 protected against nifedipine's abolishment of oscillatory contractions. Sustained contracture of uterine segments by 60 mM KCl, which stimulates voltage-dependent Ca^{2+} influx from the extracellular space, was attenuated by the SIN-1 and nifedipine treatments. Exposure of uterine segments to 5 μ M carbachol, which initiates Ca^{2+} release from $InsP_3$-sensitive intracellular stores, initially caused a sustained contracture followed by an increase in the frequency of uterine contractility as compared to the pre-treatment frequency. A 10-min pretreatment with 1 mM SIN-1 reduced the duration of the carbachol-induced sustained contracture by 50%. It is proposed that Ca^{2+} homeostasis is sensitive to oxidation by peroxynitrite and that Ca^{2+} channels are modulated by changes in cellular redox conditions.

1810 ACTIVATION OF PROTEIN KINASE C MEDIATES THE INHIBITORY EFFECT OF LINDANE ON SPONTANEOUS UTERINE OSCILLATORY CONTRACTION.

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The widely used agricultural insecticide lindane (γ-hexachlorocyclohexane) abolished spontaneous uterine oscillatory contractions in a concentration- and time-dependent manner. The present study tested the hypothesis that protein kinase C (PKC) mediates lindane-induced inhibition of uterine contraction. Isometric contractions were measured using standard muscle baths. Acute exposure to the PKC activators phorbol 12 myristate 13-acetate (PMA) (4-100 nM), 1-steroyl-2-arachidonoyl-*sn*-glycerol (1.55-15.5 μM) or 1-oleoyl-2-acetyl-*sn*-glycerol (25-250 μM) attenuated oscillatory contractions in concentration and time dependent manners. The negative control 4α-phorbol (10-250 nM) did not affect the pattern of contraction. Cumulative addition of RHC-80267 (1-150 μM), a diacylglycerol lipase inhibitior that blocks the catabolism of diacylglycerol, resulted in concentration-dependent decreases of contraction. The importance of PKC activation in inhibition of uterine contraction was further investigated by pretreating muscle strips with 50 nM PMA for 18 h, in order to deplete the tissue pool of PKC, prior to their exposure to 30 μM lindane. The PMA-pretreated strips were able to contract with greatly improved force and frequency in the presence of lindane compared to strips exposed to lindane alone. Because PKC-depleted tissues could slowly replenish their PKC pool, 50 nM of PMA was added into the bathing buffer. When PMA-pretreated strips were exposed to lindane in the continued presence of PMA, the strips maintained oscillatory contractions similar to controls. Using western blotting, the α, β, γ, δ, ε, and ζ isoforms of PKC were identified in the cytosol fraction of uterine smooth muscles isolated from midgestational rats. However, only the α, γ and ε isoforms were activated by 30 μM lindane, as indicated by translocation from the cytosol to the membrane. Inhibition of conventional PKC by Gö 6976 had no effect on lindane-mediated abolishment of contraction. However, Gö 7874, a PKC inhibitor which inhibits novel PKC and conventional PKC, partially reversed lindane's inhibition on uterine contraction. In conclusion, lindane-induced inhibition of spontaneous uterine oscillatory contraction is mediated by the activation of PKC, likely specific to novel PKC isoforms.

1811 DELTA-HEXACHLOROCYCLOHEXANE DISRUPTS THE ACTIONS OF UTEROTONINS IN VITRO.

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Oxytocin and prostaglandin $F_2\alpha$ ($PGF_2\alpha$) are key uterotonins that enhance the force of uterine contraction and determine the success of labor. This study examined the hypothesis that delta-hexachlorocyclohexane (δ-HCH) interferes with the actions of uterotonins to cause dysfunctional labor. To assess the changes in contraction, strips of uterine tissues from gestation day 10 rats were suspended in muscle baths and isometric contractions were recorded with a polygraph. After a period of equilibration, muscles were treated with δ-HCH for more than 6 hr. Exposure of uterine strips to 10, 20, 50 and 100 μM δ-HCH completely inhibited spontaneous oscillatory contractions in 64.0± 15.1, 23.3± 4.8, 16.8± 2.5 and 9.3± 1.9 min, respectively. Muscles treated continuously with ≤ 20 μM δ-HCH recovered partially within 215.0± 56.7 min, but higher doses of δ-HCH were cytotoxic. Delta-HCH (50 μM) significantly reduced the force of uterine contraction induced by 12 and 60 mM KCl, in a manner similar to that produced by 20 nM nifedipine, a voltage-sensitive calcium channel blocker. The inhibition of KCl response by δ-HCH was not due to its cytotoxic effects because the muscles recovered and developed spontaneous oscillatory contractions upon removal of δ-HCH. Pretreatment of muscles with 20 μM δ -HCH for 10 min inhibited the initial sustained and subsequent oscillatory uterine contractions induced by 8 nM oxytocin, 40 nM $PGF_2\alpha$ and 5 μM carbamoylchloride. Exposure of uterine strips to 100 μM α-tocopherol, an antioxidant, did not reverse the inhibitory effect of δ-HCH on uterotonin-induced responses, although it reversed δ-HCH-induced inhibition of spontaneous oscillatory uterine contractions. Our data suggest that δ-HCH interferes with response to uterotonins and therefore may cause improper uterine contractions resulting in delayed or dysfunctional labor.

1812 ALPHA-HEXACHLOROCYCLOHEXANE INHIBITS SPONTANEOUS AND OXYTOCIN-INDUCED UTERINE CONTRACTIONS.

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The alpha isomer constitutes 62% of the technical product of hexachlorocyclohexane (HCH) that is still used as an agricultural pesticide, and is a common environmental pollutant. Like other HCHs, α-isomer accumulates in adipose tissues, and may affect reproductive functions. The present study examined the hypothesis that α-HCH may interfere with labor by inhibiting spontaneous and agonist-induced uterine contractions. Using standard muscle baths, isometric contractions were recorded in longitudinal uterine strips from gestation day 10 rats *in vitro*. Exposure of uterine strips to 10, 50, 100 or 200 μM α-HCH induced a dose-dependent decrease in force of contraction, and completely abolished uterine contractions in 290± 11.1, 58.4± 8.8, 8.6± 1.4 and 4.5± 0.38 min. The muscles recovered and developed spontaneous oscillatory contractions in the continued presence of α-HCH, but the force and oscillatory activity of contractions were greatly reduced compared to basal uterine contractions. Furthermore, exposure of uterine strips to 20 μM α-HCH for 10 min inhibited the initial sustained and subsequent oscillatory contractions induced by 8 nM oxytocin. The duration of sustained contraction induced by oxytocin in the presence of α-HCH was 1.25± 0.25 min, which was significantly less compared to that with oxytocin alone (12.7± 2.98min). Pretreatment of the uterine strips with 100 μM α-tocopherol, an antioxidant, reduced α-HCH-induced inhibition of uterine contraction, but did not reduce the inhibitory effect of α-HCH on the oxytocin response. These data suggest that inhibition of uterine contraction is partially due to oxidative stress produced by α-HCH, but that the inhibition of oxytocin-induced uterine contraction involves a different mechanism. If these mechanisms are functional *in vivo*, then exposure to α-HCH may inhibit the effects of oxytocin, and cause dysfunctional labor without necessarily affecting the onset of labor.

1813 GAMMA GLUTAMATE CYSTEINE LIGASE (GlCl) ACTIVITY AND EXPRESSION DURING THE ESTROUS CYCLE IN THE RAT OVARY.

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Total glutathione (GSH) content of the rat ovary and *in vitro* release from perfused ovaries change during the estrous cycle, being highest on the day of estrus. Such changes may result in differential sensitivity of the ovary to toxicants during different cycle stages. However, little is known about the regulation of these changes. We hypothesized that changes in the activity and expression of the rate-limiting enzyme in GSH synthesis, GlCl (formerly known as gamma-glutamylcysteine synthetase), are responsible for the cyclic variation in GSH content. To test this hypothesis we measured GlCl activity and mRNA levels for the heavy chain, catalytic subunit of GlCl (HCGlCl) in normally cycling adult rats. The estrous cycles of female rats were monitored by vaginal cytology for at least two consecutive cycles prior to sacrifice on metestrus (MET), diestrus (DI), proestrus (PRO), or estrus (EST). Ovaries were snap frozen in liquid nitrogen and stored at -70 C. Ovarian GlCl activity was measured in the right ovaries by HPLC. RNA was extracted from the left ovaries and Northern blot analysis was performed using 32P-labelled cDNA probes to HCGlCl and to Cyclophillin, a house-

keeping gene which was used to normalize the densitometric data. Preliminary results comparing GlCl activity in ovaries from EST animals to that in MET, DI or PRO animals reveal a trend towards higher GlCl activity in estrous ovaries. In contrast, HCGlCl mRNA levels were constant from MET through PRO and then declined by about 25% on EST. In summary, the increase in ovarian GSH content on the day of estrus appears to be mediated by an increase in GlCl activity in the absence of increased expression of HCGlCl. Future studies will further investigate the regulation of GSH changes during the estrous cycle and the possible effects on ovarian sensitivity to toxicants. (Supported by NIEHS Center for Ecogenetics and Environmental Health (1 P30 ES07033-03) and by US EPA #R-825358-01-0 (to E.F.).)

1814 DIETARY GENISTEIN DOES NOT MAINTAIN SURGICALLY INDUCED ENDOMETRIOSIS IN RATS.

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Endometriosis is a disease in which uterine tissue proliferates in extrauterine sites. Exposure to chemicals with estrogenic activity via diet and environment has led to the investigation of potential links between these chemicals and endometriosis. Using a surgical model to simulate endometriosis, we explored the potential for the phytoestrogen, genistein, by injection and diet, to sustain endometriosis in rats. Uterine tissue was attached to intestinal mesentery of 8-week-old Sprague Dawley rats. After 3 weeks, the rats were ovariectomized and the implants measured. Following 3 weeks of daily injections (sc), the animals were necropsied and implants located. Injections of genistein (50 and 16.6 ug/g BW) or estrone (1 ug/rat) sustained the implants; genistein at 5.0 ug/g BW did not. Injection of the vehicle, dimethylsulfoxide (DMSO), did not support the implants. Using the same surgical protocol, we gave genistein via the diet (250 or 1000 mg genistein/kg AIN-76A diet); control rats were fed AIN-76A diet. Dietary genistein at either dose did not sustain the implants. To assess estrogenic actions of genistein in both the injection and dietary models, we measured uterine estrogen receptor alpha (ER) and progesterone receptor isoforms A and B (PR) by western blot. Rats injected with estrone or genistein (50 or 16.6 ug/g BW) had significantly reduced uterine ER, compared to DMSO treated animals, while 5.0 ug/g BW genistein did not. PR (B form) was significantly increased by estrone and genistein (50 ug/g BW). PR (A form) was significantly increased by genistein (16.6 and 5.0 ug/g BW). The dietary genistein treatments did not alter uterine ER or PR. These results suggest a critical role for ER modulation and genistein bioavailability in the maintenance of the implants.

1815 ROLE OF GLUTATHIONE IN UTERINE SECRETIONS DURING EARLY PREGNANCY.

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In this study we examined the concentration of reduced glutathione (GSH) in reproductive tract fluid of mice during the first four days of pregnancy and the effects of blocking GSH synthesis with buthionine sulfoximine (BSO). Previous studies demonstrate that, while preimplantation embryos are unable to synthesize GSH de novo until the blastocyst stage, GSH is present in reproductive tract fluid and extracellular GSH protects preimplantation embryos in vitro. In this study we explore the hypothesis that GSH in reproductive tract fluid protects preimplantation embryos from glutathione-depleting toxicants in vivo. Reproductive tracts were removed from female mice and flushed with buffer. Subsequently, GSH in reproductive tract flushings was measured with flourescent HPLC. BSO was administered four times per day to female mice (4 mmol/kg/injection) from day 0 through day 4 of gestation (day 0 being the day of vaginal plug detection). The concentration of GSH in reproductive tract fluid on day 1 (d1) and day 2 were found to be significantly higher than day 0 and nonpregnant (np) (d1 33 nmol GSH/mg of protein, d2 31, d3 12, d0 4.0, np 9.9; $p< 0.05$). Furthermore, on day 0 there was a higher concentration of GSH in the oviduct than in the uterus, while on day 2 there was a higher concentration in the uterus (Ut) than in the oviduct (Ov) (Ovd0 13 nmol GSH/mg protein, Utd0 8.2, Ovd2 26, Utd2 73; $p<0.05$). Injection of mice with BSO caused a decrease in GSH concentration in reproductive tract fluid (control (C) np 77 nmol GSH/mg protein, BSOnp 3.0; Cd2 24 nmol total glutathione/mg protein, BSOd2 6.9; Cd3 26, BSOd3 1.8; $p<0.05$). Control mice also exhibited a larger percentage of embryos developing to the 2-cell stage in vivo (C 79%, BSO 66%, $p<0.05$). BSO mice exhibited more degenerated embryos (C 11%, BSO 25%, $p<0.05$). These data indicate that there is an increased concentration of GSH in reproductive tract fluid during the period of development when embryos are unable to synthesize GSH, that inhibition of GSH synthesis by BSO causes a decrease in the concentration of GSH in reproductive tract fluid, and that this inhibition has a detrimental effect on preimplantation embryos. In conclusion, these data support the hypothesis that GSH in reproductive tract fluid has a role in protection of the preimplantation embryo.

1816 DIFFERENTIAL EFFECTS OF ATRAZINE (ATR) ON SERUM LUTEINIZING HORMONE (LH) AND PROLACTIN (PRL) IN LONG-EVANS HOODED AND SPRAGUE DAWLEY RATS.

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The chlorotriazines (i.e., ATR, simazine and cyanazine) have been shown to disrupt the control of ovarian function, advance reproductive aging and lead to an early onset of mammary gland tumors in Sprague Dawley (SD) rats. We reported previously that these effects occur as a result of altered LH and PRL secretion. We also noted that at each dose tested, ATR disrupted estrous cycles in the Long Evans (LE) hooded rats to a greater extent than in SDs. Here, we examined the effect of ATR on LH and PRL secretion to determine the dose and duration of exposure required to disrupt pituitary hormone secretion in these two strains. LH and PRL surges were induced in ovariectomized females by implanting (sc) an estrogen-filled silastic capsule. Four different doses of ATR (50, 100, 200 & 300 mg/kg) were administered 21 (21X) or 3 (3X) days prior to or on the day (1X) of the surges. LH and PRL was suppressed in 1X LE rats at 300 mg/kg, but these measures were not affected in the SD rats. The surge of both hormones was reduced at each dose level in 3X LE rats, but again 3X SD LH and PRL were not affected. After 21 days of ATR pretreatment, however, the LH and prolactin in both strains were reduced similarly. These data demonstrate a clear strain difference in the onset of the effects of ATR on pituitary hormone secretion and suggest the LE female is more sensitive to the effects of this cholotriazine. *(This abstract does not reflect US EPA policy.)*

1817 OVULATORY BLOCKADE IN THE RAT BY LOCAL OVARIAN ADMINISTRATION OF SODIUM DIMETHYLDITHIOCARBAMATE: MECHANISM OF EFFECT RELATED TO TIME OF EXPOSURE DURING THE CYCLE.

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When dosed systemically, the fungicide sodium dimethyldithiocarbamate (DMDC) can block ovulation in rats by interfering with hypothalamic activation of the luteinizing hormone surge. Local ovarian administration late on the afternoon of vaginal proestrus (pro) can also curtail oocyte release from exposed ovaries, while such dosing on diestrus 2 (di2) bilaterally blocked ovulation by suppressing the surge. In brain, the effect is likely due to a decrease in norepinephrine synthesis, but this does not appear true for ovarian exposures. Current experiments attempted to define the mechanisms underlying these local ovarian effects. Cycling Long-Evans rats were given DMDC (0 - 300 μmol/ovary) by unilateral intrabursal injection at 1900h on either pro or di2. Rats were then killed at various times, and serum samples obtained for estradiol (E2) or progesterone (P4) determinations. Groups of rats dosed on pro were also co-injected with prostaglandin F2α (PGF2α) or sodium nitroprusside (SNP). These females were then killed at 1200h on the following day and oviducts flushed for oocytes to explore whether ovarian DMDC could have affected either local prostaglandin or nitric oxide (NO) involvement in follicular rupture. In this regard, dithiocarbamates have been reported to inhibit NO synthase activity. The data showed that while the di2 exposures caused some small but statistically significant elevations in serum E2, a prompt 4-5 fold rise in P4 likely provided a down-regulatory feedback effect on the hypothalamus to block the surge. For the ovulatory suppression following pro DMDC injections, no effect was seen in response to graded PGF2α co-injections. However, co-administration of the NO donor SNP (5 μmol) & DMDC (200 μmol) was able to increase the percentage of ovulating ovaries from 25 to 66%, an effect marginally beyond statistical significance (p set at 0.05), but one that given the importance of NO in ovulatory process warrants further investigation as a potential target for the dithiocarbamates.

1818 CELLULAR AND MOLECULAR MECHANISMS OF ACTION OF THREE NOVEL ENVIRONMENTAL ANTIANDROGENS: TWO PHTHALATES AND LINURON.

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The antiandrogenic fungicide vinclozolin alters sex differentiation by competing with androgens for the androgen receptor (AR), inhibiting AR-DNA binding and altering androgen-dependent gene expression in vivo and in vitro. Several antiandrogens have been identified by their ability to induce reproductive malformations, but the mechanisms of action of these chemicals are unknown. We have developed a battery of in vitro and in vivo assays to determine if a chemical 1) is an AR ligand, 2) alters androgen-dependent gene expression in vitro or in vivo, 3) alters AR-DNA binding or 4) alters AR distribution of function in vivo. In this phase of the investigation, the potential AR-mediated action of linuron (L), a metabolite of L hydroxylinuron, dibutyl (DBP) and diethylhexyl (DEHP) phthalate and their metabolites monobutyl and monoethylhexyl phthalate were examined using competitive binding with rat AR and/or human AR (hAR) in COS cells, DHT-induced gene expression in transiently cotransfected CV-1 cells, gene expression in stably transfected CHO cells and AR-DNA binding in the promoter-interference assay. The phthalates did not bind hAR, inhibit (or stimulate) gene expression in CHO cells or inhibit AR-DNA binding in the promoter interference assay. At 10 μM, the highest dose tested, DEHP inhibited DHT-induced gene expression in CV-1 cells. As DEHP does not bind hAR, this effect may result from an interaction with cell specific coactivators or from altered cell function. Hydroxylinuron, and to a lesser degree linuron, competed with DHT for rat AR (EC_{50}=30 μM) and inhibited DHT-hAR induced gene expression in both the CV-1 (EC_{50}=0.2-0.5 μM) and CHO (EC_{50}=1-10 μM) cells, while in the COS cell, hydroxylinuron displayed hAR binding with an EC_{60} at 10 μM, the highest concentration. Although linuron, DBP and DEHP alter sexual differentiation their mechanisms of action appear to differ. (Disclaimer: These abstracts do not reflect USEPA policy.)

1819 8-METHOXYPSORALEN COMPROMISES OVARIAN FOLLICLE DEVELOPMENT IN WISTAR RATS.

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The psoralens are naturally occurring metabolites found in many crop plants, including common grocery fruits and vegetables. Synthetic forms of 5-methoxypsoralen (bergatpten) and 8-methoxypsoralen (xanthotoxin) are widely used in skin photochemotherapy. Our previous research documented for the first time that dietary bergapten and/or xanthotoxin reduced birthrate in female rats when males and females were exposed to these chemicals. Subsequent studies demonstrated that the two compounds, administered to female rats (mated to undosed males), significantly reduced levels of circulating blood estrogen, and the number of implantation sites, pups, and corpora lutea, compared with the control group, suggesting a direct effect on ovarian follicles. The present study was initiated to determine whether effects of xanthotoxin can be observed in ovarian follicles. Female Wistar rats were dosed daily (30 days) with vehicle control or xanthotoxin (30 mg/kg, p.o.). Ovaries were collected and prepared for histological evaluation. Antral follicle diameters (>600μm) were larger ($p<0.05$) in ovaries from xanthotoxin-dosed as compared with control rats. Additionally, the granulosa cell layer thickness, and granulosa cell layer:follicle diameter ratio were reduced ($p<0.05$) in dosed animals. This provides evidence that xanthotoxin compromises the granulosa cell population in large antral follicles, and is consistent with its observed reduction in circulating 17b-estradiol levels. (Supported by NIEHS/NIH #ES00288 (to MMD) and ADCRC #9808 (to PBH).)

1820 THE ARYL HYDROCARBON (AhR) RECEPTOR MAY BE INVOLVED IN THE DEVELOPMENT OF OVARIAN FOLLICLES.

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The AhR is believed to mediate the toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). However, the endogenous, physiological functions of the AhR signaling pathway are only beginning to be understood. The results reported here are part of a larger study by our group that examines the role of the AhR in the developing mammalian reproductive tract. Using AhR knockout mice and their wild-type littermates, we investigated the possibility that the AhR is important for the normal development of ovarian follicles. Ovaries were collected from knockout and wild-type mice on postnatal days 2-3, and complete serial sections were prepared for histological examination. Every tenth section was sampled to estimate the total number of primordial and primary follicles per ovary. Our results indicate that knockout ovaries contained about 65% more primordial follicles than wild-type ovaries (26,048 ± 1287 vs. 15,787 ± 2772; n = 3; p = 0.008). There was no significant difference between the number of primary follicles in ovaries from knockout and wild-type mice (n=3; p = 0.65). These data suggest that the AhR plays a role in the establishment of the primordial follicle pool, but not in the growth of primordial follicles to the primary stage by postnatal days 2-3. (Supported by the Heinz Family Foundation (JF), HD27194 (AH) and ES01332 (RP).)

1821 BIPHASIC EFFECTS OF OCTYLPHENOL ON TESTOSTERONE BIOSYNTHESIS BY CULTURED LEYDIG CELLS FROM NEONATAL RATS.

E P Murono, R Derk, J H deLen. *NIOSH, HELD, Morgantown, WV.* Sponsor: V Castranova.

Octylphenol (OP), a surfactant additive widely used in the manufacture of various detergents and other commercial products, has been reported to mimick the actions of estrogen in many cellular systems. In the present studies, the direct effects of OP on basal and human chorionic gonadotropin (hCG)-stimulated testosterone production by cultured Leydig cells from neonatal rats (6-7 days of age) were evaluated. Exposure of cultured cells to increasing concentrations of OP (1-2000 nM) and 10 mIU/ml hCG for 24 h caused a consistent biphasic pattern of testosterone response. Lower concentrations of OP enhanced testosterone levels (~10-70% above control), while higher OP concentrations (100-2000 nM) progressively decreased testosterone from peak levels to ~ 40-80% below control at the highest OP concentration. Interestingly, increasing concentrations of 17β-estradiol (1-1000 nM) were without effect on testosterone formation under the same conditions, and the biphasic pattern of testosterone biosynthesis elicited by increasing concentrations of OP and hCG was unaffected by concommitant treatment with 10 or 100 nM ICI 182,780, which is considered a pure estrogen antagonist. Therefore, these actions of OP do not appear to be mediated through the classic estrogen receptor α or β pathway. Because the biphasic pattern of testosterone biosynthesis was observed in response to exposure with increasing concentrations of OP and 0.1 mM 8-Br-cAMP, it appears that the main site(s) of action occurs following the generation of cAMP. In addition, because pretreatment of cells with increasing OP concentrations and hCG had no effect on the conversion of steroid precursors (1 μM each of 22 (R)-hydroxycholesterol, progesterone or androstenedione) to testosterone, it appears that the main actions of OP occur prior to the mitochondrial cholesterol side-chain cleavage step. Furthermore, because concommitant treatment of cells with antioxidants (α-tocopherol or butylated hydroxyanisole) did not alter the biphasic testosterone response pattern to increasing OP concentrations and hCG, it appears that OP is not acting as a pro-oxidant in producing these effects. It will be important to determine whether this dose-sensitive response to OP is observed *in vivo* and whether the maturational status of Leydig cells influences their sensitivity to OP and similar chemicals.

1822 OVOTOXIC SPECIES DIFFERENCES BETWEEN RATS AND MICE WITH 3-METHYLCHOLANTHRENE AND 7,12-DIMETHYLBENZ[A]ANTHRACENE.

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Polycyclic aromatic hydrocarbons (PAH, contaminants in cigarette smoke) destroy ovarian follicles in laboratory animals, and are therefore the likely cause of early menopause in women smokers. The purpose of this study was to compare the ovotoxicity in rats and mice caused by repeated low dose exposure to PAHs, specifically 3-methylcholanthrene (3MC) and 7,12-dimethylbenz[a]anthracene (DMBA), because this more closely mimics human exposure. Ovaries were collected from female F344 rats and B6 mice following dosing for 15d with 3-MC and DMBA, and were prepared for histological counting of oocytes. 50% pre-antral follicle loss at 15d was seen with 0.15 mg/kg 3-MC in mouse primordial follicles, (152.7± 29 total folli-

cles counted, control; 68.6± 30.6 follicles, $p<0.05$, n=5), and in rat growing follicles (11.3± 1.2 follicles, control; 6.3± 0.75 follicles, $p<0.05$, n=4). 50% loss of primordial follicles was seen with 7 mg/kg DMBA in rats (167.0± 5.4 follicles, control; 82.0± 15.3 follicles), and with 0.35 mg/kg in mice (180.0± 47.0 follicles, control; 25.1± 11.0 follicles, $p<0.05$, n=6). Loss of primordial follicles caused by 3-MC and DMBA was greater ($p<0.05$) in mice than rats. Thus, primordial follicles in rats were most susceptible to DMBA and those in mice were most susceptible to 3-MC. Additionally, 3-MC (0.15 mg/kg) targeted primordial and primary follicles in mice, but growing follicles in rats. In using low-dose exposures we have demonstrated differential ovotoxicity targeting with these two PAHs. These observations suggest that 3-MC and DMBA produce ovotoxic effects by different mechanisms. (NIEHS Center grant ES06694, ADCRC 9809.)

1823 17β-ESTRADIOL RESCUES PRIMARY FOLLICLES FROM 4-VINYLCYCLOHEXENE DIEPOXIDE-INDUCED APOPTOSIS.

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Repeated dosing with 4-vinylcyclohexene diepoxide (VCD), a known occupational ovotoxicant, accelerates atresia via apoptosis in small preantral follicles in ovaries of rats and mice. The mechanism by which VCD alters follicular apoptosis is unknown, however this destruction is particularly apparent in immature animals. Physiologic atresia, a normal process in the ovary, slows as an animal enters puberty while 17β-estradiol (E_2) increases with the onset of cyclicity. The estrogen β receptor (ER) has recently been reported to be expressed in the granulosa cells of ovarian follicles, suggesting a role of E_2 in normal ovarian function; however the specific roles of this receptor in follicular development and cell fate are unknown. The hypothesis tested was that E_2 dosing of immature rats will rescue small follicles normally destroyed in VCD-induced apoptosis. Immature rats (d28) were dosed daily for 15 days with vehicle control (sesame oil), VCD (80 mg/kg, i.p.), or VCD and E_2 (0.1 mg/kg, s.c.). Ovaries were removed and either fixed, stained, and sectioned or dissociated for follicle isolation. RT-PCR of isolated fractions of small follicles (<100 μm) in both control and VCD treated animals demonstrated expression of mRNA encoding ERβ, but not ERα. The PCR product was confirmed as ERβ by oligonucleotide sequencing. Histologic evaluation of ovarian slices showed that VCD dosing caused a significant decrease in primary follicles ($p<0.05$, control=47.3 ± 1.76, VCD=29.25 ± 1.93 follicles in the total of every 40th section counted). However, concurrent VCD and E_2 dosing reversed the VCD-induced loss of primary follicles (VCD&E_2=49 ± 3.32). Physiologic dosing of E_2 given to immature animals appears to selectively protect primary follicles from VCD-induced apoptosis and suggests that VCD may interact with the ERβ receptor. (NIEHS Center grant ES-06694, Arizona Disease Control Research Commission.)

1824 TRANSPORT OF 2,4-D INTO THE SEMINAL FLUID OF DOGS.

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Chemicals can be transported from the semen via the fluid or bound to sperm, to the gamete at fertilization, or to the developing embryo. However, little is known of the amount of toxicant that can be transmitted by the father into the environment of the conceptus, or the potential for adverse reproductive effects resulting from this exposure. Some epidemiology studies have suggested a possible association between male exposure to 2,4-D and adverse reproductive effects. In the present study, the possibility of 2,4-D entering the seminal fluid of dogs and binding to spermatozoa was assessed. Dogs were dermally exposed to 1, 10, or 20 mg 2,4-D/kg body weight. Seminal fluid was collected daily over 4 days as a split-ejaculate, separating the pre-sperm fraction from the sperm-rich and post-sperm fractions. 2,4-D was detected in both fractions indicating that it is secreted into the seminal fluid and is not merely an artifact of urine contamination. Of the applied dose, 0.0134% (±0.0113) was excreted in the seminal fluid over 96 hours irrespective of the amount applied. The percent of dose excreted in the urine increased as the dose decreased; 8.44%, 1.22% and 1.69% was excreted when the dose was 1,10 or 20 mg/kg respectively. The herbicide was detected in the seminal fluid and urine within 24 hours of dosing and was not detected in the seminal fluid after 120 hours but was still present in the urine. Statistically significant dose effects were observed in both media. Using radiolabelled 2,4-D *in vitro*, it was established that 2,4-D does not bind to spermatozoa.

1825 LACK OF EFFECTS OF NOSE-ONLY INHALATION EXPOSURE ON TESTICULAR TOXICITY IN MALE RATS.

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Reductions in testicular mass, sperm motility and mating frequency have been attributed to the stresses caused by confinement in nose-only exposure tubes. This study was designed to determine whether nose-only exposure of rats caused testicular toxicity following cooling of the exposure room and appropriate acclimation (The Toxicologist, Vol 15, 181, 1995). Twenty male rats were exposed nose-only for approximately two hours per day for 28 days prior to cohabitation and throughout a 14-day cohabitation period. To reduce thermal stress the exposure room temperature was maintained at 64° to 70°F and exposure tubes were cut to length so that not more than two inches of the tail-cooler was within the tube. Twenty control rats were housed in the same room as the exposed rats. End-points monitored were body weight, testicular weight, sperm count, sperm motility and histopathology of the testes, epididymides, prostate and seminal vesicles. The control rats gained weight more rapidly than the exposed rats. All the rats in both groups mated successfully, and testicular weights, normalized to body weight, were similar for both groups. There were no microscopic changes that could be correlated to an adverse effect on the reproductive tissues in the male rats placed in exposure tubes. Nose-only exposure for up to two hours per day for a total of 42 days did not cause adverse effects on the reproductive organs or performance of male rats.

1826 ASSESSMENT OF XANTHOTOXIN AS A REPRODUCTIVE TOXICANT IN MALE WISTAR RATS.

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The psoralens are plant metabolites found in common fruits and vegetables. The synthetic form of 8-methoxypsoralen (xanthotoxin) has been employed in combination with long-wave ultraviolet radiation, as a commercial drug since the 1940's for the treatment of psoriasis and other mitotic dermatoses. Although, an effective drug for skin photochemotherapy, xanthotoxin has been observed to cause lethal, mutagenic, and genotoxic effects in a wide variety of organisms. Previous studies revealed that xanthotoxin reduced birthrates in rats when both sexes were administered the drug. During subsequent studies, where dosed females were mated with undosed males, percent reduction in birthrate was less compared with when both males and females were treated. Thus, we hypothesized that the decrease in birthrate was due, in part, to direct effect of xanthotoxin on the male reproductive function. Male rats were dosed for 30 days with vehicle control or xanthotoxin (30mg/kg, p.o.). Animals were sacrificed and the seminal vesicles, testes, prostate gland, epipdidymis, and pituitary gland were collected and weighed. The study demonstrated that xanthotoxin significantly reduced the weights of the following organs when compared to the control; prostate, seminal vesicles and the epididymis (caput, cauda, and corpus) ($p<0.05$). The pituitary gland in the xanthotoxin-treated animals was also significantly reduced ($p<0.05$). This study provides evidence that xanthotoxin compromises normal physiological development in the male reproductive system. (Supported by NIEHS/NIH Grant ES-00288 to MMD.)

1827 (-)-FTC, AN OXATHIOLANE NUCLEOSIDE ANALOG FOR THE TREATMENT OF HIV AND HBV INFECTION, DID NOT PRODUCE ADVERSE EFFECTS ON REPRODUCTION AND DEVELOPMENT IN MICE.

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(-)-FTC is a nucleoside analog with potent activity against HIV-1 and HBV viruses, and is in Phase II/III clinical trials for treatment of HIV-1 infection in adults. The following GLP-compliant studies were conducted in CD-1® mice to characterize the potential reproductive and developmental toxicity of this candidate antiviral: 1) fertility and early embryonic development (Seg I); 2) teratology (Seg II); and 3) pre-and postnatal development with maternal evaluation (Seg III). Doses of 0, 250, 500, and 1000 mg/kg/day were tested in these experiments. There was no maternal or fetal toxicity (gestation-day 18) in a dose range-finding study in mice given (-)-FTC at doses of 0,

250, 500, and 1000 mg/kg/day. (-)-FTC had no effect on fertility, weights of reproductive organs, sperm motility or counts, and early embryonic development in the Seg I study. In the Seg II study, there were no treatment-related increases in gross, visceral, or skeletal abnormalities in mouse fetuses at any dose. Plasma concentrations of (-)-FTC in the Seg II study at 0.5 h post-dose on gestation-day 15 were proportional to dose; 50.8± 13, 88.1± 14, and 186± 14 μ g/mL (mean± standard deviation; n=5) for the 250, 500, and 1000 mg/kg/day doses, respectively. Parturition, lactation, and mothering, as well as pre-/postnatal development and fertility of the F_1 mice were not affected by (-)-FTC in the Seg III study. These favorable data support the further development of (-)-FTC for treatment of HIV-1, particularly in pregnant women and pediatric patients.

1828 THIRTEEN WEEK ORAL TOXICITY STUDY OF DIFLUOROMETHYLORNITHINE (DFMO) IN COMBINATION WITH TAMOXIFEN IN FEMALE RATS.

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DFMO is an irreversible inhibitor of ornithine decarboxylase, the rate-limiting enzyme in the biosynthesis of polyamines, and inhibits tumorigenesis in animal models. Tamoxifen (TAM), a nonsteroidal antiestrogen, is used for treatment of estrogen receptor-positive breast carcinoma. Since these agents may be used for combination therapy, the toxicity of DFMO in combination with TAM was evaluated in female rats following thirteen weeks of daily administration by gavage. Dose levels in mg/kg/day were: 0 (vehicle control), 1000 DFMO, 0.25 TAM, 2.5 TAM, 0.25 TAM + 1000 DFMO and 2.5 TAM + 1000 DFMO. No mortalities occurred. DFMO produced dermal lesions consisting of scab formation and abrasions. Decreased body weight gains were produced by DFMO and TAM. Serum albumin, total protein, cholesterol and triglyceride levels were decreased in all drug-treated dose groups, with albumin and total protein decreased to the greatest extent in the combination dose groups. TAM increased numbers of red blood cells, whereas DFMO induced slight anemia. DFMO produced intestinal lesions consisting of necrosis of crypt epithelium and crypt microabscess that were enhanced by TAM co-administration. TAM induced lesions in the ovaries, fallopian tube, vagina, cervix and uterus, indicating inhibition of ovulation and reproductive cycle arrest in the proestrus stage. Co-administration of DFMO and TAM resulted in additive toxicity. (Supported by NCI Contract No. NO1-CN055144-MAO.)

1829 EFFECTS OF INORGANIC MERCURY ON THE REPRODUCTIVE PERFORMANCE OF C57/BL6 MICE.

A Khan, A Atkinson, T Graham, S Thompson, S Ali, and S Hussain. *CVMNAH, Tuskegee University, Tuskegee, AL, USA.*

The effects of inorganic mercury (Hgcl2) treatment on the reproductive performance of mice were evaluated. Males and females were dosed for 40 and 16 days prior to mating, respectively. Pairs of male and female mice were given 0.00, 0.25, 0.50, or 1.00 mg/kg/day of Hgcl2 via oral gavage. Males were continuously dosed throughout mating and the females were continuously dosed throughout mating, gestation and lactation. Fertility, litter size, and viability indices were calculated and analyzed. The fertility indices were 44, 16, 16, and 18% and the viability indices were 91, 96, 42, and 17% at 0.00, 0.25, 0.50, and 1.00 mg/kg/day, respectively. Mice exposed to Hgcl2 had significantly reduced fertility in all dose groups and in viability among the two higher dose groups when compared to the control. The mean numbers of pups per litter were 6.49, 7.25, 8.35 and 5.68 at 0.00, 0.25, 0.50, and 1.00 mg/kg/day dose groups, respectively. Exposure to Hgcl2 did not effect the litter size. However, the number of pups born alive was significantly lower in the treated groups when compared to control. These results provide evidence which suggests that Hgcl2 produced adverse effects on reproductive performance of mice (Supported by MHPF/ATSDR Cooperative Agreement # 5R51/ATR398004.)

1830 ZINC CHLORIDE DOSE RANGE FINDING REPRODUCTIVE STUDY IN RATS.

T Graham, A Khan, A Atkinson, O Clark, S Thompson, S Ali, and S Hussain. *CVMNAH, Tuskegee University, Tuskegee, AL.*

The purpose of this study is to conduct a reproductive dose range finding study by examining the effects of zinc chloride on reproductive organs of rats. Male and female Sprague Dawley rats (40-45 days old) were administered 0.0, 60.0, 80.0, 120.0, or 160.0 mg/kg/day of zinc chloride via oral gavage for 14 days. Body weights were recorded twice weekly. Twenty four hours following the final exposure, animals were scarified. Testis, epididymis, seminal vesicle, prostate, ovary and uterus were removed, weighed and prepared for routine light microscopic evaluation. The weights of the seminal vesicle and testis were significantly decreased at the 160.0 mg/kg/day dose. There were no significant changes in the weights of female reproductive organs. Histomorphologic evaluation of the male reproductive organs revealed giant cells in the lumens of epididymal ducts at all levels of zinc treatment, along with prostatic acinar atrophy at 60.0, 80.0, and 120.0 mg/kg/day dose groups. No lesions were seen in the reproductive tissues that were examined in females (Supported by MHPF/ATSDR Cooperative Agreement # 5R51/ATR398004.)

1831 HYDRAMETHYLNON EFFECTS ON MALE REPRODUCTION IN YOUNG ADULT RATS.

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Sponsor: A K Reddy.

Hydramethylnon (HM) is an amidinohydrazone insecticide widely used by consumers and pest control operators (PCO) for the control of ants and cockroaches. Animal studies in several species have shown HM is a male reproductive toxicant. However, the mechanism as well as the exposure timeframe required to elicit adverse reproductive effects have not been well elucidated. Because of its widespread use of consumers to this insecticide, there is a concern about accidental dermal exposure to children and any possible testicular effects. To assess the potential hazard in household insecticides, a study was conducted to determine the effects of an acute exposure to young male rats. Since the dermal penetration rate of HM is relatively low (<1%), the material was administered by subcutaneous (sc) injection to assure that a higher systemic dose was achieved. Young male rats were given single sc injections of HM in an oil carrier at 100, 500, or 1,000 mg/kg of body weight. Daily clinical observations were conducted for 14 days. Clinical effects were limited to decreased body weight gain and decreased food consumption at 500 and 1,000 mg/kg. Animals were sacrificed 14 days post-exposure. Both gross and histopathological examinations revealed no apparent lesions in testes, epididymes or seminal vesicles at any dose. Relative testes to body weight ratios showed statistically significant increases only at 1,000 mg/kg dose. The study showed that single exposures even at high doses fail to elicit the testicular effects in young male rats.

1832 LACK OF EVIDENCE FOR EFFECTS OF DICOFOL ON REPRODUCTIVE HORMONE FUNCTION FOLLOWING DIETARY EXPOSURE IN RATS.

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Recently, the potential impact of environmental chemical exposure on reproduction and development via alteration of endocrine function has gained increasing attention, with particular emphasis on estrogenic and androgenic activities. Dicofol, a miticide, has been widely cited as an endocrine disruptor, due to its close structural similarity to DDT and DDE, which have known estrogenic and/or antiandrogenic activity. However, there is currently no direct evidence that dicofol alters reproductive hormone function. This study was designed to provide a comprehensive functional assessment of the effects of dicofol on reproduction and development in rats following dietary exposure at concentrations of 5, 25 or 125 ppm. This study was designed in accordance with draft EPA guidelines for reproductive toxicity studies which have been expanded to increase the sensitivity to altered reproductive hormone function. Parental animals were exposed to dicofol for 10 weeks, mated, offspring delivered, and offspring exposure continued to sexual maturation. Evaluation included, general toxicity, reproductive performance and fertility, estrus cycling, sperm analysis, age at puberty in offspring, organ weights and histopathology. Dicofol and its metabolite were detected in parental serum, neonatal tissues, milk and weanling serum, demonstrating exposure in parental animals, and gestational and lactational exposure of offspring. There were no effects of dicofol exposure on reproduction and fertility, or on survival or growth of offspring. There were no effects on estrus cycling or sperm parameters, or on age at vaginal patency, or preputial separation in female and male offspring, respectively. There were no treatment-

related effects on reproductive organ weights or histopathology at any dose. Liver weight was increased and histopathology of the liver was observed in both parental animals and offspring at 125 ppm. In conclusion, dicofol administration in the diet produced no functional effects indicative of reproductive endocrine disruption at doses which produced target organ toxicity.

1833 SPECIES-SPECIFIC NEPHROTOXICITY INDUCED BY GLUTATHIONE CONJUGATION OF EFAVIRENZ IN RATS.

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Efavirenz (EFVZ), a potent non-nucleoside reverse transcriptase inhibitor for treatment of HIV infection, produced renal tubular epithelial cell necrosis in rats within 24 hours of a single oral dose ≥ 350 mg/kg. Nephrotoxicity was not observed in cynomolgus monkeys given EFVZ for ≤ 1 year at doses yielding plasma EFVZ concentrations higher than those producing nephrotoxicity in rats. A cysteinyl-glycine conjugate of an hydroxylated metabolite of EFVZ (CG-HEFVZ) was found in the urine, and the corresponding glutathione conjugate (GSH-HEFVZ) was found in the bile of EFVZ-treated rats. To investigate the potential nephrotoxicity of GSH-HEFVZ, it's formation or catabolism was inhibited in rats *in vivo* and the effect on EFVZ nephrotoxicity evaluated. Rats were given: 1) 700 mg/kg of EFVZ, 2) 700 mg/kg EFVZ 1 h after treatment with the GGT inhibitor acivicin (10 mg/kg iv), or 3) 700 mg/kg of deuterated EFVZ (d-EFVZ; specifically reducing formation of the hydroxylated metabolite of EFVZ, the obligate precursor to GSH-HEFVZ). Total urinary CG-HEFVZ was decreased by 63% in rats pretreated with acivicin and then given EFVZ versus rats given EFVZ alone. All rats (9/9) given EFVZ had renal lesions, whereas only 5 of 9 rats pretreated with acivicin had lesions. Similar decreases in nephrotoxicity and urinary CG-HEFVZ were observed in rats given d-EFVZ compared to rats given EFVZ. Results of this study provided compelling evidence that glutathione conjugation of EFVZ was directly involved in EFVZ-induced nephrotoxicity in rats. In contrast to rats, no CG- or GSH-HEFVZ is found in urine of EFVZ-treated cynomolgus monkeys or humans.

1834 FORMATION AND METABOLISM OF HYDROQUINONE GLUTATHIONE CONJUGATES IN EKER RATS.

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The Eker rat carries a germline mutation in the Tsc-2 tumor suppressor gene which predisposes these rats to the development of renal cell carcinoma. We have used the Eker rat to study the influence of genetic susceptibility on hydroquinone (HQ)-induced nephrocarcinogenesis. Although HQ generally tests negative in standard *in vitro* mutagenicity assays, glutathione (GSH) conjugates of HQ are potent nephrotoxicants in F344 and SD rats, implying a role for cytotoxicity in the carcinogenic response. To assess whether the Eker rat is a suitable model for HQ-induced nephrocarcinogenicity, we determined the metabolism and acute toxicity of HQ in this rat strain. LC-MS/MS and HPLC-EC analysis of bile (3 h) from Eker rats treated with HQ (0.45 μmol/kg, po) revealed the presence of 2-(GSyl)HQ (792 ± 277 μmol), 2,5-*bis*-(GSyl)HQ (86±31 μmol), 2,6-*bis*-(GSyl)HQ (22 ± 2 μmol) and 2,3,5-*tris*-(GSyl)HQ [TGHQ] (20 ± 4 μmol). In addition, dose dependent (TGHQ, 7.5 - 20 μmol/kg ip; HQ, 4.5 - 6.0 μmol/kg po) increases in BUN, and in the urinary excretion of alkaline phosphatase, γ-glutamyl transpeptidase (γ-GT), glucose, LDH and GST were observed 19 h post-injection. Renal γ-GT, cysteine conjugate N-acetyltransferase and N-acetylcysteine conjugate deacetylase activities were 1.2±0.3 μmol/mg/min, 3.24±0.19 and 0.71 ± 0.04 μmol/mg/min respectively. Thus, nephrotoxic HQ-GSH conjugates are formed from HQ in the Eker rat, and the activity of the enzymes involved in the processing of these conjugates are similar to those found in SD and F344 rats. The Eker rat is therefore an excellent animal model with which to examine mechanism(s) of HQ-GSH mediated nephrocarcinogenesis. (GM39338, ES07784)

1835 CHANGES IN TUMOR SUPPRESSOR GENE AND GROWTH FACTOR EXPRESSION DURING QUINOL-THIOETHER MEDIATED NEPHROCARCINOGENICITY IN EKER RATS.

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Hydroquinone (HQ) is a rodent nephrocarcinogen that generally tests negative in standard mutagenicity assays. 2,3,5-*Tris*-(glutathion-*S*-yl)HQ (TGHQ) is a potent nephrocarcino-genic metabolite of HQ in the Eker rat. The Eker rat carries a germline mutation in the Tsc-2 tumor suppressor gene which predisposes these rats to the development of renal cell carcinoma. We now report that tumor formation in Eker rats exposed to HQ and TGHQ involves two events: the first being the inherited germline mutation of the Tsc-2 gene, and the second, induced by HQ and TGHQ, results in loss or mutation of the remaining normal allele of this gene. Loss of heterozygosity at the Tsc-2 locus, with subsequent loss in tuberin expression, the protein product of the Tsc-2 gene, occurs in tumors obtained from Eker rats treated with HQ (0.45 μmol/kg, 10 mo) or TGHQ (2.5 μmol/kg for 4 mo followed by 3.5 μmol/kg for 6 mo) suggesting that the Tsc-2 gene functions as an efficient tumor suppressor gene in normal renal tissue, and that HQ and TGHQ cause alterations in this gene. Tumor formation in TGHQ treated rats is preceded by the overexpression of transforming growth factor α (TGF-α) in preneoplastic lesions (obtained 4 mo after initiation of treatment) suggesting that TGF-α plays an important role in supporting tumor development during renal carcinogenesis. HQ and TGHQ were also capable of transforming isolated renal epithelial cells *in vitro*. Transformation frequency increased 6-20 fold and 1.8-4.0 fold in TGHQ and HQ treated cells, respectively. The data confirm that TGHQ is a nephrocarcinogenic metabolite of HQ and that loss of Tsc-2 gene function and overexpression of TGF-α play important roles in tumor development. (GM39338, ES07784, CA63613)

1836 DICHLOROETHYLENE INHIBITION OF ANION SECRETION IN MICROINJECTED HEPATOCYTE COUPLETS.

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1,1-Dichloroethylene (DCE), a contaminant in drinking water, selectively produces liver injury including damage to bile canalicular membranes and defective hepatic secretion of organic anions. In order to determine the mechanisms of DCE-induced inhibition of transport, we used an isolated cell system to examine substrate specificity, reversal agents and the role of a specific DCE-glutathione conjugate. METHODS: Isolated hepatocyte couplets were prepared by collagenase-perfusion of rat liver. Secretion of fluorescent anions into dilated canalicular spaces was determined qualitatively by fluorescence microscopy and secretion rates were quantitated using serial confocal microscopy. Microinjection was performed by impalement of one cell of a couplet and pressure injecting through intracellular micropipets. RESULTS: DCE incubation (5 mM) of hepatocyte couplets inhibited carboxyfluorescein secretion (20% for DCE vs. 61% for control; $p<0.001$) but had no effect on fluorescent bile acid secretion (53% vs. 47%). Inhibition was not observed until 30 min and became maximal by 60 min. The DCE effect was not present in couplets pretreated with dibutyryl cAMP, known to recruit transport proteins to the canalicular membrane. The thiol reducing agent, dithiothreitol (2 mM) also completely prevented transport inhibition but did not reverse inhibition after DCE exposure. These results suggested that thiol-reactive intermediates may be involved. We started to test this hypothesis by simultaneously microinjecting the known DCE conjugate S-2-chloroacetylglutathione (CAG) and a fluorescent transport substrate intracellularly into hepatocyte couplets. CAG injection had no apparent immediate effect on secretion. CONCLUSIONS: Our data suggest that DCE-inhibition of hepatic transport is not simply the result of a rapidly produced glutathione conjugate serving as a direct inhibitor of a canalicular membrane transport protein. The slow onset of the effect, as well as prevention by cAMP suggests that a more delayed process, such as DCE-attenuated recruitment of transporters to the canalicular membrane may be involved. (Supported by NIH DK 34806.)

1837 ROLE OF GLUTATHIONE IN THE BILIARY EXCRETION OF ARSENICAL DRUGS.

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After administration of the inorganic sodium arsenite or arsenate to rats, the biliary excretion of arsenic (As) is rapid, is accompanied by the biliary output of large amounts of glutathione (GSH) and is completely arrested by the GSH depletor diethyl maleate (Gyurasics et al., Biochem. Pharmacol. 41: 937, 1991; 42: 465, 1991). We have studied the biliary excretion of trimelarsan (TMA) and melarsoprol (MAP) in rats in order to determine whether GSH is also involved in the hepatobiliary transport of these trivalent organic arsenicals that are used as therapeutic agents. After injection of either drug (100 μ mol/kg iv), as was rapidly excreted in bile (up to 1 μmol/kg/min, ~ 55% of dose/100 min). Concurrently, TMA and MAP increased the biliary output of GSH 2 and 5 fold, and lowered the hepatic GSH content by 24% and 27%, respectively. In TMA-injected rats, pretreatment with diethyl maleate or buthionine sulfoximine decreased the initial biliary excretion of As by 75% and 40%, respectively, whereas in MAP-injected rats these GSH depletors diminished As output by 45% and 20%. HPLC analysis of the bile collected after administration of TMA or MAP indicated the presence of the same GSH conjugate (purportedly melarsen-diglutathione) representing ~ 80% and ~ 45% of biliary metabolites, respectively. Some TMA was also excreted in the bile unchanged, whereas MAP appeared there also as two glucuronides amounting to ~ 50% of the biliary MAP metabolites. The GSH depletors virtually prevented the biliary excretion of TMA and MAP as the GSH conjugate, but did not diminish, or even increased, their excretion in other forms. These studies indicate that the biliary excretion of TMA and MAP (1) is significant in their disposition, (2) is partially dependent on the hepatic availability of GSH, as these arsenicals are excreted in part as a GSH conjugate, and (3) is concomitant with increased appearance of GSH in bile, probably originating from dissociation of the unstable GSH conjugate of these arsenicals. Thus, conjugation with GSH is important in the elimination of both TMA and MAP, however, glucuronidation is also involved in the fate of MAP.

1838 SUSPENSIONS OF FRESHLY ISOLATED RAT HEPATOCYTES GENERATE HIGH LEVELS OF NITRIC OXIDE.

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Nitric oxide (NO) is a reactive radical and intracellular/intercellular messenger. NO can induce numerous biochemical alterations in hepatocytes including inhibition of protein synthesis, mitochondrial respiration and CYP450 activities. In addition, NO can act as an antioxidant by scavenging reactive oxygen species and inhibiting lipid peroxidation. We investigated NO production in freshly isolated rat hepatocyte suspensions. Hepatocytes were isolated by standard collagenase perfusion techniques and incubated in modified Waymouths media at 37ûC for 6 hours. Extracellular nitrite levels (a breakdown product of NO) were measured by a fluorimetric assay. The level of extracellular nitrite remained at basal levels (<0.2 nmole/10^6 cells) for approx. 3 hours post-isolation, which was followed by a rapid increase in nitrite formation reaching 5 to 10 nmole/10^6 cells by 6 hours, constituting an up to 100-fold increase in nitrite level. The formation of nitrite was blocked by the nitric oxide synthase (NOS) inhibitors, N-methyl arginine or aminoguanidine. The nitrite burst was also inhibited by a nontoxic concentration of the protein synthesis inhibitor, cycloheximide. Collectively, these findings suggest that hepatocytes in suspension produce high levels of NO and that the inducible form of NOS may be involved. The NO production by hepatocyte suspensions was also inhibited by thiol depleting agents including diethylmaleate, ethylmethane sulphonate and N-ethylmaleimide, and the absence of extracellular calcium. In addition, antioxidants such as trolox and deferoxamine, were shown to both delay and attenuate nitrite production suggesting a relationship between the formation of reactive oxygen species and nitrite. These results provide the first evidence of marked NO production by rat hepatocyte suspensions that may have important implications for both mechanistic and metabolic studies conducted with this model system. (This work was supported by NIEHS/NIH grant # R01ES 05452.)

1839 MECHANISMS OF RESISTANCE TO OXIDATIVE STRESS INDUCED BY EXPOSURE TO NITRIC OXIDE.

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Pretreatment of mammalian cells with H2O2 has been shown to induce an oxidative stress response which enables cells to resist cytotoxicity associated with further challenge by higher levels of oxidants (J. Cell. Physiol. 131:364-373; Arch. Biochem. Biophys. 318:231-240). The hypothesis that nitric oxide (NO() is capable of inducing a similar response was tested. HA1 hamster fibroblasts were pretreated with media containing approximately 1.5 mM genuine NO(, or 2.5x10-13 moles/cell H2O2 as a positive control. When challenged with H2O2 8 to 48 hrs following pretreatment, both NO(- and H2O2-pretreated cells demonstrated resistance to H2O2-induced clonogenic inactivation when compared to N2 and sham treated controls. NO(induced resistance to oxidative stress appeared to be maximal between 8 and 24 hrs following pretreatment. The accumulation of c-fos and c-jun mRNA and immunoreactive protein also increased between 0 and 8 hrs following pretreatment. Inhibition of glutathione synthesis during the first 8 hrs of recovery following NO(-pretreatment inhibited the development of resistance to H2O2-mediated cytotoxicity. These data show that nitric oxide pretreatment induces resistance to subsequent challenge with H2O2-induced oxidative stress. Furthermore, these results suggest that nitric oxide exposure is capable of inducing an adaptive response to oxidative stress in mammalian cells and this adaptive response requires the presence of glutathione. (Supported by HL51469 and ESO578101.)

1840 1,2-DICHLOROBENZENE INDUCES OXIDATIVE STRESS IN HEPATOCYTES ISOLATED FROM FISCHER-344 AND SPRAGUE-DAWLEY RATS.

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1,2-Dichlorobenzene (1,2-DCB) is used as an industrial solvent and is present in the environment. Although 1,2-DCB is a potent hepatotoxicant in male rats, the Fischer-344 (F-344) rat is far more susceptible to liver injury than the Sprague-Dawley (SD) rat. Studies performed *in vivo* indicate that 1,2-DCB-induced hepatocellular oxidative stress, marked by increased glutathione disulfide (GSSG) production, is greater in F-344 rats than in SD rats. This difference in oxidative stress between the rat strains may explain their differential sensitivity to 1,2-DCB-meditated liver injury. The objective of this study was to determine if the differences in oxidative stress between F-344 and SD rats could be observed in vitro in primary cultured hepatocytes and if this oxidative event corresponds to differences in activating protein (AP-1) transcription factor activity. Hepatocytes were isolated from naive F-344 and SD rats and treated with 1,2-DCB (0-20 nmol, in vapor phase) for 0-13 hr prior to measuring lactate dehydrogenase (LDH) activity, the concentrations of glutathione (GSH) and GSSG, and AP-1 activity. Interestingly, the cytotoxicity of 1,2-DCB in isolated hepatocytes as determined by LDH leakage into media was similar for both F-344 (LC_{50} = 6.5 nmol) and SD (LC_{50} = 7.4 nmol) rats. In contrast, treatment of hepatocytes with 1,2-DCB altered the concentrations of GSH and GSSG only in cells isolated from F-344 rats, but not SD rats. In F-344 rat hepatocytes treated with 1,2-DCB (3.55 nmol) GSSG concentrations were maximally elevated (by 15%) at 1 hr, while GSH concentrations were reduced (to 78% of control) by 6.5 hr. Enhanced translocation of AP-1 into the nucleus was observed in hepatocytes of both rat strains 4 and 6 hr after 1,2-DCB (3.55, 7.1 and 12.43 nmol) treatment, providing a molecular marker of oxidative stress. These *in vitro* results correlate with previous *in vivo* data that show the oxidative stress that occurs within the hepatocyte following 1,2-DCB treatment is greater in the F-344 rat. (Supported by NIEHS Grant (ES06095), Center Grant #P30-ES06694-02 and an AFPE Scholarship.)

1841 ACRYLONITRILE-INDUCED OXIDATIVE STRESS IN NORMAL HUMAN ASTROCYTES.

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Previous studies in our laboratory and others have indicated that Acrylonitrile (VCN) induces acute and chronic neurotoxicity and brain tumor in animal microglial cells. Glutathione (GSH) depletion was suggest-

ed as the initiator of oxidative stress in VCN-induced neurotoxicity and immunotoxicity. Astrocytes possess 30% of total brain GSH and express various immunological characteristics including secretion of cytokines. We hypothesize that astrocytes could be a primary target cells for VCN effects in the brain. Therefore, the mechanism of VCN-induced neurotoxicity was studied by exposing proliferating NHA to various doses of VCN (100- 400μ M). Oxidative stress, secretion of TNF-α, damage to cell membrane and levels of endogenous antioxidants including GSH and cattalos were assessed. The results indicate: (1) a dose dependent increase (150-300% of control, P< 0.05) in the formation of reactive oxygen intermediates (ROI), (2) levels of promutagenic DNA lesion (8OHdG) were elevated at 400μ M of VCN to 275% of control, (3) depletion of GSH and increased GSSG levels (GSH/GSSG ratios in control and treated cells were 3.15 and 1.45 respectively), (4) catalase activity was increased (135% of control) at 100μ M VCN, while at 400μ M its levels were 60% of control (P< 0.05), (5) increased secretion of TNF-α at 400μ M VCN. (6) a dose dependent increase in LDH (160-220% of control) with corresponding decrease in cell viability. The current studies suggest that VCN-induces oxidative stress in astrocytes and depletion of GSH and catalase may further enhance VCN-induced microglial damage.

1842 ACRYLONITRILE INDUCES THE RELEASE OF TUMOR NECROSIS FACTOR-α (TNF-α), ALTERS GLUTATHIONE HOMEOSTASIS AND ACTIVATES OXIDATIVE STRESS IN MOUSE ALVEOLAR MACROPHAGES.

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Acrylonitrile (VCN), widely used monomer induces lung toxicity and cancer. The mechanism by which VCN induces its pulmonary toxicity was investigated using mouse alveolar macrophages (MAM). MAM were exposed to various concentrations of VCN (50-400μ M) for 4hr. The release of TNF-α was quantitated by ELISA and formation of reactive oxygen species (ROS) was determined flourometrically. Oxidative status of the macrophages was examined by measuring intracellular glutathione (GSH/GSSG) using standard redox cycling assay. The results indicate: (1) The viability was significantly ($p < 0.05$) decreased following exposure of MAM to various concentration of VCN (75% to 37% of control). (2) A concentration dependent increase in ROS formation ($p < 0.05$) and maximum levels of ROS (240% of control) were detected at 400μ M VCN. (3) An increase in extra-cellular TNF-α levels with only 400μ M VCN induced statistically significant ($p < 0.05$) increase (118% of control). (4) A concentration dependent decrease ($p < 0.05$) in reduced GSH (85% and 74% of control). (5) A significant ($p < 0.05$) increase in the formation of oxidized glutathione (GSSG) (197% of control) indicating that the cells are undergoing oxidative stress. The data provide evidence that the mechanism of VCN induced pulmonary toxicity could be mediated via activation of alveolar macrophage functions and depletion of the intracellular antioxidant, glutathione. ROS interact with membrane lipids or DNA causing oxidative cellular damage that may be lead to pulmonary toxicity or cancer.

1843 n-PROPYLTHIAZOLIDINE CARBOXYLIC ACID (PTCA) STIMULATION OF HEPATIC GLUTATHIONE (GSH) RECOVERY AFTER DEPLETION BY DIETHYLMALEATE (DEM) IN THE RAT.

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The ability of PTCA, a cysteine (Cys) precursor, to stimulate GSH recovery after depletion by DEM was investigated using the isolated perfused rat liver. DEM in corn oil (6 mmol/kg) was injected ip 30 min before the start of surgery. After perfusion for a 30-min equilibration period (time zero), PTCA was added to the recirculating fluid (100 ml). GSH and Cys in serial liver, bile, and blood samples were determined by HPLC with electrochemical detection. In livers from untreated rats (n=5-6), GSH and Cys concentrations were 4.7 &πλυσμν; 0.6 and 0.08 &πλυσμν; 0.06 μmol/g, respectively, at time zero and 3.9 &πλυσμν; 0.5 and 0.02 &πλυσμν; 0.02 μmol/g, respectively, at 4 h. Cumulative biliary excretion (4.5 h of perfusion) of GSH and Cys was 72 &πλυσμν; 20 and 28 &πλυσμν; 11 nmol/g, respectively. DEM pretreatment (n=3-5) significantly ($p<0.05$) decreased hepatic GSH concentration (to 0.4 &πλυσμν; 0.4 μmol/g at time zero and 2.1 &πλυσμν; 0.9 μmol/g at 4 h) and cumulative biliary GSH excretion (to 15 &πλυσμν; 4 nmol/g). In livers from DEM-pretreated animals, PTCA caused dose-related increases in hepatic sulfhydryl levels. At a PTCA dose of 1 mmol (n=5-7), hepatic Cys increased to 0.4 &πλυσμν; 0.1 μmol/g at 1 h ($p<0.05$), and then plateaued. Hepatic GSH increased continuously to 10 &πλυσμν; 1 μmol/g at 4 h ($p<0.05$). There were also significant increases in blood Cys at 4 h (from 0.001 &πλυσμν; 0.002 to 0.2 &πλυσμν; 0.05 μmol/ml) and cumulative biliary Cys excretion (to 149 &πλυσμν; 52 nmol/g). The results demonstrate that PTCA stimulates GSH recovery after depletion by DEM and suggest that the effect involves the release of Cys for GSH synthesis.

1844 GLUTATHIONE DEPLETION IN PRIMARY RAT HEPATOCYTES AS AN ADDITIVE EFFECT OF DIFFERENT TOXIC MECHANISMS.

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The toxicity of acrylic and methacrylic acid esters and structurally similar compounds is often thought to be related to the electrophilic chemical reactivity of their unsaturated carbon bond. Experimental reaction rates of these compounds towards glutathione show a clear distinction between the fast reacting acrylates and the slow reacting methacrylates. However, some slow reacting methacrylates show a very high toxicity. This implies that reactivity at the unsaturated carbons is not the only toxic mechanism. The present work tested an alternative toxic mechanism of these compounds: hydrolysis of the ester moiety. Glutathione depletion was used as an effect measurement for both mechanisms. Primary rat hepatocytes were exposed in air-tight culture flasks to acrylates or methacrylates for three hours. Lactate dehydrogenase(LDH) leakage, glutathione depletion in the cells and the release of malondialdehyde(MDA) and acetaldehyde into the medium were measured at the end of the exposure period. The results show that the alcohol moiety of the reactive esters can be liberated by hydrolysis. For allyl methacrylate, allyl alcohol will be formed, known to produce lipid peroxidation(LPO) in the liver. High MDA concentrations were indeed found in cells exposed to allyl methacrylate which indicates LPO. For allyl methacrylate, GSH depletion is paralleled by LDH leakage. For the chemically reactive acrylates, glutathione depletion was found to be a more sensitive parameter than LDH leakage. In mixture experiments with different acrylates and methacrylates, glutathione depletion was shown to be a concentration additive effect.

1845 EFFECT OF CHRONIC ACRYLONITRILE ADMINISTRATION ON GLUTATHIONE STATUS IN RATS.

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We found earlier that acrylonitrile (AN) produced a dose-dependent depletion of glutathione (GSH) and cysteine (Cys) in many tissues following acute administration (Toxicologist 36:80, 1997). Little is known about GSH status following chronic exposure. The aim of this study was to determine the effects of chronic AN treatment in rats. Male Sprague-Dawley rats were exposed to AN at 10, 50, and 100 ppm in drinking water for 7, 14, and 28 days. GSH, Cys, and glutathione disulfide (GSSG) concentrations in five tissues were measured by HPLC with electrochemical detection. AN-valine adduct formation in blood was also determined. AN produced dose-dependent increases in forestomach (FS) GSH concentration. At 50 and 100 ppm, GSH concentrations in FS increased to 148 and 182%, respectively, of untreated control values after 7 days of treatment. The elevated levels persisted up to day 28. No effect was found at 10 ppm. In glandular stomach, the only significant increase in GSH was found at 100 ppm. No changes in GSH levels were found in the other tissues studied (liver, kidney and heart). FS Cys levels were increased only in animals exposed to AN at 100 ppm for 28 days. AN had no effect on tissue GSSG concentrations. AN produced time- and dose-dependent increases in AN-valine adduct formation. It was concluded that chronic exposure to AN results in increases in GSH and Cys in selective tissues. (Supported by NIEHS P20 ES06832.)

1846 EFFECTS OF AN ACUTE ETHANOL ADMINISTRATION ON HEPATIC GLUTATHIONE IN RATS.

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Factors implicated in changes of the hepatic glutathione (GSH) concentration following an acute ethanol administration were examined in rats. Adult female rats treated with either ethanol (4 g/kg, po) or an isocaloric amount

of glucose were sacrificed at 0, 2.5, 6, 12, 16 or 24 hr following the treatment. At the dose of ethanol used the hepatic GSH concentration decreased rapidly after the ethanol intoxication with the maximum response being observed at t = 6 hr. The hepatic GSH concentration was increased gradually and finally rebounded when determined at 24 hr after the ethanol ingestion. An acute dose of ethanol did not induce a significant change in the hepatic GSSG concentration throughout the period. The activity of ?-glutamylcysteine synthetase (GCS), the rate limiting enzyme of GSH synthesis, and the cysteine concentration in the liver were also measured. The GCS activity was significantly inhibited at t = 2.5 hr after the treatment, but the hepatic cysteine concentration was not altered for 24 hr. The sinusoidal efflux of GSH from the liver was determined by subtracting the GSH concentration in blood of the prehepatic inferior vena cava from that of the posthepatic inferior vena cava. Acute ethanol administration induced a remarkable increase in GSH efflux from the liver at t = 2.5 hr. The results indicate that a transient decrease in GCS activity and an increase in GSH efflux from the liver may be responsible for the decrease in the hepatic GSH concentration observed in rats treated with ethanol acutely.

1847 GLUTATHIONE DEPLETION AND HEPATOTOXICITY FOLLOWING ADMINISTRATION OF HERBICIDE ALACHLOR.

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Alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl)-acetanilide) is a chloroacetanilide herbicide with hepatotoxic effects believed to be mediated through metabolic activation to a quinone imine. The model hepatotoxicant acetaminophen also acts through a quinone imine with resultant depletion of glutathione (GSH). To determine whether a similar mechanism is involved in the toxic effect of alachlor, we compared the dose-response for alachlor hepatotoxicity with modulation of hepatic GSH levels. Male Long-Evans rats (10 wks old) were exposed to alachlor at doses ranging from 126-600 mg/kg (ip in dimethyl sulfoxide) and sacrificed 2, 4, and 24h after administration. The lowest observed hepatotoxic effect level, as evidenced by histopathology (centrilobular hepatocyte vacuolation) and increased serum enzyme levels (alanine aminotransferase and sorbitol dehydrogenase) was 400 mg/kg, while lethality occurred at 600 mg/kg. At nonhepatotoxic doses of alachlor (≤ 200 mg/kg), determination of GSH as non-protein sulfhydryls showed a transient drop to 50% of control levels at 2h, a rebound to 80% at 4h and full recovery by 24h after administration. In contrast, GSH levels remained depressed by 40% at 24h after administration of a hepatotoxic dose (400 mg/kg). These results demonstrated that depletion of GSH occurred at all dose levels with alachlor exposure, but hepatotoxicity only resulted at doses high enough to sustain GSH depletion for 24h. Thus, alachlor hepatotoxicity parallels that of acetaminophen in that both require prolonged GSH depletion. (NIH ES00044, ES07046, and North Carolina ARS)

1848 MITOCHONDRIA-SPECIFIC EXPRESSION OF GLUTATHIONE REDUCTASE.

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Glutathione reductase (GR) regenerates glutathione (GSH) from glutathione disulfide (GSSG) in a process essential for function of GSH-dependent antioxidant systems. Chinese hamster ovary (CHO) cells stably transfected with the (human) hGR cDNA have increased resistance to oxidant-mediated cytotoxicity coincident with increased cytosolic GR activities. Modification of the hGR plasmid to include the mitochondrial targeting signal (MTS) for human MnSOD increased GR activities in mitochondrial and cytosolic fractions prepared and assayed by standard procedures, and imparted even greater resistance to oxidants. As the MTS-hGR construct retains the original hGR start codon 3' to the MTS, we tested the hypothesis that initiation of translation at the ATG in the hGR sequence could be responsible for the increased cytosolic GR activities observed in cells transfected with the MTS-hGR construct. We mutated the initial ATG of the hGR cDNA and transfected CHO cells with this construct, but still observed increases in GR activities in both cytosolic and mitochondrial fractions. When cells transfected with either the original or the mutated MTS-hGR construct were examined immunohistochemically using an anti-hGR antibody, GR appeared more specifically associated with the mitochondria than was apparent from the functional assays. Additional studies revealed that differential centrifugation after careful cell disruption using a triethanolamine-based procedure demonstrated highly selective increases in GR activities more compatible with the immunohistochemical findings. Neither assay revealed clear differences between cells transfected with the MTS-hGR and the mutated MTS-hGR constructs. Similar investigations of stably transformed CHO cells containing two intact ATG codons 5' to the MTS and GR coding sequences also indicated highly preferential expression of hGR in mitochondria. These results suggest that translation of MTS-hGR begins preferentially at the MTS start site and the resultant protein is directed to the mitochondria efficiently. The greater resistance to oxidant injury imparted to cells by increased mitochondrial expression of hGR is even more striking in view of the present data indicating that these cells have limited increases in cytosolic GR activities. (Supported by NIH GM44263 and U10 HL52637.)

1849 NRF-2, SMALL MAF PROTEINS AND AP-1 FAMILY MEMBERS REGULATE THE EXPRESSION OF THE γ -GLUTAMYLCYSTEINE SYNTHETASE (GCS) GENES VIA ELECTROPHILE RESPONSE ELEMENTS (EpRE).

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Basal as well as β -naphthoflavone- (β NF) and pyrrolidine dithiocarbamate-(PDTC) induced expression of the genes encoding the catalytic (GCS_h) and the regulatory (GCS_l) subunits of γ -glutamylcysteine synthetase (GCS) are regulated by EpREs. These EpREs contain internal AP-1 binding sites, are both adjacent to AP-1 or AP-1-like binding sites and share sequence similarities to TRE-like Maf Responsive Elements (T-MARE) and NF-E2 binding sites. We hypothesized that transcription factors including members of the AP-1 and small maf families, as well as the NF-E2-related proteins, Nrf1 and Nrf2, regulate the expression of the genes via binding to EpRE sequences. Electromobility supershift studies have identified proteins binding GCS_h/EpRE4 and GCS_l/EpRE1 present in nuclear extracts isolated from HepG2 cells. Both sequences are bound by proteins recognized by antibodies to Nrf2, JunD and small maf proteins. Northern analyses revealed increases in small maf mRNA transcripts following β NF and PDTC exposure. The timing of the increases in small maf mRNA levels preceded that of GCS_h and GCS_l induction following treatment with either agent. Transient transfection with an AP-1 dominant negative mutant (AFos) reduced constitutive expression (0.5X) and β NF-induced (0.4X) expression of GCS_l 5'-luciferase reporter constructs, but the effect on GCS_h 5'-luciferase reporter constructs was minimal (0.8X, for basal and β NF-induced expression). The dominant negative small maf mutant (DNmafK) significantly decreased basal expression and eliminated β NF inducibility of GCS_l 5'-luciferase reporters. These data provide evidence for the involvement of Nrf2, small maf factors and AP-1 family members in the regulation of GCS subunit genes via EpREs. (Supported by NIH CA57549.)

1850 CORRECT STRUCTURAL ORGANIZATION AND LOCATION OF THE HUMAN MICROSOMAL GLUTATHIONE TRANSFERASE GENE (MGST1): TRANSCRIPTIONAL UP-REGULATION IN RESPONSE TO OXIDATIVE STRESS.

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The structure and regulation of the microsomal glutathione transferase gene (MGST1) is considerably more complex than originally perceived to be. We determined that the MGST1 gene is located in the 12p13.1-13.2 region and there are two alternative first exons. The region between these two first exons is not capable of directing transcription. Thus, there is one common promoter element capable of directing transcription, and RNA splicing occurs such that only one type of exon 1 is incorporated into each mRNA species with common downstream exons. MGST1 expression and regulation is therefore similar to other hepatic xenobiotic handling enzymes such as γ-glutamyl transferase and CYP19. The MGST1 gene was previously considered a "housekeeping" gene as non-oxidant inducers of cytosolic glutathione transferases had little affect on MGST1 activity. However, we discovered that the true MGST1 promoter region (region immediately upstream of the dominate first exon) is capable of transcriptionally responding to oxidative stress. In this respect, MGST1 is similar to glutathione peroxidases which also transcriptionally respond to oxidative stress. The discovery that MGST1 utilizes alternative splicing of the first exon eliminates the theoretical problem noted with the first description of MGST1 cDNA structure in which it appeared that high expression of MGST1 was in violation of the ribosomal

scanning model, due to the presence of an in-frame stop codon and an out-of-frame ATG codon upstream of the initiating ATG codon. The identification that the first exon originally noted is in fact a minor alternative first exon far downstream of the correct MGST1 promoter region and primary first exon eliminates this conundrum. The discovery that MGST1 can transcriptionally respond to oxidative stress is supportive of the idea that specific toxic compounds can activate or induce select enzymes to hasten their own demise. However, if the compound is a haloalkenes such as chlorotrifluoroethene, which after GSH-conjugate formation by MGST1 is converted to a nephrotoxin, the development of a pro-oxidant state may have adverse consequences for the organism.

1851 GROWTH RATE AND EFFECTS OF METHYL MERCURY ON SURVIVAL RATE AND MITOCHONDRIAL TRANSMEMBRANE POTENTIAL IN HEPA-1 CELLS OVEREXPRESSING GLUTAMATE-CYSTEINE LIGASE.

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GSH is important in defending against oxidative stress and heavy metal toxicants especially mercurials. MeHg can accumulate in mitochondria which results in decreased mitochondrial transmembrane potential (MTP). The rate limiting enzyme in GSH synthesis is glutamate-cysteine ligase (GLCL) which is composed of catalytic (GLCLc) and regulatory (GLCLr) subunits. We developed cells with metallothionein promoter-regulatable overexpression of either GLCLc alone, GLCLr alone or in combination to test the hypothesis that increased GSH synthetic ability can protect cells from oxidative stress and perturbations in MTP caused by MeHg exposure. Colony size distribution was evaluated as a measure of growth rate of these cells. In addition, colony forming efficiency was counted after four days of exposure to MeHg. After exposure to MeHg for 8 hours, GLCLc cells were stained with CMX-rosamine and MitoTracker Green mitochondrial stains and examined by confocal laser scanning microscopy. Cells overexpressing GLCLc were the most resistant to MeHg with or without induction by Zn^{++}. Cells overexpressing GLCLr became more resistant to MeHg when pretreated with Zn^{++} 20 hrs before the MeHg treatment. The growth rates of all GLCL-transfected cells are lower than control cells. With 25μ M Zn^{++}, the colony size of the control cells decreased while GLCL cells did not show much change. MeHg caused a dose-related decrease in mitochondrial transmembrane potential in cells transfected with the plasmid vector containing no insert. GLCLc cells were protected from this effect of MeHg. These results show that excess GSH suppresses growth rates but increased synthesis of GSH helps to protect cells from oxidative stress and perturbations of MTP caused by MeHg.

1852 EFFECT OF OVEREXPRESSION OF GLUTAMATE-CYSTEINE LIGASE ON TNF AND ACTINOMYCIN-D-INDUCED APOPTOSIS IN HEPA-1 MOUSE LIVER CELLS.

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Glutathione (GSH) has been shown to be important in free radical scavenging, cellular redox status and to influence the survival of cells to a wide variety of toxicants. The rate limiting enzyme in the synthesis of GSH is glutamate-cysteine ligase (GLCL), which is composed of catalytic (GLCLC) and regulatory (GLCLR) subunits. We have developed mouse liver (hepa-1) cell lines with increased expression of GLCLC, GLCLR, or the combination by transfecting the mouse cDNAs for these genes and selecting for cells that expressed the transgenes. Cells overexpressing the mRNA for the catalytic subunit alone have modest increases in GLCL activity, while cells overexpressing both subunits have large increases in GLCL activity. When cells overexpressing GLCLC+GLCLR are exposed to tumor necrosis factor TNFα in combination with actinomycin D, they exhibit decreased rates of apoptosis (as indicated by loss of redox potential and mitochondrial membrane potential) when compared to cells transfected with the plasmid vector alone. These cell lines should provide an interesting model to examine the influence of GSH in moderating toxicant induced necrosis and apoptosis. (This work was supported by NIH Grants ES04696, AG01571and ES07033.)

1853 REGULATION OF γ-GLUTAMYLCYSTEINE SYNTHETASE mRNA IN THE PREIMPLANTATION MOUSE EMBRYO.

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The present study examined the regulation of glutathione synthesis in the preimplantation mouse embryo in terms of γ-glutamlycysteine synthetase (GCS) expression. Previous studies indicate that glutathione concentration decreases 90% during the first four days of development, and the embryo only begins synthesizing glutathione de novo at the blastocyst stage. Consequently, the cleavage stage embryo may be very sensitive to glutathione-depleting toxicants and reactive oxygen species. GCS is the rate limiting enzyme for de novo glutathione synthesis, and its activity may be upregulated in response to toxicant exposure or oxidative stress. It is hypothesized that GCS mRNA is not normally expressed in the cleavage stage embryo, but its expression may be induced by lowering glutathione levels to blastocyst-like concentrations. To address this possibility, the pattern of GCS gene expression in both cleavage stage embryos and blastocysts was examined by use of the reverse transcriptase-polymerase chain reaction (RT-PCR). Transcripts for GCS were detected only at the blastocyst stage. Furthermore, while diethyl maleate (DEM; 65μM) exposure to deplete cellular glutathione also resulted in GCS transcript expression in blastocysts, cleavage stage embryos were not induced to upregulate expression of GCS. To compare GCS transcript expression with cellular glutathione concentration, embryos were incubated with monochlorobimane, a thiol-reactive probe that is converted to a fluorescent product when conjugated to glutathione. Fluorescence microscopy indicates that, while both cleavage stage embryos and blastocysts are depleted of glutathione by exposure to DEM, only those at the blastocyst stage are capable of glutathione recovery. These results suggest that gene expression necessary for glutathione synthesis in the blastocyst is not initiated in response to decreased glutathione concentration but may be regulated by a developmental program.

1854 DISTRIBUTION OF GLUTATHIONE, GLUTATHIONE REDUCTION, AND GLUTATHIONE SYNTHESIS CAPABILITIES IN THE TROPHECTODERM AND INNER CELL MASS OF THE PREIMPLANTATION MOUSE EMBRYO.

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This study examined reduced glutathione (GSH), glutathione reduction and GSH synthesis within inner cell mass (ICM) and trophectodermal (TE) cells of the mouse blastocyst. Previous studies indicate that cleavage stage embryos are unable to synthesis GSH *de novo* until the early blastocyst stage. Differentiation of the TE into a transporting epithelium is the first step in mammalian development, and redox status has been shown to be important in cellular differentiation. Redundant pretrophectodermal cells within the ICM are eliminated via apoptosis, whereas cells that continue development are believed to be protected via glutathione-dependent protective mechanisms. To begin to understand the role of redox status in differentiation of the blastocyst, we examined GSH recovery in ICM and TE cells after treatment with either diethyl maleate (DEM) (65 micromolar) or t-butyl hydroperoxide (tBH) (130 micromolar) and following 3.5 hours of recovery. Two-cell embryos and blastocysts were incubated with monochlorobimane, a thiol reactive probe that fluoresces after conjugation with GSH. Fluorescent microscopy with photography were then used to evaluate GSH concentration in cells of intact two cell and blastocyst stage embryos. In addition, Immnunosurgery was used to isolate ICMs, and GSH concentrations were quantified by flourescent HPLC. Both DEM and tBH depleted GSH from two-cell embryos. tBH-treated two-cell stage embryos were able to recover GSH, at times showing upregualtion of GSH concentrations. Two-cell stage embryos did not recover GSH after DEM treatment. Blastocysts depleted of GSH by both tBH and DEM demonstrated recovery. Both the cells of the ICM and TE recovered GSH after DEM or tBH. In summary, GSH concentrations, GSH reduction, and GSH synthesis capabilities did not differ between ICM and TE. In conclusion, this study does not support a role for GSH status in differentiation of the blastocyst.

1855 PURIFICATION, CHARACTERIZATION AND REGIONAL DISTRIBUTION OF GLUTATHIONE *S*-TRANSFERASE FROM CHANNEL CATFISH INTESTINE.

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Our laboratory is interested in understanding the role of the intestine in the first pass of biotransformation of lipophilic xenobiotics that are presented in the diet at very low, environmentally relevant concentrations in the aquatic environment. Studies of benzo[a]pyrene biotransformation in catfish intestine showed that glutathione conjugates were major metabolites. Our objectives were to isolate and characterize catfish intestinal GSTs, examine conjugation of BaP oxides by intestinal cytosol and purified GSTs, and investigate distribution of activity and anti-catfish GST π cross-reactive protein in segments of the intestine of catfish fed a semi-purified diet or a commercial chow for at least two weeks. Results indicate that the predominant catfish intestinal GST isolated (Mr 26,700) is closely related to the π -class enzymes. The enzyme had good activity for benzo[a]pyrene-4,5-oxide, benzo(a)pyrene-7,8-dihydrodiol-9,10-epoxide, ethacrynic acid, and 1-chloro-2,4-dinitrobenzene, but a low activity with 1,2-cichloro-4-nitrobenzene. GST activity and the percent protein cross-reacting with anti-catfish GST π showed a gradient from the proximal (highest) to the distal section in both diet groups. The cross-reacting protein was significantly higher in the proximal and medial segments in the chow-fed fish, except in the distal segment where it was higher in fish fed purified diet. These results confirm that catfish intestinal GSTs play an important role in the first pass metabolism of PAH oxides produced in the intestine from dietary PAHs and suggests they provide protection against other xenobiotics, the proximal intestine likely playing a greater role that the distal. Also, it is possible that components of the commercial chow affect the localized activity of GST and percent cross-reacting GST protein in intestine. (Supported by ES 05781.)

1856 CORRELATION OF INCREASED MORTALITY WITH THE SUPPRESSION OF RADIATION-INDUCEDIBLE EPOXIDE HYDROLASE (EH) AND GLUTATHIONE S-TRANSFERASE (GST) GENE EXPRESSION DEXAMETHASONE (DEX).

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The present study was designed to investigate the effects of DEX on the radiation-inducible expression of EH and rGST genes and on the vitamin C and E-induced radioprotective effects in association with the expression of the genes. Treatment of rats with a single dose of DEX (0.01-1 mg/kg, po) caused a dose-dependent decrease in the constitutive EH gene expression at 24 hr. The radiation-inducible EH mRNA level (3-fold increase after 3 Gy γ -irradiation) was decreased by 21%-88% by DEX at the doses of 0.1-1 mg/kg. Although DEX alone caused 2- to 5-fold increases in the hepatic rGSTA2 mRNA level, rats treated with DEX prior to 3 Gy irradiation exhibited 80%-93% suppression in the radiation-inducible mRNA level. The inducible rGSTA3 and rGSTA5 mRNAs were also significantly decreased by DEX. Vitamin C and/or E failed to enhance the radiation-inducible increases in hepatic EH and rGST mRNAs. Whereas rats exposed to 3 Gy irradiation with or without vitamin C treatment exhibited ~3-fold increases in the EH and rGSTA2/3/5 mRNA levels relative to untreated animals, DEX treatment resulted in 64%-96% decreases in the mRNA levels. The inducible rGSTM1/2 mRNA levels in the vitamin C/E-treated rats were ~50% suppressed by DEX. Although vitamin C and/or E treatment (200 mg/kg/d, po, 2d) improved the 30-day survival rates of the 8 Gy γ -irradiated mice from 39% up to 74%, the improved survival rate of γ -irradiated animals was reduced to 30% by DEX pretreatment. DEX caused no significant hematologic changes. These results demonstrate that: DEX substantially suppresses radiation-inducible EH, rGSTA and rGSTM expression; vitamins C/E exhibit radioprotective effects without enhancing radiation-inducible EH and GST gene expression; and inhibition of radiation-inducible EH and rGST gene expression in the vitamin-treated animals by DEX was highly correlated with reduction in the survival rate of γ -irradiated animals.

1857 EFFECTS OF SELECTED PCB CONGENERS ON DRUG- AND GLUTATHIONE- METABOLIZING ENZYMES IN THE RAT.

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Although Polychlorinated Biphenyls (PCBs) have been banned from production in many countries since the early 1970s, they persist in our environment and accumulate in target tissues, such as adipose and breast tissues. PCBs may induce drug-metabolism and may be substrates for the induced enzymes. Metabolism of PCBs may lead to oxidative events within the cell. The goal of this study was to determine the influence of PCBs on glutathione and glutathione related enzymes. Sprague Dawley rats were injected with 100 µmol/kg of PCBs 3, 12, 28, 77, or 153 IP, each day for four days. Rats were sacrificed on day four 3 hours after the final injection, and their livers were surgically removed, snap frozen in liquid nitrogen, and stored at –80°C. Microsomes and cytosols were prepared and EROD (1A1), MROD (1A2), PROD (2B1&2), glutathione transferase (GST), glutathione reductase (GR), and total glutathione were determined. Experimental data showed that both male and female rats, treated with PCB 77, which is a ligand for the Ah receptor, had increased hepatic P450 1A1 activity, increased GST activity as well as a decreased level of total glutathione. Rats treated with PCB 153, a PB-like inducer, showed significant increases in hepatic P450 2B activity and GST activity. GR activity was also significantly increased by PCB 77 & 153 treatments. Interestingly, male rats treated with PCB 28, a PCB that is retained in breast tissue and milk, showed a significant increase in P450 2B and GR activities but, not in GST activity. These data will be interpreted in the context of oxidative stress occurring after PCB exposure. (P42 ES 07380 and DAMD 17-96-1-6162)

1858 SUB-CHRONIC EXPOSURE OF THIODIGLYCOL ON HEPATIC-MIXED FUNCTION OXIDASE AND CYTOSOLIC GLUTATHIONE ANTIOXIDANT SYSTEM IN RATS.

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In compliance with the National Environmental Policy Act, the US Department of Defense prepared a programmatic environmental impact statement for the chemical stockpile disposal program. The US Army has proposed to neutralize sulfur mustard through a hydrolysis process to convert it to a biodegradable compound, known as thiodiglycol (2-2-thiodiethanol) (TD). Thiodiglycol has been detected as a contaminant of soil and water at certain Army installations. We studied the effect of TD on the hepatic-mixed function oxidase system and cytosolic glutathione antioxidant system in male and female rats . Liver samples were collected from Sprague-Dawley rats gavaged with neat TD (99.9% pure) at dose levels of 50, 500 and 5000 mg/kg/day for 90-days (5days/week). The exposure to TD for 90 days resulted in a significant decrease in glutathione (500 and 5000 mg/kg), glutathione S-transferase activity (in all dose groups), and glutathione peroxidase (5000 mg/kg). Decreased cytochrome b5 (500, 5000 mg/kg) and pentoxy resorufin O-dealkylase (PROD) activity (5000 mg/kg) were found in male rats but no significant differences were observed in any of these parameters in female rats. These results demonstrate the biochemical interaction of TD on the hepatic PROD and cytosolic glutathione antioxidant system in male rats. (Abstract does not reflect US Army policy.)

1859 A PREVALIDATION STUDY FOR THE ECVAM/CFN INTEGRATED TOXICITY TEST SCHEME (ECITTS) USING KINETIC SIMULATIONS AND IN VITRO TEST METHODS.

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The European Centre for Validation of Alternative Methods (ECVAM) and the Swedish Board for Laboratory Animals (CFN) have initiated a series of studies on the prediction of systemic toxicity of chemicals through integration of non-animal (e.g. in vitro) studies with kinetic models. For a prevalidation study, eight chemicals (benzene, toluene, acrylamide, lindane, parathion, caffeine, diazepam and phenytoin), having documented effects on the nervous system, were selected. Studies on general and specific aspects of neurotoxicity were performed on these chemicals using an in vitro neurotoxicity test battery. In addition, in vitro-derived data on biotransformation and body distribution were used for parameterisation of a kinetic models for each compound. From these models, target tissue (i.e. nervous system) concentrations could be simulated as a function of acute and/or (sub)chronic exposure. Thus, Lowest Observed Effect Doses (LOEDs) for acute and/or (sub)chronic exposure could be estimated for six of the eight compounds and were compared with experimental LOEDs. The discrepancy between estimated and experimental LOEDs ranged from approximately a factor two, for relatively nontoxic compounds (i.e. a high LOED), to a factor 10, for relatively toxic compounds (i.e. a low LOED). LOEDs for some compounds could only be established after additional consideration of in vitro test results

that were derived from the literature and were not reflected by the endpoints within the in vitro neurotoxicity test battery.

1860 COMPARISON OF ESTIMATED INDOOR AIR CHEMICAL CONCENTRATION RESULTS FROM FATE AND TRANSPORT MODELING VERSUS SURFACE FLUX MEASUREMENTS.

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Exposures to chemicals can result from both direct (dermal or ingestion) or indirect (vapors from chemicals in groundwater) contact. Estimation of exposure concentrations for use in health risk assessments requires calculated or actual measurements. To estimate indoor air concentrations of chemicals due to vapors migrating upwards from chemicals in groundwater, one can use environmental fate and transport modeling or perform surface flux measurements. Environmental fate and transport modeling makes use of chemical- specific and site-specific data as well as default parameters to estimate first a vapor flux (using a vapor flux model) and then an indoor air concentration (using a box model). Surface flux measurements are directly used in a box model to estimate indoor air concentrations. In this assessment, we compared the results of using default parameters and chemical-specific parameters in a vapor flux model with the results of using surface flux measurements to estimate indoor air concentrations. At the site used for this case study, tetrachloroethylene was present in groundwater located 15 feet below ground surface. The Farmer et al. model was used to estimate flux at a point above contaminated groundwater. Surface flux measurements were taken at a point above which the groundwater concentration was measured. The modeled flux was 2 orders of magnitude less than the actual flux. This resulted in a 2 order of magnitude difference in estimated indoor air concentration and, subsequently, cancer risk. Uncertainties in the modeled flux include use of default parameters for total porosity and air-filled porosity. The results of this assessment demonstrate the importance of validation of models as well as the importance of use of more site-specific parameters in models.

1861 A HUMAN HEALTH AND ECOLOGICAL ASSESSMENT OF VOLATILE COMPONENTS OF PM10 EMISSIONS.

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Approval for the construction of a fiberglass manufacturing facility under the California Environmental Quality Act required an environmental impact report concerning human health and ecological effects of likely constituents of stack PM10 emissions. Specifically, PM10 components consisted of phenol, formaldehyde and various methylol phenols (MPs) compounds. There are currently no air standards for MPs or cured phenol-formaldehyde resins. An assessment of the hazard characteristics was developed for the following compounds: 2-MP, 4-MP, 2,6-diMP, 2,4,6-triMP, and cured phenol-formaldehyde resins. A threshold air concentration for mono- and diMP, based on potential dermal sensitization (assessed as the most sensitive endpoint), was estimated to be 3100 $\mu g/m^3$ of air. Modeled air concentrations of PM10 indicated likely levels of these compounds would not exceed 16 $\mu g/m^3$. We predicted the likely PM10 emissions would not represent a threat to human health. Information on the aquatic toxicity of MP compounds is extremely limited. A no-effect level for aquatic organisms was based on toxicological information generated from mono-substituted MPs. A no-effect level of 5 mg/l was derived for MP indicating the relatively low potency of this chemical. Modeled air concentrations were not predicted to yield a water concentration in excess of this threshold. Thus, the modeled air concentrations do not represent a threat to aquatic organisms. Based on this analysis, concentrations of these compounds released as constituents of PM10 from this planned facility are not expected to pose a significant threat to human health or the environment.

1863 CHARACTERIZATION OF AROMATIC CANDLE EMISSIONS AND ITS SIMILARITY TO DIESEL ENGINE EXHAUST.

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At least fifty documented incidences of severe soot deposition in Florida residences, believed to be caused by aromatic candle emissions, have occurred since 1996. Concerns of occupant exposures and potential health impacts were raised. Submicron particulate has been recognized as carcinogenic in health assessments of diesel emissions. The estimated clearance time for these insoluble carbon particles from the human respiratory system is up to 1 year, providing adequate time for desorption of adsorbed organic compounds. Aromatic candle emissions resemble those of diesel engine exhaust in particle size and organic compounds, while their use in confined spaces may constitute a significant occupant exposure. An assessment of the emissions from aromatic candle usage was performed using a 45 liter stainless steel chamber with high air exchange rates to reduce losses to chamber surfaces. Exhaust was collected for analysis of particulate matter and gaseous emissions. Particulate and gaseous phase emission rates were calculated. To determine the quantity, and identification, of adsorbed compounds GC/MS and GC/FID analyses were performed. Initial characterization and quantification of particulate matter (PM) emissions revealed emission of particles ranging from 0.06 to 0.1 μm, determined by scanning electron microscopy (SEM), at rates up to 3.5 mg/min. Gas phase emissions contained compounds including benzene, 2-butanone, chloroform, carbon tetrachloride, xylenes, and other volatile and semivolatile compounds. This study determined that certain candles produce particulate and gaseous emissions similar to those of diesel engine exhaust, and when used indoors, can result in occupant exposures that may pose a significant risk. (Supported in part by NIOSH Grant T42/CCT412874.)

1864 HIERARCHICAL APPROACH TO PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS.

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Historically, many physiologically based pharmacokinetic (PBPK) models have been analyzed through commercial simulation and optimization software such as SimuSolv®. During the optimization process, model parameters are adjusted to maximize the value of the objective function, the log likelihood function (LLF). Optimization of these PBPK models is usually based on repeated measurement data from multiple individuals. Under the normality assumption, the optimization method implemented in SimuSolv® is correct for data from a single individual when statistical inference is focused solely on that individual. When data from multiple individuals are pooled, however, a problem arises in using SimuSolv®. Used to derive the LLF, the error model in SimuSolv® is not appropriate because it confounds two sources of variation: intraindividual variability, which is variation among measurements within a given individual, and interindividual variability, which is random variation among individuals. If sources of variation are not taken into proper account, misleading estimates of the parameters and uncertainty in those estimates may result. These two variation components can be taken into appropriate account in a statistical hierarchical or staged model. We used the PBPK models for tert-amyl methyl ether (TAME) and tert-amyl alcohol (TAA) to contrast inference based on the incorrect error model implemented in SimuSolv® and inference based on a hierarchical model. To fit a hierarchical model, maximization of a more complex objective function than that found in SimuSolv® is required. SAS® software is very efficient in implementing hierarchical model fitting for PBPK models. With a hierarchical approach to current PBPK models, the model that most accurately accounts for variation can be developed, which will allow a more precise extrapolation to humans.

1865 ROUTE-TO-ROUTE EXTRAPOLATION WITH A PHYSIOLOGICALLY-BASED PHARMACOKINETIC (PBPK) MODEL FOR CUMENE.

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EPA's inhalation Reference Concentration (RfC) and oral Reference Dose (RfD) for cumene are both based on renal effects observed in separate studies where exposures were by the respective route. As some pharmacokinetic data are available, cumene toxicity could thus be examined for dosimetric route-to-route relationships. A PBPK model was developed in which the chemical-specific partition coefficients and metabolic parameters were estimated from structurally-related chemicals. The resulting model was used to convert the inhalation concentrations of the RfC study to equivalent oral doses based on the predicted area under the concentration curve for cumene metabolites in the blood. Estimates from this dose conversion, in mg/kg-day, are 78 (from 100 ppm inhalation), 408 (from 496 ppm inhalation) and 955

(from 1202 ppm inhalation) with the route-converted NOAEL (no observed adverse effect level) at 408 mg/kg-day. The effect levels and doses from the original oral study were the NOAEL at 154 and the LOAEL (lowest observed adverse effect level) at 462 mg/kg-day. The route-converted NOAEL was higher than the oral NOAEL, but less than the oral LOAEL, showing the relationship between the doses and effect levels across separate, independent studies. This exercise demonstrates that reasonable route-to-route extrapolations may be projected with PBPK models that utilize non-specific (but related) chemical parameters. However, chemical specific parameters would better validate the model and lessen the uncertainty of the model projections.

1866 RELATIVE BEHAVIORAL SENSITIVITY OF RATS AND HUMANS TO ACUTE TOLUENE EXPOSURE.

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The relative sensitivity of rats and humans can be empirically described by a cross-species map (CSM), a continuous function of human and animal dose pairs which produce equal effects in the two species. In previous work, literature on acute toluene exposure was used to fit dose-effect functions for behavioral data in rats and in humans. Effect magnitudes were transformed to percent-of-baseline to permit pooling data. Dose (arterial blood toluene) was estimated with a physiologically-based pharmacokinetic model. Two kinds of behavioral data in rats (avoidance and activity) and choice reaction time (CRT) in humans were analyzed. The rat and human dose-effect curves from the previous work were used here to estimate the CSM and the 95% confidence limits. A CSM was estimated for equating equal effect sizes for avoidance behavior in rats and CRT in humans. Another CSM was estimated for equating activity level in rats to CRT in humans. The CSM using rat avoidance behavior was an exponential function. From this CSM it was estimated that upper-range human doses were about 10% of equal-effect rat doses, but at ED-10, human doses were about 4% of ED-10 rat doses. The CSM for rat activity level and human CRT was nonlinear and slightly negatively accelerated. In contrast to avoidance, the ED-10 human dose was only about 33% of the equivalent rat dose. This means that activity level data produced the most sensitive index of effects in rats (almost as sensitive as humans). The CSM procedure is based on purely empirical data. Even behaviors that are not biologically or functionally similar can be numerically compared for sensitivity to toxicants. Such comparisons are useful for estimating relative sensitivity, but do not necessarily contribute to biological understanding. The CSM procedure is a precursor to empirical interspecies extrapolation for the frequent case where insufficient biological theory exists. This procedure estimates best for wide-dose-ranges in both species and gives dose confidence limits in the target species. (This is an abstract of a proposed presentation and does not reflect EPA policy.)

1867 HAZARD CHARACTERIZATION OF NONCANCER HEALTH EFFECTS OF BENZENE.

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The U.S. EPA conducted a comprehensive review of the published literature on noncancer health effects of benzene to derive an inhalation reference concentration (RfC) and an oral reference dose (RfD). It was concluded that benzene exposure results in adverse noncancer health effects by all routes of exposure to test animal species. Hematotoxicity and immunotoxicity have been consistently reported to be the most critical noncancer effects both in limited studies in humans and experimental animals. The bone marrow is the most sensitive target organ for the expression of benzene hematotoxicity and immunotoxicity. The human occupational exposure epidemiological study of Rothman et al. (Am J. Ind. Med. 29:236, 1996) and the 28-day drinking water exposure study of Hsieh et al (Arch Environ. Toxico. 17:157, 1988) were considered to be the co-principal critical studies for derivation of the RfC and RfD. The Rothman et al (1996) showed significant reductions in absolute lymphocyte counts in Chinese factory workers exposed to a median 8-hr TWA concentration 7.6 ppm(24mg/m^3) and was identified as a lowest observed effect level (LOAEL). A LOAEL ($_{HEC}$) was adjusted for the occupational ventilation rate and for intermittent work week schedule for 24 hr exposure, 7 days/week to 8.6 mg/m^3. The adjusted LOAEL ($_{HEC}$) and 1000-fold uncertain factors (for lack of NOAEL, subchronic to chronic extrapolation and data deficiencies) were used to derive RfC of 9 E-3mg/m^3. The human chronic dose of ingested benzene considered to be without any appreciable risk (RfD) is 1E-3mg/kg-day and it is supported by and RfD value of 8 E-4 mg/kg-day, which is 1/10,000 of the LOAEL identified for hematological effects in subchronic drinking water study of Hseih et al (1988). The overall confidence in the RfC/RfD is medium. (The opinions expressed in this abstract are that of the authors and should not be constructed as the US EPA policy).

1868 CANCER RISK ASSESSMENT OF BENZENE.

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Recently, we updated the EPA 1985 interim characterization of human carcinogenic risk of exposure to benzene. The study of pliofilm rubber workers (Rinsky et al (Am J Ind Med 2:217-245, 1981; N Engl J. Med 316: 1044-1050; 1987) continues to provide the basis for evaluation of human cancer risks from exposure to benzene. Benzene affects the hematopoietic system, especially, bone marrow cells in several different ways and the effects are produced by interactive action of multiple metabolites at DNA level. Both genotoxic and epigenetic effects, are critical components of the leukomogenic properties of benzene. However, data are not sufficient at this time to state precisely which of the various documented effects, genotoxic or otherwise, are critical ones for benzene induced leukomogenicity. Several risk estimates based on Rinsky's cohort have become available with individual risk estimates using various assumptions and/or models, with outcomes ranging more than six orders of magnitude. However, none of the approaches have a greater scientific validity than any other. The arguments in favor of benzene induced leukemia being nonlinear at low doses can be matched by opposing views as a most likely occurrence. There is not sufficient evidence currently to reject a linear dose-response curve for benzene in the low-dose region. Therefore, the EPA default approach of using a model with low-dose linearity is still recommended. Of the various approaches employing a linear assumption, the risk at 1 ppm exposure ranges from 7.1 x 10^{-3} to 2.5 x 10^{-2} within which any calculated risk estimate would have equal scientific validity. (The opinions expressed in this abstract are that of the authors and should not be constructed as the U.S. EPA policy).

1869 DETECTION OF THE DEPURINATING DNA ADDUCT, 7-(BENZO-[*A*]PYREN-6-YL)ADENINE (BP-6-N7A) IN HUMAN URINE: A NEW CLASS OF BIOMARKERS FOR CANCER RISK ASSESSMENT.

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Exposures to complex mixtures, *e.g.*, cigarette and coal smoke, containing polycyclic aromatic compounds (PAC), are associated with increased incidences of lung cancers. Carcinogen-adducted DNA bases comprise the primary carcinogenic lesions which are transformed into gene mutations. Studies of stable PAC-DNA adducts indicate broad correlations with PAC exposures and large inter-individual variability. Recent studies have identified PAC-adducted purine bases which are rapidly and spontaneously released from the DNA. These have been termed "depurinating adducts". The goal of the present study was to determine whether BP-6-N7A, a depurinating DNA adduct of benzo[*a*]pyrene (BP), could be detected in the urine of individuals exposed to cigarette and coal smoke. Urine was collected from 5 smokers, 5 nonsmokers, 7 coal smoke-exposed women, and 7 non-exposed women. PAC exposure due to coal smoke was *ca.* 20 times that due to cigarette smoke. Each sample was extracted with a Sep-Pak cartridge eluted with an acetonitrile-water gradient. The extract was fractionated by reverse-phase high-pressure liquid chromatography, and the adduct fraction was analyzed by capillary electrophoresis inter-faced with fluorescence spectroscopy (CE/FS), and by liquid chromatography coupled with mass spectrometry (LC/MS). Analyses of the first 4 samples identified BP-6-N7A in the PAC-exposed individuals. The adduct was not detected in non-exposed individuals. Daily excretion of BP-6-N7A was 5 and 0.9 pmol, by LC/MS, and 14 and 0.7 pmol, by CE/FS, for coal and cigarette smoke exposure, respectively. This work has identified a new class of biomarkers for PAC-associated cancer risk assessment.

1870 INTERSPECIES DOSIMETRY OF FINE-MODE AEROSOLS.

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Laboratory animals may be used as surrogates in inhalation exposure experiments to estimate the potential threat to human health of fugitive fine-mode aerosols derived from the combustion of fossil fuels (e.g., residual oil fly ash, ROFA). Interspecies dosimetry models may be used in a complementary manner with these animal experiments to: 1) aid in the determination of appropriate experimental conditions *a priori* which permit animal tests to simulate prescribed human exposures to air pollutants; and, 2) assist in the analysis and interpretation of laboratory data by providing a quantitative assessment of particle deposition in animals and humans. We have performed computer simulations to study factors affecting ROFA disposition within the lungs of humans and a selected surrogate (rat). In the animal tests CO_2 levels were regulated to simulate different degrees of respiratory intensity. A specific observation of this investigation is that the resting rat is a poor surrogate for the resting human. Moreover, the simulations suggest that the active rat, as described by the respiratory profile produced by a 4% CO_2 chamber atmosphere, is a suitable surrogate. (DISCLAIMER: This is an abstract of a proposed presentation and does not necessarily represent EPA policy. ACKNOWLEDGMENTS: C J Musante was funded by the EPA/UNC Toxicology Research Program, Training Agreement CT902908. R A Segal was funded by the NCSU/EPA Cooperative Training Program in Environmental Sciences Research, Training Agreement CT826512010.)

1871 CANCER AND NONCANCER ASSESSMENTS OF SOLUBLE NICKEL.

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A substantial body of occupational epidemiology data has associated exposure to mixtures of water-soluble and insoluble nickel compounds-with the development of lung and nasal cancer. However, due to coexposure to soluble and insoluble forms, and limitations in exposure measurements, the contribution of soluble nickel is difficult to determine. Soluble nickel was negative for tumorigenicity in an NTP inhalation bioassay, while there was some evidence for tumorigenicity in rats for insoluble nickel oxide, and there was clear evidence for tumorigenicity of insoluble nickel subsulfide in rats. Results of parenteral assays follow a similar pattern, but provide evidence of weak carcinogenicity of soluble nickel. The weight-of-evidence from the animal and epidemiology data, indicate that soluble nickel is not carcinogenic by itself, but appears to enhance the lung or nasal cancer risks associated with exposure to other forms of nickel. Therefore, the carcinogenic activity of insoluble nickel compounds should not be used to predict the carcinogenic potential of water-soluble nickel salts. A chronic oral RfD was derived based on decreased body weight and kidney effects in rats as the critical effects. A chronic inhalation RfC was derived based on a benchmark dose for fibrosis in male rats observed in an NTP bioassay. As part of an effort to foster cooperation between industry and government, this work was cosponsored by to the Metal Finishing Association of Southern California and the U.S. EPA. (Conclusions do not necessarily reflect the opinions or policies of U.S. EPA.)

1872 PREDICTIVITY OF PHARMACEUTICALS TOXICITY IN HUMANS FROM ANIMAL DATA.

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This investigation describes the procedure for compiling data and presents the results to-date from a multinational pharmaceutical company survey of the predictivity of the toxicity of pharmaceuticals in humans from animal data. The main aim of this project is to better understand the usefulness of animal studies to identify toxicity biomarkers for pharmaceutical-associated human toxicity (HT), and to identify opportunities to improve detection of HT. The strategy for developing the database for this survey resides in two sets of participant pharmaceutical company records: (1) significant HTs for therapeutics identified during clinical development, and (2) based on this data, the determination of whether animal toxicity studies did or did not identify biomarkers for the relevant HTs. A standardized tabular format was developed to collect information including: identification of therapeutic category, the type (organ system) of HT that was identified, and the duration of animal studies in which the corresponding toxicity was first identified or not observed. To-date the survey includes input from 7 pharmaceutical companies with data compiled from approximately 130 compounds with more than 160 human toxicity events reported. Interim results demonstrate the true positive prediction rate of animal models for human toxicity of 69% across therapeutic classes evaluated. The results support the predictive value for identifying HTs in non-clinical studies up to one month in duration. Study results from non-rodent (dog, primate) species have shown good potential to identify HTs from many therapeutic classes. The predictive value of toxicities from rodent studies bears further examination to determine if other factors (drug metabolism, toxicokinetics) play a significant role. While additional results are being included in updates, these survey results support the value of in vivo studies to predict for many significant HTs.

1873 PESTICIDE REGISTRATION: A NEW PARADIGM ON THE INCORPORATION OF MECHANISTIC CONSIDERATIONS WITHIN THE FRAMEWORK OF REQUIRED DESCRIPTIVE TESTING.

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Within the context of conducting "guideline" testing to support registration of new chemical pesticides, mechanistic studies are typically performed after the emergence of some effect (usually tumors). Such an effect may lend itself to further clarification through testing of a mechanistic hypothesis that provides compelling evidence that the findings in the animal are not relevant to the human. However, despite the improved regulatory picture in terms of potentially achieving a more appropriate risk assessment, the fact remains that the chemical has often been linked to an undesirable outcome, such as tumors. Moreover, the mechanism work, which provided evidence to mitigate the guideline finding, was likely conducted at the same level of unrealistic exposure that in all probability promoted/contributed to the tumorigenic response seen in the original bioassay. With this brief introduction as background, we have developed a scheme or approach in which to merge the fundamental interests or requirements of the mechanistic study with the practical and time-driven characteristics that are fundamental to descriptive guideline testing. First, the approach focuses on developing a mechanistic "game plan" at the beginning of the product development process - not at the end. The key step is dose selection, which in turn is driven by the data from preliminary pilot studies, a toxicokinetic assessment, and a mechanistic screening study incorporating an endocrine focus (consistent with emphasis of FQPA). Currently, we are at different stages with respect to incorporating each of these components into the pesticidal development process. The toxicokinetic protocol is not a classic kinetic protocol (metabolites, plasma concentration etc.) but rather a dose and time-driven design intended to provide general evidence of the likelihood of a dose-related change in a given metabolic profile that can then be correlated to alterations in key endocrine endpoints. By combining the data obtained from each of these areas, one can initiate guideline studies at dose levels that in all probability are representative of the linear portion of the dose response curve. Furthermore, this approach facilitates the conduct of additional follow-up mechanistic work, employing a dosing regimen that is not only comparable to that selected for the guideline work but likewise positioned squarely in the linear segment of the dose response curve.

1874 APPROACH FOR ESTIMATING THE POTENTIAL AGGREGATE EXPOSURES TO ORGANOPHOSPHATES.

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The Food Quality Protection Act (FQPA) of 1996 requires a new approach for pesticide exposure and risk assessment. Instead of assessing potential risk by identifying dominant exposure routes for a single compound in a single source scenario, FQPA calls for the aggregation of exposure routes (i.e., dermal, inhalation and ingestion) from multiple sources, and adding the exposures of compounds that have a common mechanism of toxicology to estimate overall potential cumulative exposure and risk. Multiple sources

include diet, drinking water, and non-dietary residential (indoor and outdoor) sources. To address this task, a case study group focusing on organophosphates (OPs) was assembled. The case study group is working to prepare aggregate assessments for individual OPs and then to develop cumulative assessments for the group of OPs. The work of the non-dietary group will be presented. The non-dietary group began by identifying the product use patterns and associated exposure scenarios for a cluster of OPs. The group then considered the exposure potential of each scenario, and the plausible aggregation of the exposure scenarios for each compound. Basic algorithms quantifying exposure were established, and algorithm input parameters (including point estimates and distributions) were identified, characterized, and summarized for each exposure scenario. Non-proprietary exposure data were identified and categorized including mixer/loader/applicator, post-application, and use information. The model is flexible to permit the addition of proprietary data.. Standard operating procedures (SOPs) were developed for characterizing and summarizing the exposure data. The availability of data and information for age-specific activity patterns was reviewed: such information was then characterized and summarized. These efforts resulted in a screening-level residential exposure assessment model. This case study shows how spatial, temporal, and demographic data can be used to estimate potential exposure more precisely than point estimates.

1875 UNCERTAINTY ASSOCIATED WITH CARCINOGENIC POTENCY OF ETHYLENE DIBROMIDE.

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Uncertainties associated with cancer potency values are often the result of model choice. An example is the cancer potency of ethylene dibromide (EDB, or 1,2-dibromoethane), which was formerly used as a pesticide and gasoline additive and is found in groundwater at some contaminated sites. Uncertainty associated with the carcinogenic potency of EDB is considerable. The U.S. Environmental Protection Agency (EPA) calculates a cancer slope factor (q*) of 85 per mg/kg× d (oral administration) using an unpublished model applied to a male rat gavage study conducted by the National Cancer Institute in 1978. A standard multistage model with a Weibull time-to-tumor component represents the most similar structure to the unpublished model. We were not able to reproduce the EPA value using the same data and assumptions listed in EPA documentation in the multistage and a number of other commonly-used linear (no threshold) cancer models. We calculate a maximum likelihood estimate (MLE) slope factor as 5 per mg/kg× d, and a 95% upper bound slope factor as 7 per mg/kg× d using the multistage model. MLE results from other models give a maximum value of approximately 40 per mg/kg× d, and a minimum of less than 1 mg/kg× d. Rather than select one model's results, we use weighted Monte Carlo sampling to estimate uncertainty distributions reflecting intra- and inter-model uncertainty in the EDB slope factor. Estimates of cancer risk, potential remediation levels, and remediation costs are directly dependent upon slope factor values. Therefore, careful assessment of cancer potency values and model uncertainty is important in the context of public safety and the costs of environmental remediation.

1876 COMPARISON OF A PROBABILISTIC APPROACH TO RISK ASSESSMENT BASED ON EMPIRICAL AND MODELED DATA.

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Perhaps the greatest uncertainty in human-health risk assessment lies in measuring individual differences in susceptibility. A method has been developed to quantify individual differences (Glowa et al., 1986) in laboratory animals, but the necessary baseline approach in experimental design is incompatible with most standard toxicity tests (e.g., developmental, chronic exposures). A modification was therefore created, based on randomization testing, to make the approach applicable to the more traditional types of toxicity data. Adult male Long Evans rats were used to establish dose-effect functions for two pesticides (triadimefon, carbaryl) and motor activity in each rat. For each pesticide, rats (N=36) were divided into 4 groups of 9, with group assignment determining the order of dosing. Triadimefon produced dose-related increases in motor activity and carbaryl produced dose-related decreases in activity. ED10s were determined by interpolation from each rat's dose-effect data, and then the group-mean and SD of ED10s were calculated. A similar analysis used randomization testing (with replacement) on the initial, group-defined (i.e., N=4x9) dose-effect functions, and the results were then compared to the empirical distributions of pesticide ED10s. Means and SDs of ED10s based on randomized testing closely matched those derived from the empirically determined distributions (for triadimefon: 12.1 and 1.3 mg/kg vs. 8.4 and 1.7 mg/kg, respectively; for carbaryl 2.5 and 1.8 mg/kg vs. 2.6 and 2.2 mg/kg, respectively). These results suggest randomization testing is a viable alternative to quantifying individual differences in chemical susceptibility in the absence of dose-effect data for individual subjects.

1877 EMPIRICAL vs. MECHANISTICALLY-BASED DOSE-RESPONSE MODELING OF ORGANOPHOSPHATE PESTICIDE TOXICITY.

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Dose-response assessment generally relies on empirical models. However, such models do not incorporate existing knowledge relating to mechanisms of toxicity. Biologically-based dose-response models have been proposed as a means to improve estimates of behavior outside the observed range of existing data. We explore the use of both empirical and biologically-based models as applied to dose-dependent acetylcholinesterase (AChE) inhibition by organophosphate pesticides (OPs). The mechanisms of organophosphate toxicity are well characterized and involve enzymatic interactions resulting in AChE inhibition. Thirteen data sets were used to examine the activity of seven OPs. Models used to fit the data included three empirical models (linear , power law and log-linear) and four models that have been developed based on the understanding of the mechanism of enzyme interactions and receptor theory (Sigmoid-Emax , Emax, modified Emax, and indirect response). Mean response (i.e. AchE inhibition) weighted by 1/SD were used to assess fit across all models and to estimate the dose at which 10% response is observed (ED_{10}). Dose-response model predictions are also evaluated at ED_{20}, and ED_{50}. Model fits were assessed using appropriate statistics including r^2, squared error, and uncertainty of parameter estimates. The best fits were provided by the various Emax-models and the Power Law function, consistent with the known mechanisms of OP activity. Estimates of ED_{10} using the Emax models and Power Law functions were generally lower, and in some cases more than an order of magnitude lower, than the study-derived LOAEL. This analysis demonstrates that model selection is critical when deriving benchmark dose estimates, and that using models based on described biologic processes can provide a credible rationale for dose-response assessment. (Work supported by the Consortium for Risk Evaluation with Stakeholder Participation (CRESP) through the Department of Energy, AI#DE-FC01-95EW55084 and the EPA/NIEHS Center for Child Environmental Health Risks Research R826886-01-0.)

1878 RELEVANCE OF DOG TOXICITY DATA FOR EVALUATING HUMAN HEALTH EFFECTS FROM EXPOSURE TO CHLORINATED PHENOXYACETIC ACID HERBICIDES AND RELATED ORGANIC ACIDS.

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Compared to other species, the dog is particularly susceptible (lower NOEL) to the effects of chlorinated phenoxy herbicides such as 2,4-dichlorophenoxyacetic acid (2,4-D) and related organic acids. It is proposed that this sensitivity is due to a lower capacity to excrete organic acids that share common pharmacokinetic (PK) properties. These common properties include (1) extensive but reversible plasma protein binding; (2) high ionization of weak acids at physiological pH; (3) limited metabolism of the parent acid; and (4) urinary clearance involving saturable active secretion. To test this hypothesis the 2,4-D PK parameters, volume of distribution (V_d), renal clearance (Cl_r) and plasma half-life ($t_{1/2}$) were evaluated across species and the results compared to previously published data on 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), triclopyr and 1-amino-cyclopropanecarboxylic acid (ACPC). Cross species comparisons were done by scaling the PK parameters utilizing an allometric approach based on the equation $Y=\alpha W\beta$. The variable parameter Y (V_d, Cl_r, $t_{1/2}$) was fit by log-linear regression, where body weight, and the allometric coefficient and exponent were represented by W, α and β , respectively. Comparisons of 2,4-D, V_d, Cl_r, and $t_{1/2}$ versus W for mice, rats, dogs, pigs, calves and humans were conducted. The V_d for 2,4-D was well described for all species ($V_d=0.12W^{1.11}$). Whereas, the relationship for Cl_r and $t_{1/2}$ versus W were well described by the equations $Cl_r=37W^{0.86}$ and $t_{1/2}=2.14W^{0.27}$ for all species, except the dog. The dog had a significantly slower Cl_r and longer $t_{1/2}$ relative to other species, including humans. The

results of this analysis are consistent with the allometric relationships observed with 2,4,5-T, triclopyr and ACPC in which the dog was an outlier, relative to other species. This PK evaluation of 2,4-D and related organic acids questions the relevance of using dog toxicity data for the extrapolation of human health risk. Based on the lack of a pharmacokinetic concordance between dogs and other mammalian species it is suggested that the dog is not a reasonable surrogate for humans.

1879 CHARACTERIZING NONCANCER RISKS ABOVE THE REFERENCE DOSE (RfD): CHLORDANE AND METHYL MERCURY AS EXAMPLES.

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Noncancer health risks above the RfD were characterized for methyl mercury (MeHg) and chlordane using a method that combines dose response modeling and Monte Carlo simulation of uncertainty factor (UF) distributions. The risks are based on the critical effect given in EPA's IRIS database. Dose response modeling was used to estimate the dose causing a 50% response (ED_{50}). Probability distributions were employed for each UF used by EPA in setting the RfD. A reference distribution (Swartout et al., 1998) was employed for UF values of ten and the square root of the values from the reference distribution were used for UFs of 3. The ED_{50} for chlordane was based on a study of liver toxicity in mice. Reference distributions were used for the interspecies and interindividual UFs and the square root of the reference distribution was used for the database UF. Dose response curves illustrate that at a dose 50 times greater than the RfD only 2% of individuals would be expected to respond (median) with 90% certainty that less than 10% would respond. The ED_{50} for MeHg was calculated from the neurodevelopmental data on exposed mother-child pairs from Iraq. Pharmacokinetic data was used to generate a distribution for human variability for MeHg (J. Swartout, pers. comm.) and the square root of the reference distribution was used for the database UF. The dose response curves were steeper for MeHg, with a 90% certainty that 4% of the population might respond at doses only 5 times the RfD. Risks above the RfD could not be calculated for PCBs, DDT, or dioxin due limitations in the dose response data. (EPA Co-Operative Agreement #CX825499-01 entitled "Comparative Dietary Risk.")

1880 COMPARATIVE DIETARY RISKS: BALANCING THE RISKS AND BENEFITS OF FISH CONSUMPTION.

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Analyses of fish in water bodies throughout the United States have confirmed the presence of chemicals in fish. In many cases the concentrations of chemicals have been high enough to warrant the posting of fish consumption advisories. In virtually all cases, these advisories are based solely on the potential adverse effects posed by the chemicals in fish. Yet some recent publications have suggested that the health benefits of eating even contaminated fish may outweigh the potential risks caused the by the presence of contaminants. If contaminated fish also confer health benefits to people eating fish, then prior to setting of advisories, public health officials might estimate the magnitude of those benefits and consider altering advisories that are strictly risk-based. A framework for comparing the risks from chemical contamination of sport-caught fish to the reduction in the relative risk of several adverse health endpoints from eating fish is developed. The framework includes a simple method based on a four category severity scale and a novel approach to determine the risks from chemical exposures above the RfD in order to combine the health risks from cancer and non-cancer toxicity. A similar severity construct is used to compare these risks with the benefits of eating fish. The framework accommodates scales for perceived severity and cultural benefits, if desired. The framework is able to compare the risks and benefits of fish consumption over a wide range of fish consumption rates, illustrated with three case studies. People using the framework should be able to apply it to many contaminants at various concentrations. Data limitations, and assumptions used to develop the framework, are highlighted.

1881 RELATIVE DEVELOPMENTAL RISKS OF *FUSARIUM* MYCOTOXIN, DEXOYNIVALENOL (DON) and BENOMYL (BEN) IN WHEAT.

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Since 1984, the wheat supply has had elevated DON levels from fungal infections which can be suppressed with BEN. DON and BEN are developmental toxins. As such, evaluation of the relative developmental risks is warranted. The dietary NOAELs for decreased pups body weights for DON, and BEN are 0.375 mg/kg/day in mice and 5 mg/kg/day in rat, respectively. Human exposure to DON was estimated from surveys of wheat grain and finished products. Mean DON concentration, 0.86 ppm, estimated from survey data and the tolerance level for BEN in grain, 0.2 ppm are used to estimate exposure. Presence of DON and BEN in wheat at these concentrations would not impact intake. A modified margin of exposure (modMOE) was developed using "*a*" kg (food) /kg (body weight) as the constant for wheat consumption. The modMOE for DON is 28/*a* and that for BEN is 1667/*a*. Comparative relative risks can be expressed as the ratio of BEN risks to DON risks (60). Treatment with BEN at the most efficacious time decreased DON levels to 34 % of controls, and decreased the comparative risk ratio to 27. The potential for fungicides to decrease mycotoxin residues is important in the evaluation of fungicides. This approach improves on current practices by including the expected reductions in risks from naturally occurring mycotoxins.

1882 ART MATERIALS (LHAMA) RISK ASSESSMENT FOR ACRYLIC STAINS.

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An acute can chronic human health risk assessment was performed on an acrylic stain used as an art material according to the guidelines of the American Society for Testing and Materials (ASTM D-4236) to satisfy the Labeling of Hazardous Art Materials Act (LHAMA) requirements for art materials. Each ingredient in the product was identified by its CAS number and searches performed in the National Library of Medicine data bases. For pigments and dyes, the Color Index was used. Hazards for individual ingredients and the final product were evaluated by estimation of high-use hobbyist exposure rates for acute and chronic exposure and comparison made to acceptable intake levels. Exposure assessment for both brush and spray application was performed based on use rates for similar products and actual measurements of spray rates. Duration of exposure was assumed 6 years for the child and 30 years for adults. Acceptable Daily Intakes were derived from acute toxicity data using the EPA safety factor approach. Chronic exposure hazard was calculated using the EPA Human Health Evaluation Manual route specific equations for calculating chemical intakes. The product consisted of various acrylic polymers in aqueous solution with pigments. The oral ADI calculated from individual toxicities using a 100 fold safety factor to account for species and route differences, was 40 mg/kg or 13 ounces for a 10 kg child. The acute dermal hazard was below the 1.0 safety threshold but the acute hazard for inhalation was 2.9 for the child. Chronic hazard quotients for oral and dermal exposure were below the 1.0 threshold for dermal and oral exposure but 5.5 for inhalation by the child due to ammonia. Mild eye irritation was predicted based on Draize tests of ingredients. Sensitizers were identified among the acrylic monomers. Labeling for eye irritation and precautions for use by children were recommend.

1883 INTERIM ESTIMATES OF THE REFERENCE DOSE (RfD) FOR KEY BREAKDOWN PRODUCTS OF CHEMICAL AGENTS.

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There is a need to evaluate the health risks associated with chemical warfare (CW) agents and their degradative products. This need has been heightened by the present program of planned agent destruction. Provisional estimates of Reference Dose (RfD), for non-cancer toxicity, have been made for five key breakdown products for which toxicity data are either limited or lacking: thiodiglycol (TDG), methylphosphonic acid (MPA), ethyl methylphosphonic acid (EMPA), S-(2-diisopropyl amino) ethyl methylphosphonothioic acid (EA2192), and lewisite oxide/ 2-chlorovinyl arsonous acid. Three approaches were used for estimating RfD. (1) Experimental chronic or subchronic LOAEL or NOAEL, (2) LOAEL or NOAEL for a closely related compound,

and (3) An estimate of rat chronic LOAEL made using a commercially available computational toxicology system for Quantitative Structure Activity Relationships (QSAR). We used the TOPKAT ™ system (Health Designs, Inc., Rochester, NY). In all cases, calculation was based on the use of Uncertainty Factors (UF), adhering where possible to USEPA guidelines. Laboratory data suitable for RfD development were available only for TDG. For EA2192 and lewisite oxide/ 2-chlorovinyl arsonous acid, an experimental subchronic LOAEL for the chemical agent VX and an experimental NOAEL for lewisite were used, respectively. Similarly, for EMPA, a published LOAEL for isopropyl methylphosphonic acid (IMPA) was used. RfDs for TDG, MPA, and EMPA were developed using both actual data and TOPKAT estimates of the rat chronic LOAEL. The estimated RfDs for MPA, EMPA, EA2192, and lewisite oxide were 40, 28, 0.0006, and 0.1 :g/kg/day, respectively. For TDG, an RfD estimate is being developed based on recent 14-day and 90-day rat studies at this Center. These provisional values are intended for screening purposes and as an aid in cleanup decisions. (Abstract does not reflect U.S. Army policy.)

1884 IDENTIFICATION OF PERSISTENT AND TOXIC CHEMICAL WARFARE (CW) AGENT DEGRADATION PRODUCTS.

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The primary agents within the United States CW agent inventory include several vesicant or blister agents (sulfur mustards: H, HD, HT; nitrogen mustards: HN1, HN2, HN3; and the organic arsenical, lewisite: L), four nerve agents (VX, GA, GB, GD), and the blood agent, cyanogen chloride. With the occasional exception of the sulfur mustards, most of the CW agents are not persistent in the environment as they are subject to a variety of degradation mechanisms including hydrolysis and oxidation. The authors evaluated fate and toxicity data from field and laboratory studies as well as reports of storage container analyses at burial or unitary stockpile sites in order to identify degradation products that are highly persistent and/or exhibit high mammalian toxicity. The emphasis was on those potential degradation products resulting from agent contact with soil or water, especially from buried chemical weapons and wastes. The degradation products of primary interest include thiodiglycol for HD; lewisite oxide for lewisite; and *S*-(2-diisopropylaminoethyl) methylphosphonothioic acid (EA 2192), ethyl methylphosphonic acid, and methylphosphonic acid (MPA) for VX. Methylphosphonic acid is also a product of both GB and GD degradation. The GB contaminant diisopropyl methylphosphonate is of interest because of its environmental persistence; the closely related isopropyl methylphosphonic acid is a GB hydrolysis product that slowly hydrolyzes to MPA. Of these degradation products, only lewisite oxide and EA 2192 possess high mammalian toxicity.

1885 UPTAKE OF METALS AND POLYCYCLIC AROMATIC HYDROCRABONS FROM CONTAMINATED SEDIMENT INTO EDIBLE BLUE CRABS.

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Estuarine areas of southeast Texas commonly provide habitat for a variety of species of commercial and recreational importance, including blue crabs (*Callinectus sapidus* Rathbun). Many of these areas provide drainage for a variety of municipal and industrial facilities, which may result in release of chemicals to surface water or sediment. Prior sampling of a drainage canal from which commercial fishing for crabs is known to occur adjacent to a refinery identified metals and polycyclic aromatic hydrocarbons (PAHs) in sediment at concentrations that might result in uptake by crabs to levels of concern for people who eat them. To confirm this, crabs of edible size were collected from the canal with emphasis on areas in which sediment concentrations were highest. Sediment samples were collected to determine the bioavailable fraction of the simultaneously extractable divalent metals (SEM; cadmium, copper, lead, nickel, zinc) by comparing the total molar concentration of SEM to the total concentration of acid volatile sulfide (AVS). Edible crab tissue (32 composite samples of 5 crabs each) was analyzed for PAHs and metals, and the 95 percent upper confidence limit of the mean concentration (95 UCL) of each analyte was compared to conservative risk-based values. None of the individual analyte concentrations or 95 UCLs exceeded the risk-based value. Results of the SEM/AVS determination indicated that the five divalent metals were not present in a bioavailable form; this finding was independently confirmed by the low concentrations of these metals in edible crab tissue.

1886 DEVELOPMENT OF FISH MODEL FOR ENVIRONMENTAL CANCER RISK ASSESSMENT; INTERRELATIONSHIP OF MUTATION, APOPTOSIS AND CANCER.

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We tried to develop fish model to get more information for the interrelationship of mutation, apoptosis and cancer, then eventually to use for environmental cancer risk assessment. Fish (medaka, *Oryzias latipes*) was investigated following a 28 day, 3 times /week pulse exposure to N-methyl-N'-nitro-N-nitrosoguanidine(MNNG). 5-week-old fishes were exposed to MNNG at concentrations of 0, 0.5, 1.0 mg/L and reared for 3, 5, 7 more months from the last day of exposure. Glutathione (GSH) and lactate dehydrogenase (LDH) levels from whole body samples were measured by spectrophotometer. GSH level was decreased while LDH level was increased in the samples of the higher dose groups and longer time groups. Mutational responses were detected by arbitrarily primed polymerase chain reaction (AP-PCR) fingerprinting method using whole body DNA samples as templetes and PBR primer. AP-PCR fingerprinting revealed dose- and time-dependent mutational changes by band density, loss and gain. A band from normal fish samples was disappeared after treatment of MNNG, then, the band disappeared was excised and sequenced. The band has 869 base pair-long sequence, however, there was no putative protein coding region based on analysis by Genbank. Flow cytometric analysis revealed that fishes from 3 and 5 month -reared group were under the severe apoptotic changes in the dose- and time-dependent manner, but those from 7 month-reared group were under less apoptotic, rather showed more necrotic and carcinogenic alterations. However, terminal deoxynucleotidyl transferase (TdT) assay showed that fish carrying tumor were still under the severe apoptotic cell changes. In deed, tumors were detected on the whole body sections from 4 individuals of 10 fishes and immunohistochemical analysis showed that tumor cells were actively proliferating. This study does not only show the interrelationship of mutation, apoptosis and cancer at a glance, but indicate that medaka can be used as a good fish model for environmental cancer risk assessment.

1887 HEALTH RISK ESTIMATES FOR "POLYBROMINATED DIPHENYL ETHERS" (PBDEs) ASSOCIATED WITH CONSUMPTION OF FISH AND HUMAN BREAST MILK.

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Recent reports in the scientific literature describe the detection of "polybrominated diphenyl ethers" (PBDEs) in human breast milk, human adipose tissue, fish, and marine mammals. Among the 209 possible congeners and three commercial PBDE products, the reports largely indicate the presence of 2,2',4,4'-tetrabromodiphenyl ether and, to lesser extent, tri- through hexa-brominated congeners. The most widely used PBDE, decabromodiphenyl ether (DPBDE), has not been detected in marine mammals, fish, or milk. Ultra-trace HRGC/HRMS analysis indicate levels of penta-, hexa-, and hepta- brominated congeners are generally below 0.1 ng/g in freshwater fish; substantially lower levels are reported in marine fish. Swedish studies report similar low levels in human breast milk and adipose tissue. However, little is understood about the source(s), the level of human exposure, or the toxicological significance of the different congeners found in the environment. Using the limited available exposure data and conservative assumptions in a risk assessment model, noncancer risks were calculated for the reasonably exposed and maximally exposed individual. Exposures to adults and infants through consumption of contaminated fish and breast milk were examined. For some congeners, references doses for noncancer effects were extrapolated from the available literature.

1888 HUMAN HEALTH RISKS OF ARSENIC IN MARINE ANIMALS AND REGULATORY APPROACHES.

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High levels of arsenic are detected in marine animals. Despite the high concentration of arsenic in the edible portions of aquatic animals, no reports were found on the actual health effects of arsenic in people consuming large amounts of seafood. This may be because the arsenic found in marine animals exists in various methylated forms, which are not bioaccumulated and

have low toxcities. No legal limits exist for arsenic in fishery products in the U. S. However, the U.S. Environmental Protection Agency has calculated an ambient water quality criterion value of 0.140 μ g/L (inorganic arsenic) for waters containing edible aquatic animals representing a 1 x 10-6 incremental increase of cancer risk. This concentration of arsenic is lower than the average normal levels of arsenic (0.96 - 2 μ g/L) detected in marine waters. We used an alternative approach to determine the human health risk based on the actual inorganic arsenic concentration detected in seafood. Compiled data on the concentration of total arsenic in marine animals found in the world seas were used. The inorganic arsenic content in marine animals was estimated based on the reported average inorganic arsenic body burden ranging between 0.5 and <6 % of the total arsenic levels. These calculated inorganic arsenic levels were then used with estimates of average seafood ingestion rate (6.5 g/day) and apportioned total daily seafood consumption (44% fish, 44% crustaceans and 12% bivalves) to determine a lifetime average daily dose (LADD) of inorganic arsenic. The estimated excess lifetime cancer risk (ELCR) using the LADD and the inorganic arsenic slope factor (1.5 $mg/kg/day)^{-1}$ ranged between 1.5 $x10^{-6}$ and 1.2 x 10^{-6}. The results suggest that marine animals harvested from the world seas (containing up to 2 μ g/l arsenic) may pose minimal arsenic-related health hazards in the average consumer. Since data on arsenic speciation is abundant, it seems more appropriate to set limits to protect public health for arsenic in seafood based on the inorganic arsenic levels in marine organisms rather than using marine water arsenic concentrations.

1889 EXPOSURE FACTOR AND TOXICITY DATA FOR CALIFORNIA WILDLIFE: TRENDS, DATA GAPS AND SOURCES OF UNCERTAINTY FOR ECOLOGICAL RISK ASSESSMENTS.

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Evaluating wildlife exposure to toxicants in ecological risk assessments requires knowledge or estimation of a diverse array of species-specific information that includes various normalizing factors (e.g., body weight, metabolic rate), contact factors (e.g., ingestion rate, dietary composition), population dynamics (e.g., population density, fecundity) and seasonal activities. To meet this information need, a relational database containing such information for sixty-five California mammals, birds, reptiles and amphibians was created from the existing literature. Available toxicological data were also collected for these species to provide information relevant to interpretation of exposure data. A qualitative analysis was conducted to identify the relative abundance of various types of data for species and groups of species. Physiological exposure factors, except for body weight, were underrepresented compared to ecological factors (e.g., population dynamics) which tended to be well studied. Exposure factor information was limited for many species but data were most abundant for mammals, followed by birds and herpetiles. Wildlife toxicity data were considerably less abundant than exposure factors and were clustered within a few commonly studied species. Toxicity data on other, less "traditional", study species were available with data abundance greatest for birds, followed by mammals and herpetiles. Overall, this analysis may assist in the selection of "data-rich" receptors, as well as provide quick identification of exposure factor and toxicity information gaps that may be sources of uncertainty in ecological risk assessments.

1890 A MODEL FOR ASSESSING AIRBORNE EXPOSURES TO *LEGIONELLA* BACTERIA

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Assessing exposures to airborne *Legionella* bacteria and the potential human health risks for developing disease depends on several factors. Factors considered for such exposure assessments should include not only the concentration of *Legionella* bacteria at the source, but also the characteristics of the pathway to potentially exposed individuals and how the original source concentration is modified by that pathway. We developed a model to estimate airborne concentrations of *Legionella* bacteria following release of an aerosol from a contaminated cooling tower in a hospital. Inhaling these bacteria may cause Legionnaires' disease, a serious pneumonia, or Pontiac fever, a less severe, flu-like illness. Several different exposure scenarios were developed to compare exposure estimates. The model incorporated a variety of factors affecting airborne concentrations of *Legionella* bacteria between the cooling tower source and exposed individuals. Variables affecting source concentrations included *Legionella* concentration in the cooling tower water, water flow and air flow rates in the tower, and the percentage of tower drift. Pathway variables affecting the aerosol concentration between the cooling tower and potentially exposed individuals included distance from the tower, wind conditions, moisture level in the air, and filters in the ventilation system. Evaluation of one exposure scenario determined that the time needed to inhale a dose of only one viable *Legionella* bacterium was 200,000 times longer for a hospital patient compared to a maintenance worker on the roof near the cooling tower. Development of an exposure assessment model that incorporates actual exposure parameters provides a useful tool for estimating health risks associated with exposures to *Legionella* bacteria.

1891 POLLUTANT-INDUCED IMMUNOSUPPRESSION AS A CAUSE OF DWINDLING AMPHIBIAN POPULATIONS.

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Recently, a decrease in the populations of marine toads (*Bufo marinus*) and whistling frogs (*Eleutherodactylus johnstonei* and *E. gossei*) has been noted on the island of Bermuda. In the current study, we investigated whether this decline was related to altered immune functions. During August 1998, a significant proportion (25%) of the toads exhibited deformities. Analysis of soil samples revealed presence of chlorinated pesticides and PCBs. Spleen cells from toads collected from more polluted areas exhibited a decrease in B cell proliferative response to lipopolysaccharide when compared to the responsiveness of B cells from toads collected from less polluted areas. In contrast, the T cell responsiveness to mitogens in these two groups was not significantly altered. Analysis of major parenchymatous organs in marine toads and whistling frogs showed alterations in hepatic and splenic morphology, indicative of exposure to toxicants. Together, the current study demonstrates that the immune functions of toads from contaminated sites of Bermuda are significantly altered, which may account for the decline in their population. (Supported in part by grants from NIH and EPA.)

1892 CANCER RISK ASSESSMENT OF METHYL T-BUTYL ETHER (MTBE) FOR CALIFORNIA DRINKING WATER.

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MTBE, an oxygenate in California's cleaner burning gasoline, has appeared in ground water and drinking water sources, primarily from leaks in underground fuel storage tanks. The California Local Drinking Water Protection Act of 1997 mandates the establishment of primary and secondary drinking water standards for MTBE. As part of this process OEHHA has developed a public health goal (PHG) for MTBE based solely on human health criteria. OEHHA evaluated the toxicity data on MTBE and concluded that the animal carcinogenicity data was an appropriate basis of the PHG. Carcinogenicity of MTBE has been observed in both sexes of the Sprague-Dawley rat in a lifetime gavage study, in male F344 rats in a 24-month inhalation study, and in male and female CD-1 mice in an 18-month inhalation study. The tumor types observed in rats were Leydig interstitial cell tumors, renal cell tumors and combined lymphoma/leukemia. Evaluation of genotoxicity and mechanistic data did not identify the modes of action for carcinogenicity at these sites; however the relevance to human cancer risk could not be discounted. A physiologically-based pharmacokinetic model was used to estimate an internal dose metric (AUC for unchanged MTBE) in the rat which allowed cross-route extrapolation (inhalation to oral equivalent). A polynomial curve was fit to the tumor incidence data and adjusted doses, and the 95% lower confidence limit on the dose for 10% incidence estimated for each site (LED_{10}). The cancer slope factors (CSFs) for the rat data were calculated as $0.1/LED_{10}$, yielding a geometric mean CSF of 1.8 x 10^{-3} $(mg/kg\text{-}d)^{-1}$.

1893 ENDCORINE DISRUPTOR KNOWLEDGE BASE (EKB).

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The NCTR's Endocrine Disruptor Knowledge Base (EKB) is intended to assist in risk assessment and regulatory decisions for exogenous compounds that may alter responses and disrupt vertebrate endocrine systems. This integrated multidisciplinary program comprises three primary elements: 1) *in vitro* and *in vivo* experimentation 2) an Internet-based bibliography and bioactivity database; and, 3) computer-based predictive models. *In vitro* competitive binding assays have been done for about 100 active and 50 inactive compounds. The assays are for not only the estrogen receptor, but also Testosterone-Estradiol Binding Globulin and Alphafetoprotein, respectively, the human and rodent serum binding proteins that are major determinants of estrogen potency. The EKB provides a cross-linked database and bibliography that is searchable with an Internet browser. An ORACLE database is designed for *in vitro* and *in vivo* data, including complex assays such as the multi-generation protocols of the NIEHS-supported endocrine disruptor studies. The EKB will be made universally available via the Internet. Users will be able to obtain and contribute data, conduct data mining and structure search, share annotations among a defined audience such as for data peer review, and view or download data and chemical structure files. Thus, the EKB will provide an environment for virtual collaboration and knowledge mining facilitating identification of data gaps and research hypotheses, and development of QSAR, chemometric, and simulation predictive models. The overarching goal of our computational modeling efforts is to assess, develop and validate a broad spectrum of analytical tools for predicting a compound's endocrine disrupting potential at the mechanistic level. QSAR models include 3D Comparative Molecular Field Analysis, Hologram QSAR, as well as pharmacophoric models. Modeling is guided with structural diversity measures using descriptors selected with genetic algorithms. Classification models include unsupervised learning approaches such as Principal Components and Hierarchical Cluster Analysis, and supervised learning approaches with K Nearest Neighbor, SIMCA and neural networks.

1894 USING CLASS BASED ACTION LEVELS (CBAL) TO EVALUATE POTENTIAL CHEMICAL EXPOSURES IN DRINKING WATER.

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NSF International is a not-for-profit group dedicated to the protection of public health and the environment through standards development and third-party certification of products impacting the quality of drinking water. In accordance with ANSI/NSF Standards 60 and 61, NSF international uses available chemical and toxicological data to establish chemical specific limits for drinking water. Often times however there is insufficient toxicological data available to establish a chemical specific Maximum Allowable Level (MAL), or chemicals are only tentatively identified by Gas Chromatography/Mass Spectrometry. For these evaluations, NSF International has established chemical Class Based Action Levels (CBAL). CBAL can be used to evaluate tentatively identified chemicals, as well as chemicals that do not have sufficient information to develop an MAL. A chemical class is defined by similarities in structure, functional group, and molecular weight. Prior to the establishment of CBAL, NSF created the Toxicology Evaluation Database (TED), which is a summary of toxicological information on over 780 chemicals. This database was constructed with relevant toxicological information available from sources such as the USEPA, Health Canada, NSF International, and various State and Federal Drinking Water Standards and Guidelines. In conjunction with OncoLogic® Structure Activity Program developed by EPA, the TED database was used to establish the various CBA> The use of this strategy is presented for the alkylated phenolic class.

1895 DEVELOPMENT OF AN AQUATIC TOXICITY DATABASE: CRITERIA FOR DETERMINING CRITICAL ENDPOINTS.

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A database has been developed to provide a standardized resource of aquatic toxicity data for use in ecological risk assessment, environmental health and safety, and hazardous chemical evaluation. The dynamic list is comprised of data derived from a large amount of acute toxicity testing conducted on freshwater aquatic species for over 250 commonly used industrial chemicals. Data selection is in compliance with global harmonization efforts, based on information and guidelines established by the Toxic Substance Control Act (TSCA) and the Organization for Economic Cooperation and Development (OECD), as well as various reference texts. Entries in the database contain toxicity data from three species of fish, a representative invertebrate (*Daphnia magna*), a luminescent bacterium, and alga, where available. All entries in the database contain the test endpoint, the common name of the species of the aquatic organism, and experimental value. The entries also contain test parameters including age of test fish species, test method, and information on water conditions. The chosen parameters are considered to be the most commonly used and accepted to provide the most useful assessment of toxicity and to allow for comparisons between data sets. This data is particularly useful for incorporation into Material Safety Data Sheets (MSDS) in sections dealing with ecological information as recommended by ANSI Z400.1 and as measures of effect for assessment endpoints in ecological risk assessment.

1896 PEER REVIEW PROCESS FOR RISK ASSESSMENT DOCUMENTS AND METHODS.

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High quality human health risk values can be assured by independent peer review. Traditionally, governments have assessed the human health hazards of pollutants and derive risk values (e.g., RfCs, MRLs, slope factors, TDIs). Increasingly industry, states, consulting firms, trade groups and consortiums are estimating risk values and preparing supporting documentation. Toxicology Excellence for Risk Assessment (*TERA*) instituted a program in late 1996 to make independent peer review of risk values available. A balanced panel of scientists from government, industry, consulting, academia, and environmental groups is brought together for quarterly meetings. After approval, a risk value can be included on the International Toxicity Estimates for Risk (*ITER*) database (http://www.tera.org/iter) for use by other risk assessors world-wide. Participants have included Health Canada, the International Copper Association, ChemRisk, Dow AgroSciences and U.S. EPA. Summaries of discussions and results are made available on the Internet (http://www.tera.org/peer). Results include an acrylonitrile inhalation unit risk developed by *TERA* of 1.1E-2 per mg/cu.m, and a site-specific reference dose developed by ICF Kaiser International of 0.0003 to 0.001 mg/kg-day for people eating methylmercury contaminated fish. The latter risk value uses a PBPK model and Monte Carlo analysis, which results in a distribution of ingestion rates and the RfD. Both values were approved by expert panels and are now available on *ITER*. A hexavalent chromium RfC, developed by ChemRisk, and Telone II cancer and noncancer assessments, prepared by Dow AgroSciences, have also been approved by expert panels and will be loaded on *ITER* shortly.

1897 COMMUNICATING REGULATORY INFORMATION ABOUT ENVIRONMENTAL CONTAMINANTS TO THE PUBLIC.

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To contribute effectively as citizens and to make individual risk decisions, the public needs to have at least a rudimentary understanding of the different rules and regulations that govern environmental contaminants in different media; e.g., pesticides in foods. Although government agencies have made significant efforts to make information about regulations available, particularly on the WWW, it generally requires a great deal of time and effort to locate this information, even for those familiar with both the WWW and the way government functions. It is especially burdensome if a citizen wants to know regulations governing a pesticide in different media; e.g., drinking water and air. This requires visits to several WWW sites each requiring a different search strategy. To overcome this difficulty, we have summarized all of the critical federal laws and rules in lay language and put this information in one place on the WWW. In addition, these summaries are linked to the specific pages on government sites that provide regulatory values; e.g., MCLs in drinking water. Thus, this information is always current. Further, these pages are linked to information about the implementation of the regulations; e.g., lists of Superfund sites. The summaries are supplemented by

glossaries that provide definitions of terms that must be used to be accurate but which may not be familiar to the public. This WWW site is currently being expanded to include similar information relating to the rules and regulations in the states in the Great Lakes region. It is hoped that this will provide a model that can be used in other parts of the country.

1898 THE AIR TOXICS HOT SPOTS PROGRAM RISK ASSESSMENT GUIDELINES, PART II: TECHNICAL SUPPORT DOCUMENT FOR DESCRIBING AVAILABLE CANCER POTENCY FACTORS.

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A cancer unit risk and potency factor database for 120 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). Unit risk and cancer potency 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 Assessment 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), and other OEHHA documents. A hierarchy of choice was developed for selecting cancer potency values available from multiple sources which gives preference to sources which 1) include external peer review and public comment procedures for publishing cancer potency values, and 2) use the most recent available data sets and scientific methodology. The database includes a summary of the key studies and methodology used to derive the cancer unit risk and potency factors for each compound listed. Changes from prior draft versions include 1) the addition of lead and lead compounds and diesel exhaust to the database; 2) adoption of new US EPA unit risk values for polychlorinated biphenyls (PCBs); 3) the addition of oral cancer potency factors where appropriate; 4) a discussion of how harmonization with the new draft US EPA cancer risk assessment guidelines will occur. This database has undergone external peer review (CARB Scientific Review Panel), public comments at workshops, and is accessible via the Internet. This Hot Spots database will facilitate Hot Spots cancer risk assessments performed in the state of California by providing a single, easily available source for cancer unit risk and potency factors.

1899 CLONING, HETEROLOGOUS EXPRESSION, AND CHARACTERIZATION OF HUMAN CYP2A13.

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Three members of CYP2A subfamily, CYP2A6, CYP2A7, and CYP2A13, have been identified in humans. CYP2A6 is the major coumarin 7-hydroxylase and is involved in the metabolic activation of numerous procarcinogens, whereas CYP2A7 may be inactive. A cDNA for CYP2A13 has not been isolated; a putative coding sequence (CYP2A13*1) was previously deduced from *CYP2A13* gene sequence. It is not known whether CYP2A13 is functional or where it is expressed. In this study, the coding region of a CYP2A13 cDNA, named CYP2A13*2, was cloned from human nasal mucosa. Sequence alignment with CYP2A13*1 indicated that the two allelic variants share 98.6% amino acid sequence identity. Preliminary genotyping studies showed that CYP2A13*2 may be the predominant allele. CYP2A13*2 mRNA was detected by RNA-PCR in liver, in addition to nasal mucosa, and in a number of other extrahepatic tissues. Microsomal preparations from CYP2A13*2-expressing SF9 cells were active in a reconstituted system in metabolizing coumarin to 7-hydrxycoumarin, albeit with a lower activity than that of CYP2A6. In addition, CYP2A13*2 was also active in the metabolic activation of several xenobiotic compounds, such as hexamethylphosphoramide and N-nitrosomethylphenylamine. CYP2A13*2 may play important roles in xenobiotic toxicity in humans. (Supported in part by NIH grant ES-07462.)

1900 BIOTRANSFORMATION OF COUMARIN BY RODENT AND HUMAN CYTOCHROMES P450: METABOLIC BASIS OF TISSUE-SELECTIVE TOXICITY IN THE OLFACTORY MUCOSA OF RATS AND MICE.

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Coumarin was previously found to cause tissue-selective toxicity in the olfactory mucosa (OM) of rats and mice, with rats being the more sensitive species. The aim of this study was to explore the role of target tissue biotransformation in OM-selective toxicity and the metabolic basis of the species differences in coumarin toxicity. At least six coumarin metabolites were detected in OM microsomal reactions, with *o*-hydroxyphenylacetaldehyde (*o*-HPA) being the most abundant. The rates of metabolic activation of coumarin, as indicated by the formation of *o*-HPA, were comparable in OM microsomes of rats and mice, but were about 25- and 3-fold higher in OM than in liver microsomes of rats and mice, respectively. Thus, target tissue activation seems to play an important role in the tissue-selective toxicity, whereas differences in the rates of hepatic metabolism may be responsible for the species difference in olfactory toxicity. Purified, heterologously expressed mouse CYP2A5 and CYP2G1 produced 7-hydroxycoumarin and *o*-HPA as the predominant products, respectively. Kinetic analysis and immunoinhibition studies indicated that the OM-specific CYP2G1 plays the major role in metabolic activation of coumarin. Furthermore, of 13 human P450s examined, five were active in the metabolic activation of coumarin, suggesting a potential risk of coumarin toxicity in humans. (Supported in part by NIH grant ES-07462.)

1901 EXPRESSION OF BIOTRANSFORMATION ENZYMES IN HUMAN FETAL NASAL MUCOSA.

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High levels of P450 are present in the olfactory mucosa (OM) in mammalian animals and contribute to the known tissue-selective toxicity of numerous chemical compounds. Olfactory toxicity in perinatal period may have greater impact on behavior, growth, and development than in adults. To establish a molecular basis for determining the risk of developmental toxicity in OM, the expression of a number of P450 isoforms and other biotransformation enzymes was examined in hepatic and nasal microsomes prepared from aborted human fetus at gestational day 91-125 (G91-G125). The relative tissue levels of various biotransformation enzymes were determined on immunoblots. Expression of CYP2A, CYP2B6, CYP2J, NADPH-P450 reductase, and microsomal epoxide hydrolase was detected in both OM and liver at as early as G91. The level of expression in OM is lower than that in liver, except for the CYP2A-related protein, which was expressed in OM at much higher levels. OM expression of CYP2A6, CYP2A13, CYP2B6, and CYP2J2 mRNA was confirmed using RNA-PCR. The prenatal expression of biotransformation enzymes in human OM may have detrimental consequences upon transplacental exposure to xenobiotics. (Supported in part by NIH grant ES-07462.)

1902 THE EFFECT OF *o*-XYLENE INHALATION ON CYTOCHROME P450 ISOZYMES AND TOXICITY IN RAT LUNG AND NASAL MUCOSA.

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The respiratory tract represents the initial site of contact for inhaled toxicants such as xylene. The lung and nasal mucosa receive the highest dose of inhaled xenobiotics and are known to have high mixed function oxidase capability. Exposure to *o*-xylene by inhalation (300 ppm for 6 hours) inhibited numerous cytochrome P450 isozymes in the lung and nasal mucosa. Pulmonary CYP2B1 activity, as measured by O-dealkylation of benzyloxyresorufin (BROD), and CYP2E1, as measured by NDMA demethylase activity, were inhibited, 50% and 13%, respectively, immediately following exposure to *o*-xylene. However, pulmonary CYP2B1 and CYP2E1 activity recovered to control values 24 hours following cessation of exposure. Pulmonary CYP4B1 activity, as measured by 2-aminofluorene N-hydroxylase activity, was inhibited by 59% immediately following exposure and remained inhibited (40%) 24 hours following cessation of exposure.

Isozymes of the nasal mucosa were also affected by exposure to *o*-xylene; CYP1A1 activity, as measured by O-dealkylation of ethoxyresorufin (EROD), as well as CYP2B1, CYP2E1, and CYP4B1 were inhibited by 69%, 44%, 40% and 49%, respectively. With the exception of CYP2B1 activity, none of these isozymes of the nasal mucosa recovered 24 hours following cessation of exposure. Although P450 isozymes in the respiratory tract were markedly affected by exposure to *o*-xylene, analysis of bronchoalveolar lavage fluid and nasal lavage fluid did not demonstrate evidence of cellular damage. Levels of gamma-glutamyl transferase (GGT), a membrane-bound enzyme, and lactate dehydrogenase (LDH), a cellular enzyme, were not significantly elevated in lavage fluid at either time point following *o*-xylene exposure. Therefore, a decrease in the metabolic capacity of the respiratory tract was not accompanied by overt toxicological changes at this site.

1903 METHYL-PARATHION (Me-PA) IS ACTIVATED IN RAT BRAIN HOMOGENATES BY CYTOCHROME P450 (CYP) 2B1.

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The role of cytochrome P-450 on the activation of Me-PA, a widely used phosphorotioate pesticide and its inhibition by phenobarbital (PB), monoclonal anti-rat CYP antibodies and CO was studied in brain post-mitochondrial homogenates of adult male Wistar rats. The activation of Me-PA (20 – 160 nM) was monitored by the inhibition of acetylcholinesterase (AChE) using the hydrolysis of acetylthiocholine and colour development with 5,5'-dithio-bis-2-nitrobenzoate. As expected, Me-PA irreversibly inhibited the AChE activity (Vmax decreased 36%, whereas Km_{app} barely change). Previous bubbling with CO or addition of anti-2B1 antibodies totally prevented the inhibition of AChE by Me-PA, but anti-CYP 1A1 antibodies showed no effect. PB (80 μ M), which acts as a CYP 2B1 substrate, decreased 20% the inhibitory effect Me-PA (20 nM) on AChE. The protective effect of anti-2B1 antibodies and PB towards AChE inhibition by Me-PA indicates that CYP 2B1 is responsible for pesticide activation in the brain. However, Me-PA shows a higher affinity for CYP 2B1 than PB. (Supported by the European Commission: Biomed 2, BMH4-CT96-0184 and CONACyT and CNR, Mexican and Italian governments.)

1904 INDUCTION OF HEPATIC CYP2B1/2 FOLLOWING INHALATION EXPOSURE TO OCTAMETHYLCYCLOTE TRASILOXANE (D4): A DOSE RESPONSE STUDY.

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Exposure to D4 increases liver size and induces hepatic CYP2B1/2 enzymes in a manner similar to phenobarbital. The purpose of the present study was to characterize the pharmacodynamics of liver enlargement and hepatic CYP2B1/2 enzyme induction over a wide range of exposure concentrations. Female Fischer 344 rats were exposed to 0, 1, 7, 30, 70, 150, 300, 500, 700, and 900 ppm D4 via whole body inhalation 6 hr/day, for 5 days. Following exposure, liver was removed and microsomes were prepared. Liver-to-body weight ratios increased in an exposure- and liver concentration-dependent manner relative to controls. The response portion of the curve was linear with no apparent plateau. A maximum increase in liver weight of 24% over controls was achieved after exposure to 900 ppm D4. Hepatic CYP2B1/2 activity (as determined by PROD) increased in a D4 exposure- and liver concentration-dependent manner. Maximum activity occurred at 500 ppm D4, with decreasing activity between 500 and 900 ppm D4. Induction of CYP2B1/2 immunoreactive protein reached a plateau between 500 and 900 ppm D4. The lowest concentrations of D4 that resulted in significant liver enlargement and CYP2B1/2 induction were 150 and 7 ppm, respectively. These data indicate that the induction of hepatic CYP2B1/2 enzymes is an early biochemical response to D4 exposure in the rat and that a maximum response can be achieved following 5 days repeated inhalation exposure to 500 ppm D4. Supported in part by the Silicones Environmental Health and Safety Council.

1905 EVALUATION OF OCTAMETHYLCYCLOTETRASILOXANE (D4) AS A POTENTIAL INHIBITOR OF HUMAN HEPATIC CYTOCHROME P450 ENZYMES.

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D4 is a low molecular weight (296 Da.) cyclic siloxane with industrial and consumer applications. Exposure to D4 increases liver size and induces hepatic CYP2B1/2 enzymes in a manner similar to phenobarbital. In addition, *in vivo* experiments with rats indicated that the enzymes induced by D4 are also involved with its metabolism. The purpose of the present study was to evaluate the ability of D4 to inhibit rat and human hepatic P450 enzymes *in vitro*. D4 inhibition of rat CYP2B1/2 activity in the presence of pentoxyresorufin (Km/2, Km, 2Km, and 4Km) was assessed by measuring the rate of dealkylation (PROD activity) with increasing D4 (0.095 to 2.4 μ M) concentrations. In these experiments D4 inhibited rat PROD activity in a concentration-dependent manner with a Ki value of approximately 0.11 μ M. To evaluate the ability of D4 to directly inhibit the major P450 enzymes in human liver, as well as determine the type of inhibition, microsomes from a pool of seven individuals were incubated with marker substrates at concentrations equal to Km/2, Km, 2Km and 4Km in the presence or absence of D4 (0.037 to 2.9 μ M). D4 appears to be a weak noncompetitive inhibitor of human CYP2B6 (Ki = 3.6 μ M), 2D6 (Ki=14 μ M) and 3A4/5 (Ki=11μ M) and a weak competitive inhibitor or CYP1A2 (Ki=12 μ M). Overall, these data indicate that D4 does not significantly inhibit the activity of the human CYP enzymes evaluated and therefore biologically relevant chemical-chemical interactions in humans would not be expected. Supported in part by the Silicones Environmental Health and Safety Council.

1906 SAR STUDIES OF CYP 2B INDUCTION IN PRIMARY CULTURED RAT HEPATOCYTES.

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Induction of cytochromes P450 (CYP) by drugs can lead to drug-drug interactions. Work in our laboratory has focused on the application of primary cultured hepatocytes to determine CYP induction potential of drug candidates in support of drug discovery. To further determine the usefulness of this model, a series of structurally related compounds from our COX-2 inhibitor program were evaluated for their ability to induce CYP 2B, in order to determine any structure activity relationship. Freshly isolated rat hepatocytes were cultured on Matrigel for 2 days to allow for culture adaptation followed by exposure (24 h) to test compounds. Cells were then harvested and CYP 2B analysis was performed by Western Blot using anti-rat CYP 2B1 antibodies. Nontreated cells as well as cells exposed to phenobarbital were used as negative and positive controls, respectively. Induction was defined as a percentage relative to positive control (phenobarbital treated cells). Results showed that CYP 2B inducibility by our model compound could be dramatically decreased by the simple modification of the R-group from an ethyl to a methyl or hydrogen moiety (from 80% to <5% CYP 2B induction). These studies clearly demonstrate that the use of such an *in vitro* cell model can significantly aid our ability to carry out SAR studies within a structurally related class of compounds.

1907 REVERSE TRANSCRIPTION-POLYMERASE CHAIN REACTION CHARACTERIZATION OF EXPRESSION OF CYP2B7 AND CYP2B8 (P450 hIIB2) mRNAs IN HUMAN LIVERS.

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The human CYP2B subfamily computes three mRNAs, namely CYP2B6, CYP2B7 and CYP2B8 (P450 hIIB2). In contrast to the already characterized, predominantly hepatic, expression of CYP2B6, our knowledge of the expression of CYP2B7 is limited, and the expression of CYP2B8 has not been evaluated at all. CYP2B7 mRNA has been found in lung, lung tumors, and bronchial epithelial cells. The mechanism of translation of CYP2B7 and CYP2B8 and the properties of the enzymes remain to be determined. We evaluated the extent of CYP2B7 and CYP2B8 mRNA expression in human livers. Detection of CYP2B7 mRNA was based RT-PCR amplification of a 578bp fragment of the mRNA. The CYP2B7 primers amplify the CYP2B6

mRNA as well, but the latter fragment is 704 bp long. The CYP2B8 forward primer hybridizes to a sequence unique to the CYP2B8. The detection of CYP2B8 mRNA is based on amplification of 480bp fragment. The RT-PCR analysis of 30 human livers was performed. Evidence for presence of trace amounts of CYP2B7 mRNA in human livers was found while strong bands representing CYP2B6 were also amplified. The expression of CYP2B8 was demonstrated in the majority of specimens analyzed. The data agree with lung-specific expression of CYP2B7. Expression of CYP2B8 mRNA in most human livers is a novel finding. Further studies are required to determine whether CYP2B8 mRNA encodes a functional enzyme. (Supported by NIH grant ES03765.)

1908 DIFFERENTIAL REGULATION OF THE CYP2B10 PHENOBARBITAL RESPONSIVE ENHANCER MODULE (PBREM) AND THE CYP2B1 PROMOTER IN HEPG2 CELLS.

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Negishi and colleagues recently identified a 51 base pair phenobarbital-responsive enhancer module (PBREM) in the 5' -flanking region of the mouse *CYP2B10* gene (Honkakoski, P., *et al*; *Mol. Pharmacol.*, 53, 597, (1998)). This sequence significantly enhances reporter gene expression (~10-fold) in transfected mouse hepatocytes treated with either phenobarbital (PB) or 1,4-bis[2-(3,5-dichloropyridoyloxy)]benzene (TCPOBOP). A putative PBREM is found in the 5' regulatory region of the rat *CYP2B1* gene. This sequence shares 98% identity with the *CYP2B10* PBREM. We hypothesize that humfFan CYP2B genes contain similar regulatory sequences. The purpose of this study was to determine if the *CYP2B10* PBREM is activated in the human hepatoma-derived HepG2 cells. The PBREM and 3.9 kb of the CYP2B1 5' flanking region were subcloned into vectors with firefly luciferase (luc) as a reporter gene; pGL2-Promoter and pGL2-Basic, respectively. A CMV/β-galactosidase reporter was used as a control for transfection efficiency. Treatment of transfected HepG2 cells with either PB or TCPOBOP significantly activated the PBREM/luc construct (2-4 fold) but did not induce the expression of the CYP2B1/luc construct. These results suggest that HepG2 cells activate the PBREM in response to prototypical CYP2B inducers. Contrasting the response of PBREM/luc with that of CYP2B1/luc suggests the presence of one or more negative regulatory elements within the CYP2B1 promoter region. Further studies will address this hypothesis. (Supported by NIH grant ES03765)

1909 INSULIN-MEDIATED MODULATION OF CYTOCHROME P450 GENE INDUCTION PROFILES IN PRIMARY RAT HEPATOCYTE CULTURES.

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In this investigation, we examined the effects of insulin on gene induction responsiveness in primary rat hepatocytes in light of recent studies which have questioned the role of this hormone in the maintenance of cytochrome P450 (CYP) gene expression. Cells were cultured for 72h either in the absence or presence of 1 μ M insulin and then exposed to increasing concentrations of phenobarbital (PB; 0.01-3.5 mM). Culturing in the absence of insulin produced 1.5-2-fold increases in the induction magnitude of CYP2B1 and CYP2B2 mRNA expression resulting from PB exposures, without altering the bell-shaped dose-response curve characteristic of this agent. However, for the CYP3A1 gene, insulin deprivation led to a pronounced shift in both the PB induction magnitude and dose-response relationships of the induction response, with higher levels of CYP3A1 expression resulting from exposures to lower concentrations of inducer. Insulin deprivation also reduced the time required for maximal induction of CYP2B1/2 and CYP3A1 gene expression. The insulin effects were not specific for PB induction, as insulin deprivation similarly enhanced both dexamethasone- and β -naphthoflavone-inducible CYP3A1 and CYP1A1 expression profiles, respectively. In contrast, the level of liver-specific albumin mRNA expression was considerably reduced in cells deprived of insulin. We conclude therefore, that insulin is an important regulator of inducible and liver specific gene expression in primary rat hepatocytes and that its concentration in media formulations should be more physiologically defined. (Supported by USPHS Grant GM 32281 and the Burroughs Wellcome Fund.)

1910 BONE MARROW STROMAL CELL EXPRESSION OF DMBA-METABOLIZING CYP1B1 IS REQUIRED FOR PRE-B CELL APOPTOSIS.

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We hypothesize that the bone marrow toxicity of DMBA requires metabolism by CYP1B1. Previous studies in our laboratory have demonstrated that bone marrow stromal cells constitutively express high levels of CYP1B1 that metabolizes DMBA, and that the expression of CYP1B1 is not dependent on the AhR. To investigate the potential roles of CYP1B1 and the AhR in bone marrow toxicity, primary bone marrow stromal cell cultures were established from mice deficient or heterozygous for CYP1B1, and from mice deficient or heterozygous for the AhR. These primary bone marrow stromal cells were co-cultured with pre-B cells in the presence or absence of DMBA. Pre-B cells apoptosis was used as a measure of toxicity and was evaluated by TUNEL labeling and PARP cleavage methods. DMBA treatment resulted in a significant increase ($p<0.02$) in pre-B cell apoptosis when co-cultured with CYP1B1 heterozygous, AhR deficient, and AhR heterozygous bone marrow stromal cells. However, DMBA treatment did not result in pre-B cell apoptosis when co-cultured with CYP1B1 deficient bone marrow stromal cells. These findings suggest that CYP1B1, but not the AhR, is required for the bone marrow toxicity of DMBA.

1911 THE ARYL-HYDROCARBON RECEPTOR AND CYP1B1 ARE NEGATIVE REGULATORS OF ADIPOCYTE DIFFERENTIATION.

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Mouse embryo fibroblast (MEF) lines and primary embryo cells undergo differentiation to adipocytes when exposed to the appropriate hormonal stimulus. We have generated MEF lines derived from wild-type C57BL/6 mice (WT MEF) and from mice that are deficient in the aryl-hydrocarbon receptor (AhR(-/-)MEF). WT MEFs exhibit a typical cascade of adipogenesis that is blocked by TCDD in its early stages (first 48 hrs). TCDD treatment suppresses differentiation markers such as PPARγ and C/EBPα, G3PDH activity, and lipid accumulation. Subconfluent AhR(-/-)MEFs show enhanced lipid synthesis in standard medium. Given the appropriate stimulus, these cells also undergo adipogenesis that is completely resistant to TCDD. AhR(-/-)MEFs are also deficient in CYP1B1, the major xenobiotic metabolizing P450 found in fibroblasts that is under the control of the AhR. MEFs derived from CYP1B1-deficient mice (1B1(-/-)MEFs) show greatly enhanced spontaneous and induced adipogenesis. However, adipogenesis in these cells is sensitive to TCDD. Based on these results, we hypothesize that constitutively expressed CYP1B1 represses adipogenesis. AhR activation and the subsequent induction of CYP1B1 may further repress this pathway. However, AhR activation independent of CYP1B1 is sufficient to supress adipocyte differentiation. We propose that CYP1B1 activity determines the steady state level of an early lipophilic mediator of adipogenesis.

1912 LACK OF INVOLVEMENT OF cAMP AND PKA IN THE PHENOBARBITAL (PB) MEDIATED INDUCTION OF THE RAT CYTOCHROME P450 (CYP) CYP2B GENES IN PRIMARY HEPATOCYTE CULTURES.

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The effects of PB on the liver are quite diverse and include cellular hypertrophy, tumor promotion, perturbations of gap junctional intercellular communication, as well as gene induction. Previous research, from our laboratory and others, has focused recently on evaluating the signal transduction processes that regulate the inductive effects of PB on the CYP2B genes. These studies have examined the effects of diverse kinases, phosphatases, and cyclic adenosine monophosphate (cAMP) analogs on PB inducible CYP family members. The present studies were conducted to examine the potential role of second messenger pathways in the initial phases of induction, specifically addressing the effects of PB on the expression of intracellular cAMP levels and associated protein kinase A (PKA) activity. Using a highly differentiated primary rat hepatocyte system, cells were exposed to PB and examined for expression of intracellular cAMP and subsequent PKA activity. Cells were harvested at timepoints as early as 30 seconds and up to 48 hrs after treatment with PB. Intracellular cAMP was measured using a cAMP immunoassay and PKA activity was assessed by measuring transfer

of a labeled phosphate to a biotinylated Kemptide substrate. Although PB markedly induced CYP2B mRNA expression, there were no detectable increases in cAMP levels or PKA activity at any time following PB additions. These results demonstrate that the initial PB mediated induction of the CYP2B genes is not associated with cAMP dependent pathways or effects. (This work was supported by NIH grants GM32281 and ES07032, and the Burroughs Wellcome Fund.)

1913 REGULATION OF CYP1B1 TRANSCRIPTION IN MCF-7 AND HEPG2 CELLS.

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Cytochrome P450 1B1 (CYP1B1) is expressed constitutively and is inducible by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the human breast adenocarcinoma cell line MCF-7, but not in the human hepatoma cell line HepG2. Genomic DNA isolated from both cell lines was digested with the methylation-sensitive restriction enzyme isoschizomers *Msp*I and *Hpa*II and subjected to Southern analysis with a CYP1B1 *Xho*I fragment containing the region -1311 to +216 relative to the transcription start site. Although differences were observed in methylation patterns for the CYP1B1 gene from MCF-7 and HepG2 cells, treatment with the demethylating agent 5-azacytidine (10μM for six days) did not activate CYP1B1 mRNA expression in HepG2 cells. Treatment of HepG2 cells with the histone deacetylase inhibitor Trichostatin A (100 nM for 24h) also did not produce an increase in CYP1B1 mRNA. Analysis of DNA-protein interactions at the CYP1B1 minimal promoter region (-101 to +25) indicated no differences in protein binding patterns with nuclear extracts prepared from either MCF-7 or HepG2. Comparative analysis in MCF-7 and HepG2 cells of the constitutive expression of CYP1B1/luciferase reporter constructs containing a series of deletions in the 5' CYP1B1 enhancer region indicated the potential involvement of cell-specific *cis*-acting elements located within the region from -1336 to -911. Taken together, these findings indicate that the observed differences in CYP1B1 constitutive expression are not mediated by histone acetylation or DNA promoter/enhancer methylation. The results suggest that constitutive expression of CYP1B1 is controlled by the presence of negative regulatory factors, absence of positive regulatory factors, or both, acting in a cell-specific manner at a DNA element located at least 800 bp 5'-ward of the minimal promoter.

1914 USE OF THE YEAST 'ONE-HYBRID' APPROACH TO INVESTIGATE THE MOLECULAR MECHANISM OF TRANSCRIPTIONAL REGULATION BY PHENOBARBITONE.

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Phenobarbitone (PB) is a potent inducer of CYP2B10 in mouse liver at the transcriptional level, and the gene regulatory elements that mediate this induction have been identified previously. The so-called Phenobarbitone-Responsive Enhancer Module (PBREM) is also present in the promoter regions of the rat PB-responsive CYP2B1 and 2B2 genes, and the PBREM sequences confer PB responsiveness on reporter genes in transient transfection assays using primary mouse hepatocytes. Our aim with these experiments was to obtain cDNA clones for the proteins that interact with the PBREM sequences, thereby improving our understanding of PB action at the molecular level. The yeast 'one-hybrid' system is designed to screen for DNA binding proteins, and is well suited to this situation where regulatory DNA sequences have been mapped but the interacting proteins have not been identified. Yeast 'reporter' strains were generated using three tandem of the PBREM sequences from the mouse CYP2B10 promoter, linked to the yeast HIS3 gene (to allow growth selection), and separately to the LacZ gene (to allow selection with X-Gal substrate). These reporter strains were screened with BALB/c mouse liver 'Activation Domain' (AD) libraries. Such libraries consist of plasmids containing cDNA fragments linked to a yeast transcription activator protein. Those cDNAs encoding proteins able to bind to CYP2B10 PBREM sequences will permit growth on medium lacking histidine, and/or produce blue colonies on exposure to X-Gal. To detect 'false positive' clones, yeast reporter strains have also been made using PBREM sequences from the closely related but PB-unresponsive CYP2B9 gene. The strategy is to screen for clones that are positive with the 2B10-based reporter strains, but negative with those containing 2B9 PBREM sequences. The results indicate that sequence-specific DNA binding proteins can indeed be identified in this way, and that this system represents a useful means of identifying factors involved in transcriptional regulation by toxic compounds.

1915 EFFECT OF PHENOBARBITAL ON SERUM ESTRADIOL LEVELS IN SPRAGUE DAWLEY RATS.

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Phenobarbital (PB) is known to induce hepatic P450 enzymes, some of which are involved in the metabolism of 17β -estradiol (E_2). *In vitro* studies using high E_2 concentrations have suggested a role for the 2B1/2 enzymes in E_2 metabolism. The objective of this study was to evaluate the effect of phenobarbital (PB) on circulating levels of E_2 in female Sprague Dawley rats. A radioimmunoassay was utilized to monitor total E_2 levels at comparable stages of the estrous cycle. Microdialysis techniques were used to determine the percentage of total E_2 which existed as free hormone. Both techniques showed that PB treatment did not significantly decrease E_2, either as total hormone or alter the percentage that exists as free hormone. Follow up *in vitro* metabolism studies of estradiol by control and PB induced hepatic microsomes provided the following information: 1) The rate of disappearance of E_2 varies with substrate concentration, with the major PB inducible P450 enzymes, 2B1/2 appearing to have a role in E_2 metabolism only at concentrations that far exceed physiological levels (>500 nM). The induction of the 2B1/2 enzymes by PB was confirmed by PROD analysis, showing a >25 fold induction. 2.) Microsomes containing only recombinantly expressed rat CYP2B1 enzyme and reductase showed no metabolism of E_2 even at very high substrate concentration and high specific activity of radiolabeled E_2. 3.) CYP3A enzymes are involved in E_2 metabolism, however other P450 enzymes are capable of metabolizing E_2 as competition experiments with 100 fold excess of dexamethasone resulted in only a 50% reduction in the rate of metabolism. These *in vitro* metabolism studies illustrate the need for careful selection of substrate concentrations when *in vitro* experiments are used to predict *in vivo* events.

1916 MONO-SPECIFIC ANTIPEPTIDE ANTIBODY TO HUMAN CYP2B6.

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To study the CYP2B6 contribution to methoxychlor metabolism within human liver microsomes and to initiate an investigation of CYP2B6 protein expression, we developed a highly sensitive polyclonal antibody targeted to a 20 residue CYP2B6 peptide. Though many immunological studies have described the absence or low expression CYP2B6 in human livers, in the present investigation we have found this not to be the case. Using our antibody, we immunoquantified CYP2B6 apoprotein expression in a panel of 28 livers and found concentrations ranging from 2 to 82 pmol/mg protein, with a mean value of 25 pmol/mg protein. Five livers (~18%) displayed relatively high levels of CYP2B6 (> 40 pmol/mg protein). There were no sex-related differences, though the highest level was observed in a one-week postpartum donor given several medications. A marked diminution in variability was found in individuals aged 56 or older (n = 12), but there were no age-related trends in mean CYP2B6 content. The antibody inhibited ortho-hydroxylation of methoxychlor catalyzed by cDNA-expressed CYP2B6 more than 90% but inhibited this reaction only 37% in human liver microsomes, indicating additional catalysis by other isoforms. We suggest that CYP2B6 represents a significant portion of total P450 in human liver. The exquisite sensitivity of our antibody (fmol quantities are easily detected on immunoblots) may explain our detection in 100% of livers versus the detection of CYP2B6 in a limited number of livers by certain other investigators.

1917 CONTRIBUTION OF CYTOCHROME P-450 2E1 AND P-450 2B1/2 TO THE HEPATOTOXICITY CAUSED BY KEPONE AND CARBON TETRACHLORIDE.

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The contribution of cytochrome P-450 isoenzymes P-450 2E1 and P-450 2B1/2 to hepatotoxicity induced by carbon tetrachloride (CCl_4) alone and Kepone/CCl_4 in combination was investigated in adult F344 male rats. Expression of P-450 2E1 and P-450 2B1/2 were evaluated in rats pretreated

with Kepone (single gavage dose at 16 mg/kg in corn oil, 8 days before sacrifice) by immunohistostaining and Western-blot. Hepatic damage was evaluated by histopathological examination in rats at 6 and 12 hr after the treatment of CCl_4 with or without the pretreatment of Kepone. In immunohistostaining study, we found that Kepone significantly induced P-450 2B1/2 in liver centrilobular and midzonal regions, however, it did not affect P-450 2E1 expression, which is mainly distributed in centrilobular region. Preliminary results from Western-blot studies suggested that Kepone could induce P-450 2B1/2 and did not have any significant effect on P-450 2E1. The above results can be used to explain the histopathological injury caused by CCl_4 alone and Kepone/CCl_4 interaction. In CCl_4 treated rats, liver histopathological change is mainly in centrilobular region, which is consistent with the centrilobular distribution of P-450 2E1, the dominant enzyme responsible for CCl_4 bioactivation in normal rats. In Kepone/CCl_4 treated rats, liver histopathological injury is throughout lobules with higher degree of injuries in centrilobular and midzonal regions. The induction of P-450 2B1/2 by Kepone in cetrilobular and midzonal regions might have caused the formation of free radicals from CCl_4 and contributed to the toxicity in these regions. We concluded that both P-450 2E1 and P-450 2B1/2 may play important roles in Kepone and CCl_4 interactive hepatotoxicity. (This study was supported by NIEHS Superfund Basic Research Program Project P42 ES05949.)

1918 INSULIN-MEDIATED DECREASE IN CYP2E1 EXPRESSION IN PRIMARY CULTURED RAT HEPATOCYTES: ROLE OF SRC KINASE AND PI3-KINASE.

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We have previously demonstrated that insulin negatively regulates CYP2E1 mRNA and protein expression in primary cultured rat hepatocytes (Chem.-Biol. Interact., 1997, 107:75). In order to delineate the signalling pathways involved in the insulin-mediated decrease in CYP2E1, we have employed pharmacological inhibitors of various kinases involved in insulin-mediated signalling. Primary rat hepatocytes cultured in modified Chee's medium on Vitrogen substratum were maintained in the absence of insulin for 48 h. The addition of 1 nM, 10 nM or 1 μM insulin for 24 h resulted in a 65, 85, and >95% decrease in CYP2E1 mRNA levels, respectively, relative to untreated cells. Addition of the src kinase inhibitor geldanamycin (0.1 μM) 1 h prior to 1 nM, 10 nM or 1 μM insulin addition, diminished the insulin-mediated decline in CYP2E1 mRNA to 25, 70, and 85%, respectively. Addition of the phosphatidylinositol (PI)3-kinase inhibitor wortmannin (1-10 μM) 1 h prior to insulin addition also ameliorated the insulin-mediated decrease in CYP2E1. Wortmannin (10 μM) prior to 10 nM insulin addition maintained CYP2E1 mRNA levels at 75% of the level in untreated cells, whereas 10 nM insulin alone decreased CYP2E1 mRNA to 15% of the level in untreated cells. In contrast, the MAP kinase kinase inhibitor PD98059 (10-100 μM), and the p38 MAP kinase inhibitor SB202190 (1-100 μM), failed to affect the insulin-mediated decrease in CYP2E1 mRNA. Hence, the metabolic insulin signalling pathway involving Src kinase and PI3-kinase, and not the mitogenic pathway involving MAP kinases, is implicated in mediating the negative effect of insulin on CYP2E1 expression. (Supported by NIH Grant ES03656 and NIEHS Center Grant P30 ES0 6639.)

1919 GLUCOCORTICOID-MEDIATED EXPRESSION OF CYP2E1 AND CYP3A mRNA IN PRIMARY CULTURED RAT HEPATOCYTES.

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The effects of dexamethasone (Dex), on CYP2E1 and CYP3A expression was examined in primary cultured rat hepatocytes. Cells were plated on Vitrogen-coated 60 mm dishes and grown in modified Chee's medium (0.1 nM insulin and 0.1 μM Dex) for 18-22 h prior to glucocorticoid addition. Northern blot analysis revealed that hepatocytes treated with 0.1 nM to 10 μM Dex for 24 to 72 h gave a maximum induction of 20-fold (0.1 μM) for CYP2E1 mRNA levels, and 90-fold (10 μM) for CYP3A mRNA levels at 72 h. Furthermore, when Dex (0.1 μM) was incubated with the glucocorticoid receptor antagonist, RU486 from 1.0 nM to 10 μM for 48 h, CYP2E1 mRNA levels decreased by 80%, compared to a 2- to 3-fold stimulatory affect observed for CYP3A mRNA levels. Since gene expression can be influenced by mRNA stability, of which the latter is regulated (in part) by cytoplasmic mRNA binding proteins, constructs containing fragments of the CYP2E1 gene (coding sequence = 1.65 kb total) were cloned into appropriate vectors. Construct 2E1-R#3 (824-1648 bp, including the 3'UTR) was linearized and used to make 32P-labeled mRNA (*in vitro* transcription) that was incubated with cytosolic extracts of Dex-treated cells and 1 μg/μlRNase. Gel retardation assays revealed a band with increased intensity at 0.1 μM compared to 1.0 nM Dex treated hepatocytes. These data show that CYP2E1 and CYP3A are differentially induced by glucocorticoids and suggest that the Dex-mediated increase in CYP2E1 may be the result of mRNA stabilization. (Supported by grants ES03656 and EHS Center Grant ES06639.)

1920 COMPARISON OF CYP2E1 CONTENT IN LYMPHOCYTES OF ALCOHOLICS AND AMONG ETHNIC GROUPS.

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Expression of the ethanol-inducible P450, CYP2E1 (2E1), in humans is influenced by several factors including xenobiotics and genetic polymorphisms. Monitoring 2E1 levels in individuals holds appeal because it may provide a means for risk assessment. We demonstrated that expression of 2E1 in lymphocytes reflected *in vivo* activity of the hepatic enzyme. The present investigation compares 2E1 content and metabolic parameters between alcoholics and non-alcoholics and among ethnic groups, including Native Americans (NA) and Hispanics (Hisp). In addition, previously described polymorphisms were examined. Individuals were administered the 2E1 substrate, chlorzoxazone (CZX) and lymphocytes isolated from whole blood samples. 2E1 protein and mRNA were measured in lymphocytes by western blotting or quantitative RT-PCR, respectively. Alcoholics exhibited elevated lymphocyte 2E1 mRNA (4-fold) and protein (2-fold) when compared to non-alcoholics. CZX clearance rates were 2.3-fold higher and AUC values were 2-fold lower in alcoholic individuals than in non-alcoholics. Furthermore, CZX clearance rates correlated ($r=0.40$, $p<0.005$, $n=52$) with 2E1 mRNA content of lymphocytes and mRNA further correlated ($r= 0.59$, $p<0.001$) with 2E1 protein content of white cells. The *Rsa*I + *Pst*I and *Dra*I polymorphisms were examined in the study participants, however the rare alleles were not identified. Nonetheless, 29% of NA and 13% of the Hisp were Type B (c1/c2) for the *Rsa*I + *Pst*I polymorphism. In contrast, Type B individuals were not identified in the Caucasian population. Interestingly, two Caucasians exhibited an unlinked *Rsa*I + *Pst*I allele, with the polymorphism occurring at the *Pst*I site. Individuals possessing the *Dra*I CD allele, included 22% of Hisp, 19% of NA and 8% of Caucasians. Results suggest that 2E1 mRNA in lymphocytes predicts corresponding protein levels and *in vivo* activity. Moreover, genotypic differences were observed among the two ethnic groups which differed from Caucasians. (Supported by DHHS grant AA08990.)

1921 THE ROLES OF CYTOCHROMES P450 2E1 AND 2B10 IN THE STEREOCHEMICAL BIACTIVATION OF 4-VINYLCYCLOHEXENE.

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4-Vinylcyclohexene (VCH), an intermediate in the production of rubber and styrene, is converted to toxic epoxides [1,2-vinylcyclohexene monoepoxide (1,2-VCHE), 7,8-vinylcyclohexene monoepoxide (7,8-VCHE), and, ultimately, vinylcyclohexene diepoxide (VCD)] by multiple isoforms of cytochrome P450. VCH has been shown to be ovotoxic in female $B6C3F_1$ mice, but not in female Fischer-344 rats. A crucial factor for the differences in toxicity can be attributed to a greater formation of the monoepoxides, and ultimately VCD, from VCH in the mice as compared to rats. Therefore, studies determining which cytochrome P450 isoforms are responsible for VCH bioactivation were performed to better explain the species-dependent toxicity. Hepatic microsomes isolated from mice treated with acetone (1% in drinking water for 5d) showed elevated levels of CYP2E1 and CYP2b10 as measured by densitometric analysis of blots. Following microsomal incubations, this treatment group demonstrated 2-fold increases in bioactivation of racemic VCH, (*R*)-VCH, and (*S*)-VCH to 1,2-VCHE as compared to controls. There was no increase in 7,8-VCHE formation. VCH pretreatment itself (7.5mmol/kg per day for 10d, ip) increased the formation of 1,2-VCHE (3-fold), 7,8-VCHE (2-fold), and VCD to detectable levels as compared to controls, all non-stereospecifically. Interestingly, VCH-induced microsomes showed approximately a 2-fold increase in CYP2b10 protein levels, but no increase in CYP2E1 levels. These data were supported by a lack of increase in aniline hydroxylase activity or p-nitrophenol activity (both CYP2E1 substrates) in VCH-induced microsomes as compared to control. Therefore, the increased epoxidation following repeated administration of VCH can be attributed to increased levels of CYP2b10 and not CYP2E1. (This research was supported in part by NIEHS (ES07091), Center Grant (ES06694) and Chemical Manufactures' Association.)

1922 VARIABLE CHANGES IN CYP2E1 ACTIVITY AFTER ENDOTOXIN ADMINISTRATION IN HUMANS.

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Administration of E. coli lipopolysaccharide (LPS) has been shown to significantly reduce CYP2E1 protein and activity in the rat. To further investigate this phenomenon in humans, IV LPS (U.S. Standard Reference Endotoxin, CC-RE-Lot2) was administered to healthy volunteers for chlorzoxazone(CZN) pharmacokinetic and metabolite (6OHCZN) formation clearance analysis. Methods: Eight healthy nonsmoking male volunteers were enrolled in a balanced crossover design to evaluate the effect of endotoxin on the pharmacokinetics of chlorzoxazone. A 500mg oral dose of chlorzoxazone was given 1 hour following the final LPS or saline dose. Serum and urine CZN, 6OHCZN, and serum C-reactive protein (CRP) were quantified. In addition, serum protein binding of chlorzoxazone was determined via ultracentrifugation. Results: LPS administration produced a significant rise in temperature (3.1 ± 1.0 ° F), pulse (30 ± 9 bpm), and CRP (3.1 ± 0.8 mg/dL); thereby, demonstrating LPS induced an acute phase response. Endotoxin treatment failed to show a statistical change in CZN oral clearance (LPS; 4.2 ± 1.1 ml/min/kg vs. control; 4.1 ± 1.1 ml/min/kg) or formation clearance of 6OHCZN (LPS; 2.6 ± 1.1 ml/min/kg vs. control 2.5 ± 1.0 ml/min/kg). Upon closer inspection, intraindividual comparisons reveal 5 subjects which demonstrate an increase of 37 ± 30% in Clo and a 52 ± 41% increase in Clf. The remaining 3 subjects all demonstrate a reduction of 35 ± 3.5% in Clo and a 43 ± 30% decrease in Clf. Chlorzoxazone protein binding was not altered by endotoxin administration (LPS; 98.2 ± 0.6 vs. control 98.1 ± 0.4 %). Conclusions: Unlike the rat, humans demonstrate great interindividual response in 6OHCZN formation following endotoxin administration. This variability is not due to alterations in protein binding and is not well correlated with CRP alterations.

1923 CYTOCHROME P450 2E1 CONTENT OF ISCHEMIC LIVER IS MAINTAINED AT PRE-ISCHEMIC LEVELS BY DIMETHYL SULFOXIDE (DMSO).

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Many potential transplant patients die waiting for organs. Using organs from non-heart-beating donors (NHBD) might reduce the current donor shortage. Warm ischemia in NHBD produces damage that can lead to graft failure in recipients post-transplantation. DMSO has several well-known biological activities that might prevent or reduce such damage. Male F344 rats were pre-treated with DMSO (2-6 ml/kg, IP) or saline (controls) and were made NHB for various periods. Organs were removed, cored, precision-cut and incubated *in vitro* for 4-96h. Potassium (K+) and lactate dehydrogenase (LDH) content determined viability of the tissue. Induction of heat shock proteins (HSP) was analyzed as they have been shown to increase in cells undergoing stress, heat or chemical damage. In another series, one lobe of the liver was clamped and, after an interval, reperfused. Animals were sacrificed or allowed to survive 24-48h. Microsomes were analyzed for CYP2E1 activity using the paranitrophenol hydroxylase assay. The K^+ and LDH content indicated that slice viability was significantly decreased in organs subjected to 15 minutes or more warm ischemia. Pretreatment with DMSO maintained tissue viability equal to control, non-ischemic organs. HSP were higher in the ischemic tissue compared to controls but were not induced in the DMSO treated group. CYP2E1 activity was maintained at non-ischemic levels in microsomes from rat livers pre-treated with DMSO and made ischemic for varying periods as well as in microsomes from rats given DMSO 24h post-ischemia. This suggests that DMSO pretreatment would prevent tissue damage in NHBD livers, thus providing additional organs suitable for transplantation. (Supported by the ADCRC (JBU, PZN), the UA UBRP (JLB, RR, JJW, PWJ), the Richard Siegel Foundation, the Armenis Family Trust and NIEHS Center Grant P30-ES-06694 (JBU).)

1924 BENZENE INDUCES CYP 2E1 mRNA IN RAT PERIPHERAL LYMPHOCYTES.

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Benzene is an environmental contaminant classified as a carcinogen which is activated mainly in the liver, by the cytochrome P450 monooxygenases, into reactive metabolites. Peripheral lymphocytes are very accessible cells which can be used for monitoring the exposure to xenobiotics. In the present study we investigated the expression of CYP 2E1 in lymphocytes of male rat intraperitoneally treated with benzene (5, 10, 15 mmoles/kg body weight), using the reverse transcriptase-polymerase chain reaction (RT-PCR). Fresh whole blood (about 8-10 mL collected from each rat) was mixed with Ficoll Hypaque and lymphocytes separated by centrifugation at 1000 Xg. Total RNA was extracted with TRIZOL Reagent and cDNA was obtained and amplified with specific rat CYP 2E1 primers. The resulting amplified product was electrophoresed through agarose gels and the expected 617 bp product was observed with Ethidium Bromide staining. Lymphocytes of benzene treated rats was higher expression of CYP 2E1 compared to controls. In addition, we used liver as a positive control, where also an increased expression of CYP 2E1 was detected in the treated animals. These results suggest that lymphocytes are suitable for monitoring the exposure to benzene and could be an adequate system to be used as a marker of effect *in vivo*. *Supported by the European Commission (Biomed-2 Contract N° BMH4-CT96-0184), CONACyT /CNR, Mexican and Italian governments.*

1925 EFFECTS OF LOW-DOSE CARBON TETRACHLORIDE EXPOSURE BY INHALATION OR DRINKING WATER ON CYTOCHROMES P450 2E1 AND 2B EXPRESSION.

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Rats, mice and hamsters were exposed to 0.5 and 5 ppm CCl_4 in drinking water for 1 and 4 weeks or by inhalation to 5, 20 or 100 ppm CCl_4 for 1 or 12 weeks. Effects on CYP2E1 and 2B protein levels and catalytic activities were examined. Hepatocellular necrosis and increased serum alanine amino transferase levels were observed in all species at 100 ppm and in mice at 20 ppm inhaled CCl_4. Microsomal protein content increased in rats exposed by inhalation for 1 week and in hamsters exposed by either route, but decreased in rats exposed by inhalation to 100 ppm CCl_4 for 12 weeks. CYP2E1-associated chlorzoxazone 6-hydroxylase (CHZ 6-OHase) was increased 16 and 23% in rats (but not mice or hamsters) exposed for 1 week to 0.5 and 5 ppm CCl_4, respectively, in the drinking water. In all species, inhaled CCl_4 at 100 ppm or, in some species, 20 ppm suppressed CHZ 6-OHase and two CYP2B-associated activities, benzyloxyresorufin and ethoxytrifluorocoumarin O-deethylase activities. Western blot analyses indicated that subtoxic doses of CCl_4 typically induced CYP2E1 and CYP2B apoprotein levels but that toxic doses generally decreased levels of these proteins. An exception was observed in the rats exposed by inhalation, where P450 apoprotein levels increased at doses that decreased catalytic activity. Overall, these results show that toxic doses of CCl_4 suppress CYP2E1 and 2B levels but that lower doses can stimulate microsomal proliferation and increase P450 apoprotein levels. The increase in apoprotein levels, however, is rarely associated with increased catalytic activity, presumably due to suicide inactivation by CCl_4. (Supported by US DOE contract DE-ACO6-76RLO 1830.)

1926 PULMONARY METABOLISM OF ETHYLBENZENE FOLLOWING EXPOSURE IN RATS.

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Previous studies have shown that exposure of Sprague Dawley rats to ethylbenzene vapor (300 ppm) for one or three days (six hours/day) results in a decline in the level of this compound in both blood and liver between the two exposure time points. *In vitro* rates of ethylbenzene metabolism in hepatic microsomes from ethylbenzene-exposed animals increase in this same time frame. In this study, levels of ethylbenzene in lung tissue were quantified and were also found to decrease, from 18.2 ug/g at one day of exposure to 9.4 ug/g at three days, a 48% decline. In contrast to the liver however, where the activity of cytochrome P450 (CYP P450) enzymes was found to increase

with exposure to ethylbenzene, activity of CYP P450 enzymes in the lung was reduced. Pulmonary CYP 2B1-associated benzyloxyresorufin dealkylase activity (BROD) was 40% of control values after one and three days exposure. However, CYP 2B1 protein levels did not similarly decline. CYP 4B1–associated 2-aminoflourene N-hydroxylase activity was 34% and 69% of control values at one and three days, respectively. CYP 2E1-associated N-nitrosodimethylamine demethylase (NDMA) activity was unchanged. *In vitro* rates of ethylbenzene metabolism were determined in pulmonary microsomes from control and treated animals. At a substrate concentration of 0.05 mM, the rate of 1-phenylethanol formation was 22.4± 1.5 pmol/min/mg protein in control microsomes. In tissues from animals exposed for one and three days, rates were significantly decreased compared to controls; 17.7± 1.1 and 13.6± 3 pmol/min/mg protein, respectively. The data indicate that hepatic metabolism of ethylbenzene dominates clearance of this compound following inhalation exposure, even in respiratory tissue.

1927 EFFECTS OF C-TERMINAL TRUNCATION ON THE TURNOVER AND FUNCTION OF CYTOCHROME P450 2E1.

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Cytochrome P450 2E1(CYP2E1) is an ethanol-inducible monooxygenase regulated posttranslationally. The C-terminal eight amino acids of CYP2E1 are completely conserved among species. In this study, the function of the conserved C-terminal region of rabbit CYP2E1 was studied by deleting nine amino acids (485-493) from the C-terminal end (CYP2E1CD). The steady state turnover of the mutant was determined by pulse-chase analysis and compared to wild type CYP2E1. The half-life of CYP2E1CD was 5.4 fold shorter than the full length enzyme (0.72 vs 3.9 h). 4-Methylpyrazole, a CYP2E1 ligand that normally stabilizes the enzyme and prevents degradation, did not alter the rapid turnover of CYP2E1CD. 1-Aminobenzotriazole, which inactivates P450 isozymes predominantly by N-alkylation of the heme and leads to formation of apoprotein, increased the half-life of the wild type enzyme but did not affect the half-life of CYP2E1CD. Wild type CYP2E1 is degraded predominantly by the proteasome pathway. Using selective inhibitors of lysosomal, Ca^{++}-dependent calpains and the proteasome proteolytic pathways, it appears that the C-terminal truncation did not alter the degradation pathway of the mutant. The mutant had no chlorzoxazone 6 hydroxylase activity and the reduced carbon monoxide complex was not detectable. These results suggest that the carboxyl terminus of CYP2E1 plays an important role in the turnover of this enzyme and may have a role in heme binding. (Supported by AA08608 and AA05462.)

1928 DEVELOPMENT OF A 96-WELL PLATE ASSAY FOR P4503A4 INHIBITION.

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The standard way to evaluate P4503A4 inhibition is to study the conversion of the specific P4503A4 probe testosterone to its 6ß-hydroxy metabolite in human liver microsomes, in the absence and presence of potential inhibitors. Quantification of the 6ß-hydroxy metabolite is achieved by HPLC resulting in a tedious and time-consuming assay. In order to increase the P450 inhibition assay throughput, efforts were made to find a P4503A4 probe that would produce a fluorescent metabolite. It was found that L-781,440, a COX-II inhibitor compound, was significantly metabolized in human microsomes to give the fluorescent compound L-788,706. Incubations conducted with Gentest Supersomes™ indicated that P4503A4 played a major role in the metabolism of L-781,440. Inhibition studies with specific P4501A1, 1A2, 2C8/9, 2C19, 2D6, and 2E1 chemical inhibitors did not suppress L-781,440 activity. However, troleandomycin, ketoconazole and nifedipine, three known P4503A4 inhibitors, completely abolished the formation of L-788,706. Also, there was also a good agreement between the formation of L-788,706 and formation of 6ß-hydroxytestosterone when L-781,440 and testosterone were incubated with various human liver microsome preparations. In order to use L-788,706 as a fluorescent P4503A4 marker in a 96-well plate format, it was important to remove the excess NADPH at the end of the incubation because the fluorescence of NADPH interferes with L-788,706 detection. This was achieved by adding oxidized glutathione and glutathione reductase to convert NADPH to $NADP^+$ which is not fluorescent. In this presentation, the optimization of the incubation conditions as well as the automation of the incubations in a 96-well plate with direct measurement of L-788,706 production will be described. Application of this new fluorescent HTS assay to test P4503A4 inhibitors will be presented.

1929 EFFECTS OF SOY PROTEIN ISOLATE ON EXPRESSION AND GLUCOCORTICOID-INDUCIBILITY OF CYP3A AND CYP2B ENZYMES IN MALE RAT LIVER.

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The constitutive and glucocorticoid (dexamethasone; 50 mg/kg) inducible expression of hepatic cytochrome P450 enzymes in gene family CYP3A and CYP2B1 was studied in male Sprague-Dawley rats fed semi-purified diets (AIN93) where the protein source was casein (AIN) or changed to soy protein isolate (SPI). Dietary consumption of SPI resulted in significantly ($p<0.05$) increased constitutive expression of CYP3A1(CYP3A23) mRNA, CYP3A-apoprotein and testosterone 6b-hydroxylase and increased constitutive expression of CYP2B1 mRNA. Dietary consumption of SPI also resulted in significantly ($p<0.05$) increased expression of CYP3A1(CYP3A23) and CYP3A2 mRNA following dexamethasone induction. This was accompanied by significant ($p<0.05$) differences in CYP3A apoprotein expression and metabolism of CYP3A-substrates erythromycin, testosterone, ethylmorphine and corticosterone in dexamethasone-induced SPI-fed compared to AIN-fed animals. In contrast, diet and dexamethasone had no effect on expression of CYP3A9 or CYP3A18 mRNA or on the 6b-hydroxylation of the CYP3A18 substrate lithocholic acid. Dexamethasone induction of CYP 2B1 apoprotein and pentoxyresorufin O-depentylase activity was also increased in SPI-fed compared to AIN-fed animals. These results may partly explain the chemoprotective effects of soy consumption on tumorigenesis following exposure to dietary or environmental procarcinogens and suggest potential effects of soy consumption on metabolism and clearance of clinical medications. (Supported by USDA-ARS-Arkansas Children's Nutrition Center.)

1930 DIFFERENTIAL EFFECTS OF DEXAMETHASONE (DEX), PHENOBARBITAL (PB) AND CLOTRIMAZOLE (CLO) ON CYTOCHROME P450 (CYP) 3A AND MICROSOMAL EPOXIDE HYDROLASE (mEH) EXPRESSION IN MALE AND FEMALE RATS.

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CYP3A biotransforms numerous xenobiotics to epoxides which are further metabolized by mEH to their corresponding trans-dihydrodiols. This pathway detoxifies as well as activates procarcinogens to carcinogens. We have examined CYP3A and mEH protein levels in both male and female rats following treatment with DEX (10 mg/kg/day, i.p., 4 d), PB (100 mg/kg/day, i.p., 3 d) and CLO (80 mg/kg/day, i.p., 2 d). In untreated rats, CYP3A and mEH levels were lower in females than males. Following DEX treatment, CYP3A23 levels increased while mEH levels decreased in both sexes. Interestingly, CLO and PB treatment produced differing responses in males and females. In male rats, CLO and PB treatment resulted in comparable increases in CYP3A levels. In females, CYP3A increased ~20 fold following CLO treatment, but only one tenth of that increase was observed following PB treatment. The differential expression of CYP3A and mEH observed between untreated male and female rats, and male and female rats following CLO and PB treatment suggests that bioactivation of procarcinogens may be gender-dependent. (Supported by NIH grant N43 ES 81003 (H.K.) and ES 03656 (R.F.N.).)

1931 CHRONIC ETHANOL TREATMENT DIFFERENTIALLY AFFECTS THE EXPRESSION OF CYP3A ENZYMES IN THE LIVER OF MALE RATS.

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The CYP3A subfamily is the most abundant of the human hepatic cytochromes. CYP3A isoforms mediate the biotransformation of many drugs, including a number of psychotropic, cardiac, analgesic, hormonal, immunosuppressant antineoplastic, and antihistaminic agents. We are studying diet/ethanol interactions using total enteral nutrition (TEN) for alcohol

delivery to adult male Sprague-Dawley rats in diets containing 16% protein, ethanol (> 12.5 g/kg), corn oil (25-45%) and high (21%) or low (1%) carbohydrate. Using this model we have reported that ethanol produced a decrease ($p<0.05$) in CYP3A activity (testosterone-6β-hydroxylation) and apoprotein levels (Western blot) and this effect was independent of the dietary carbohydrate content (Hakkak *et. al.*, Toxicological Sciences, 42:90, 1998). To further study the mechanism of this ethanol-induced inhibition of CYP3A, we have used specific antisense oligonucleotides against CYP3A1, CYP3A2 and CYP3A18 and a CYP3A9 cDNA to measure ethanol effects on CYP3A mRNA's using Northern blots. In all the animals studied, there was detectable mRNA for all the hepatic CYP3A enzymes with no differences in mRNA steady-state levels expressed for CYP3A1 and CYP3A18 in animals fed ethanol compared with animals fed control diets. In contrast, there was a large decrease (84%) in CYP3A2 mRNA in the animals fed ethanol versus control animals. Interestingly, in the livers of these same animals, ethanol in the low or high carbohydrate diets caused a 1.7 and 2.5 fold increase in CYP3A9 mRNA levels, respectively. These results indicate that the decrease in CYP3A activity and apoprotein levels reported earlier are most likely due to the ethanol-induced decrease in CYP3A2 mRNA levels. Moreover, ethanol differentially affects the expression of CYP3A gene family and this regulation can be modulated by dietary carbohydrate. (Supported in part by AA08645.)

1932 UNDERSTANDING P450 3A4 COOPERATIVITY: A ROLE FOR RESIDUE F304.

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P450 3A4 is the most abundant cytochrome P450 in the liver, is responsible for the metabolism of endogenous and exogenous compounds of diverse structure, and exhibits both homotropic and heterotropic cooperativity. Our laboratory has successfully utilized molecular modeling to better understand structure-function relationships of cytochromes P450 and has applied this methodology to a rigorous study of P450 3A4 cooperativity. Using predictions from docked molecules of substrate and effector into the P450 3A4 active site, site-directed mutants were constructed and analyzed. Harlow and Halpert (PNAS 95, 6636-6641, 1998) demonstrated that mutant L211F/D214E displayed altered testosterone hydroxylase cooperativity, showing elevated rates of metabolite formation in the absence of effector and lower stimulation in the presence of effector when compared to P450 3A4. This presentation extends this work and analyzes a third residue, F304, which modeling studies also predicted to lie near a docked molecule of α-naphthoflavone. Mutant F304A, unlike L211 and D214, exhibited an altered ratio of the major progesterone metabolites in the absence and presence of α-naphthoflavone, without any change in the level of stimulation. F304W, however, displayed higher production of both metabolites in the absence of α-naphthoflavone, but lowered stimulation in its presence. The triple mutant, L211F/D214E/F304W, showed an even more dramatic loss of α-naphthoflavone stimulation. These results suggest that residue F304 may be positioned near both the substrate and effector binding pockets, while L211 and D214 appear to be involved exclusively in effector activity. (This work was supported by NIH grant GM54995 and NRSA GM19058.)

1933 INDUCTION OF CYP3A BY 24(S),25-EPOXYCHOLESTEROL IN PRIMARY CULTURED RAT HEPATOCYTES.

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Sterols modulate cholesterol homeostatic gene expression through diverse mechanisms involving sterol regulatory element binding proteins (SREBP) and LXR nuclear receptors. Several lines of evidence suggest that sterols may also modify hepatic cytochrome P450 expression. We examined the effects on P450 expression of treating primary cultured rat hepatocytes with either of two oxysterols, 25-hydroxycholesterol (a potent modulator of SREBP-regulated gene expression, but weak activator of LXR) or 24(S),25-epoxycholesterol (a potent activator of LXR). Treatment of 48 h-old rat hepatocyte cultures for 24 h with 25-hydroxycholesterol at doses of 3×10^{-5} M or higher produced slight elevations in CYP3A mRNA and immunoreactive protein levels. By contrast, treatment of hepatocyte cultures with 24(S),25-epoxycholesterol at doses of 10^{-5} M or higher produced marked dose-dependent elevations (up to ~50% of the increases observed with 10^{-5} M dexamethasone) of CYP3A mRNA and immunoreactive protein levels. CYP1A, CYP2B and CYP4A mRNA and immunoreactive protein levels were unaffected by sterol treatment. Treatment with 24(S),25-epoxycholesterol significantly elevated luciferase reporter gene expression in cultured rat hepatocytes transiently transfected with a plasmid containing a CYP3A23 gene fragment spanning bases -175 to +25 (containing both a DR4 and DR3 nuclear receptor motif) or bases -148 to +25 (containing the DR3 motif only), or containing four copies of the DR3 motif and driving reporter gene expression through the SV40 promoter. These results demonstrate that the naturally-occurring oxysterol 24(S),25-epoxycholesterol is an effective inducer of CYP3A expression, and suggest that activation of transcription through the DR3 motif represents one component of the regulatory mechanism. (Supported by NIH Grants HL50710 (TAK) and HL52069 (TAS) and NIEHS Center Grant ES06639.)

1934 REGULATION OF CYTOCHROME P450 ISOZYMES BY MIREX AND CHLORDECONE AND CLONING AND EXPRESSION OF CYP3A25 GENE IN *E.COLI.*

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Mirex and chlordecone (kepone) were studied for regulation of cytochrome P450 (Cyp) isozymes 1a2, 2b10, 2e1 and 3a in mouse liver. Adult male and female CD-1mice were treated with either mirex or chlordecone at 20mg/kg/day for 3 days, respectively. Although mirex is structurally similar to chlordecone, these compounds exhibit different regulatory effects on P450 isozymes. Our results show that both mirex and chlordecone induce Cyp isozyme levels and catalytic activities, but there are gender differences, including increases in the ratio of liver weight to body weight, and Cyp content. *In vitro* enzyme activity assays indicate that mirex and chlordecone significantly increase pentoxyresorufin O-dealkylation (Cyp2b10) in either gender. Metabolism of methoxyresorufin O-dealkylation (Cyp1a2) was highly induced in males and slightly induced in females by either compound. No obvious induction was observed by the use of the p-nitrophenol hydroxylase assay (PNP) (Cyp2e1). Testosterone 6β –hydroxylase activity (Cyp3a) shows a different regulatory pattern: induction in females and inhibition in males. Immunoblotting results shows that both mirex and chlordecone induce males to a greater extent than females for isozymes Cyp1a2 and Cyp3a while females were induced more than males with respect to Cyp2b10. To further study Cyps, which are associated with sex hormone metabolism, a cDNA encoding a novel form of the Cyp3a subfamily, Cyp3a25, has been isolated from mouse liver. The sequence contained 2010 base pairs and encoded a protein of 503 amino acids. The amino acid identity with the murine Cyp3a13 and Cyp3a16 is 71% and 76%. To facilitate highly heterologous expression of Cyp3a25 in *E.coli,* the 8 amino acids in the N-terminus were modified. The expressed protein has a slight faster mobility than Cyp3a4 from human liver on SDS-PAGE. Cyp3a25 was expressed both in male and female mouse livers. Functional characterization of Cyp3a25 is currently being undertaken with use of testosterone, estradiol, and erythromycin.

1935 IMMUNOHISTOCHEMICAL LOCALIZATION OF CYTOCHROME P450 LMC5 (CYP3A27) IN RAINBOW TROUT G-I TRACT.

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CYP3A27(LMC5), a cytochrome P450 3A subfamily member, is expressed in liver, intestine and other tissues. Immunohistochemical staining (IHS) procedures were used to investigate the location of its expression in the gastrointestinal tract in untreated male and female rainbow trout. Northern and Western blotting techniques were used to determine the transcriptional and translational expression of LMC5 in corresponding sections of intestine that were stained by the IHS procedure. Sections of esophagus, pyloric stomach, pyloric caeca, jejunum, and rectum were examined. Paraffin-embedded sections were incubated with rabbit polyclonal antibodies confirmed to be specific for trout LMC5 and detected by color substrate 3,3'-diaminobenzidine tetrahydrochloride in an avidin-biotin complex protocol. The greatest staining intensity was observed in the pyloric caeca and staining intensity decreased along the intestinal tract with little staining observed in rectum. The intensity of IHS for CYP3A27 was closely correlated with Western and Northern blot results. Western and Northern blot techniques showed the greatest expression in decreasing order as follows : pyloric caeca, jejunum, rectum, and pyloric stomach. Sex related differences in CYP3A27 expression were observed with higher expression in female G-I tract than male's

especially pyloric caeca in both Northern and Western blot results whereas higher amounts were found in male liver than female liver.

1936 ROLE OF CYTOCHROME P450 4B1 IN 1,3-BUTADIENE (BD) OXIDATION IN LUNG MICROSOMES OF HUMANS, RATS, AND RABBITS.

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Previously we have shown that 4B1 is the major cytochrome P450 isozyme involved in BD and butadiene monoxide (BMO) oxidations in the male mouse kidney. P450 4B1 also contributes to BD metabolism in male and female mouse lung microsomes. Since human exposure to BD in the petrochemical and synthetic rubber industries is mainly through inhalation, we examined the role of 4B1 in BD bioactivation with lung microsomes from the rat, rabbit and human. Inclusion of a polyclonal antibody prepared to rabbit lung 4B1 (2 mg IgG/mg protein) in BD incubations with male and female rat lung and male rabbit lung microsomes inhibited BMO formation by 73%, 81%, and 33% respectively. When the 4B1 antibody (2 mg IgG/mg protein) was included in BMO incubations with male rabbit lung microsomes, both meso- and (±)-diepoxybutane were inhibited by nearly 55%. Human lung microsomes did not catalyze BD oxidation; this may be due to low expression levels, different substrate selectivity, or inactivation of the P450s during isolation and storage of the human lung samples (brain dead and cadaver) since *p*-nitrophenol hydroxylase and 7-ethoxycoumarin deethylase activities were also not detectable. Immunoblotting showed that 4B1 was present in the lung microsomes of four human donors as well as in male and female rat and male rabbit lung microsomes. These results show that 4B1 is involved in BD bioactivation in the lungs of rats and rabbits and may contribute to BD activation in human lung. (Supported by NIH grant ES 06841.)

1937 THE STRUCTURE OF *CYP2F1*: A GENE THAT CODES FOR A HUMAN PULMONARY-SELECTIVE CYTOCHROME P450 ENZYME.

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The selective bioactivation of a number of pneumotoxicants and pulmonary carcinogens is catalyzed by cytochrome P450s that are selectively expressed in lung cells. A human P450 gene *CYP2F1*, encodes a P450 capable of metabolizing several lung-selective toxicants. CYP2F1 expression is tissue-selective, showing the highest expression in the lung compared to the liver, kidney, and gastrointestinal tract. We hypothesize that expression of CYP2F1 is under *cis*-regulatory control and responds to specific activating factors that control the tissue-selective expression of CYP2F1 in lung cells. To determine the factors that regulate the expression of CYP2F1 in lung cells, it is necessary to identify the regulatory region of *CYP2F1*. Three cosmid clones containing the gene were previously identified (Hoffman et al., J Mol Evol 41: 894-900, 1995) from a human genomic library by Southern hybridization to *CYP2F1* cDNA. The cosmid clones have been restriction fragmented and probed with a 5' 298 bp fragment from *CYP2F1* cDNA to identify the 5'-most sequence. Sequencing to date has identified several intron/exon junctions of the *CYP2F1* gene but has not identified the transcriptional start site or TATA box. Further sequencing is required and will be performed on all three cosmid clones. This should permit the identification of the transcription start site and any *cis*-regulatory elements within the sequenced 5' region. Additional studies will attempt to characterize the *cis*-regulatory domains that control the tissue-selective expression of CYP2F1. These studies will use deletion analysis of the 5' flanking region with luciferase constructs, *in vitro* electrophoretic mobility shift assays, and DNase footprinting to analyze and identify *cis*-regulatory domains. These studies will provide novel information on the biochemical mechanisms of cytochrome P450 expression and the tissue-selective expression of CYP2F1 in lung cells. (Support provided by USPHS Grant #HL13645.)

1938 IMPORTANCE OF MURINE *cYP2F2* IN THE METABOLIC ACTIVATION OF NAPHTHALENE AND IN THE METABOLISM OF OTHER XENOBIOTICS.

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Despite their substantially lower levels relative to the hepatic tissues, pulmonary cytochrome P450 monooxygenases play an important role in the metabolic activation of substrates that cause lung injury. The target- and species-selective toxicity of a number of pulmonary injurants has been attributed to distribution of enzyme and to the presence of enzymes with high k_{cat} in target airways of the susceptible species. However, experimental demonstration of these concepts and quantitative assessment of the contribution of individual P450 isoforms is lacking. This study was undertaken to characterize the catalytic activities of *Cyp2f2* with naphthalene, a murine Clara cell toxicant, as well as other xenobiotics that either undergo metabolic activation to cytotoxic intermediates or that are 'isoform-selective' substrates. *Cyp2f2* was generated using the baculovirus expression vector system. Recombinant *Cyp2f2* produced in Sf9 and Tn5 cells accounted for approximately 20% of total cellular protein. Incubations of Tn5 cell lysates, containing recombinant *Cyp2f2* with NADPH-cytochrome P450 reductase and NADPH, metabolized naphthalene with a high degree of stereoselectivity to the 1R,2S-naphthalene oxide (66:1 enantiomer ratio). The K_m and k_{cat} values along with the specificity constant for naphthalene metabolism by recombinant *Cyp2f2* were 4.7 μM, 118 min^{-1}, and $4.2x10^5$ $M^{-1}sec^{-1}$, respectively. The recombinant *Cyp2f2* also metabolized ethoxyresorufin, pentoxyresorufin, p-nitrophenol, 4-ipomeanol and 1-nitronaphthalene at easily detectable levels. These studies suggest that *Cyp2f2*: 1) plays a key role in the species- and cell-selective toxicity of naphthalene, and 2) efficiently metabolizes a number of other substrates, including some thought to be isoform-selective. (Supported by NIEHS 04699, 08408, 05711, 05707.)

1939 AN ADDITIONAL TEN-FOLD SAFETY FACTOR FOR CHILDREN: IS THIS NEEDED?

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The Food Quality Protection Act of 1996 (FQPA) represents a major overhaul of pesticide regulations in the United States. A key section of the Act denotes that children exhibit an especially high susceptibility to pesticide-induced toxicity. This leads to a provision requiring the Environmental Protection Agency (EPA) to employ an additional 10-fold safety factor for infants and children. The EPA Administrator is, however, provided the option of using a different margin of safety if it can be shown that it will protect this subpopulation. In order to broaden access of children to essential medical treatments, the Food and Drug Administration Modernization Act of 1997 (FDAMA) provides incentives to pharmaceutical manufacturers who conduct acceptable studies in children with drugs identified by the FDA for which pediatric information would be beneficial. At the present time, following the establishment of safety in adults, safety in children is usually demonstrated by sequential clinical testing in children of decreasingly younger age groups. The FQPA's regulatory position that children are inherently more susceptible to the adverse effects of chemicals may carry over to pharmaceuticals (and other chemicals). This might engender alarm unnecessarily and discourage the proper use of pharmaceuticals in this age group. What factors should be considered in demonstrating the relative susceptibility or resistance of children to the potential adverse effects of pesticides or drugs? What is the basis for assuming that children exhibit an exceptionally high susceptibility to the potential adverse effects of chemicals? Should decisions be made on a case-by-case basis? What is the scientific basis for establishing pesticide exposure levels for children, bearing in mind that exposure does not equal dose? What is the scientific basis for establishing pharmaceutical dose levels in children?

1940 ENDOGENOUS ESTROGENS AS CARCINOGENS: METABOLIS ACTIVATION THROUGH OXIDATIVE METABOLISM.

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The major risk factors for human breast cancer include early age of menarche, late first full term pregnancy, nulliparity, late menopause, and to some degree, hormone replacement therapy. A common factor in all is prolonged and/or elevated estrogen exposure. The main mechanism proposed for estrogen carcinogenesis has been estrogen receptor-mediated, persistent cell proliferation associated with spontaneous replication errors. While estrogen-induced cell proliferation undoubtedly has a central role in the carcinogenic process, mounting evidence supports a hypothesis for a concomitant process in estrogen carcinogenesis involving indirect and direct genotoxicity originating from estrogen metabolites such as 16α -hydroxyestrone and the 4-hydroxy estradiol catechol. This symposium will address evidence in sup-

port of this hypothesis by summarizing the pathways of estrogen oxidative metabolism, addressing the formation of DNA damage caused by estrogen quinone metabolites and by reactive oxygen species arising from redox cycling processes supported by the estrogen catechols, and exploring evidence supporting a role for these metabolites in estrogen carcinogenesis. The symposium will include discussion of the possible effects of environmental chemical exposures on, and/or genetic polymorphisms in phase I and phase II metabolism of estrogens in breast cancer.

1941 GOOD DISRUPTERS, BAD DISRUPTERS.

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A variety of molecules, dietary changes, and activities, can act to alter estrogen metabolism in opposing directions. Whether the results are protective or risk promoting depends on the metabolic responses which are induced. Compounds (e.g. I3C, EGCG) which increase 2-hydroxylation of estrogens appear to be protective against breast cancer and recurrent respiratory papillomatosis, while substances which increase 4 or 16(hydroxylation of estrogens (carcinogens, oncogenes, and viruses) appear to be risk promoting for breast or cervical cancer and recurrent respiratory papillomatosis. In many cases these act by inducing phase I enzymes capable of carrying out 2,4 or 16(-hydroxylation. More recently we have discovered that I3C, in addition to inducing 2-hydroxylation also markedly inhibits 4-hydroxylation. This shows that good disrupters can act both by enhancing a risk protecting pathway like 2-hydroxylation but also can decrease a risk increasing event like 4-hydroxylation. In addition to their effects on estrogen metabolism as a risk modulating event good disrupters also have direct effects on Er binding, cyclin activity, and apoptosis. Good disrupters can act to oppose the harmful effects of bad disrupters (e.g. reversing the decreased 2/16(metabolite ratio observed after carcinogen administration). Cancer risks from the environment may represent a balance between good disrupters causing favorable responses while bad disrupters cause opposite actions in terms of altering estrogen metabolism, apoptosis, cyclin action, etc. Increasing the role of good disrupters should be one of the goals of dietary and environmental studies in the coming years.

1942 XENOBIOTIC-INDUCED CATECHOL ESTROGEN FORMATION IN BREAST EPITHELIAL AND BREAST CANCER CELLS.

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Estrogens have long been associated with Carcinogenesis in the uterus and breast. In addition to cell-proliferative effects leading to tumor promotion, there is mounting evidence supporting a role for metabolic activation of steroidal estrogens by means of 4-hydroxylation in the initiation of carcinogenesis in experimental animals. In humans, elevated rates of estradiol 4-hydroxylation were observed in tumors of the breast and uterine myometrium. Our studies have focused on the regulation of estradiol 2- and 4-hydroxylation in human breast epithelial and breast cancer cells and the effects of xenobiotics on these activities. We found that exposure to Ah-receptor agonists including halogenated dibenzo-p-dioxins and dibenzofurans, polychlorinated biphenyls, and indolo {3,2-b} carbazole caused marked stimulation of both 2- and 4-hydrozylation in human breast cells. Ah-receptor-regulated estradiol 2- and 4-hydroxylases were identified as CYP1A1 and CYP1B1, respectively. Differential regulation of CYP1A1 and CYP1B1 in human breast cells was evident, as CYP1A1 inducibility was only observed in breast cell lines displaying epithelial morphology, whereas CYP1B1 inducibility was observed in cell lines displaying either epithelial or mesenchymal morphology. CYP1B1 expression and inducibility predominated over CYP1A1 in several estrogen receptor-negative cell lines representative of advanced breast cancer. Differential regulation of CYP1B1 and CYP1A1 was also observed in MCF-7 breast cancer cells in response to 12-O-tetradecanoylphrobol-13-acetate, as exposure to this compound augmented CYP1B1 but depressed CYP1A1 inducibility. These observed differences in CYP1B1 expression may reflect the same molecular mechanisms that give rise to the elevated rates of estradiol 4-hydroxylation observed in human breast tumors.

1943 FORMATION, ACTIVATION, AND DNA DAMAGE BY 4-HYDROXY-ESTROGENS.

J G Liehr. *Stehlin Foundation for Cancer Research, Houston, TX, USA.* Sponsor: J D Yager.

Estrogen-induced tumors in rodents such as in kidneys of hamsters, uteri of mice, and pituitaries of rats are used to study the mechanism of hormone-induced carcinogenesis. Increasing evidence suggests a tumor-initiating role by metabolic activation of estrogens in addition to hormonal responses mediated by receptors. For instance, an elevated 4-hydroxylation of estradiol was detected in organs of rodents (listed above) where estrogens induced tumors and also in normal and neoplastic human breast and uterus. In contrast, in human and rodent livers and other organs, 4-hydroxyestradiol is formed only as a minor by-product (15-20%) of total catecholestrogens. Both 2- and 4-hydroxyestradiol may undergo metabolic redox cycling between hydroquinone and quinone forms, a mechanism of free radical generation. Consistent with this argument, direct and indirect free radical-mediated DNA damage has been induced by estrogens in vitro and in vivo. These forms of DNA damage have been detected in organs of rodents treated with estradiol and in mammary DNA of breast cancer patients and include: 8-hydroxylation of guanine bases of DNA and DNA adducts formed by malonaldehyde, a decomposition product of free radical-induced lipid peroxides. Steroidal estrogens may be complete carcinogens and induce tumors in specific tissues capable of metabolic activation and of stimulating growth of cells depending on the receptor content. (Supported by the NIH, NCI (CA 63129).)

1944 DECREASED CATECHOL ESTROGEN PHASE II METABOLISM BY CATECHOL-O-METHYL TRANSFERASE (COMT) AS A RISK FACTOR FOR BREAST CANCER: MOLECULAR EPIDEMIOLOGIC AND EXPERIMENTAL EVIDENCE.

J D Yager and J A Lavigne. *Division of Toxicological Sciences, Department of Environmental Health Sciences, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD, USA.*

Catechol metabolites of estrogens may contribute to the development of estrogen-induced cancers. Their inactivation is catalyzed by COMT which is polymorphic in the human population. We conducted a molecular epidemiology study to test the hypothesis that low activity COMT (COMTLL) may be a risk factor for human breast cancer using a PCR-based RFLP assay to determine COMT genotype in a cohort of 112 matched, nested case-control samples (Cancer Res., 57: 5493-5497, 1997). Postmenopausal COMTLL women had a greater than two-fold increased risk of developing breast cancer (OR=2.18, CI=0.93-5.11). The association of COMTLL with the development of postmenopausal breast cancer was stronger and statistically significant in those women with a body mass index >24.47. Combining COMTLL with either glutathione S-transferase (GST) M1 null or with GSTP1 intermediate/low activity genotypes, the risk for developing postmenopausal breast cancer was also significantly increased. These findings suggest that the allele encoding low activity COMT may be an important contributor to the postmenopausal development of breast cancer in certain women in this study population. Ongoing laboratory studies show that inhibition of COMT in human liver and breast epithelial cells causes an increase in cellular reactive oxygen species. However, a molecular epidemiology study in a different study cohort (Cancer Res., 58: 2107-2110, 1998) found that COMTLL was associated with an increased risk in premenopausal women and that the risk was greatest in the heaviest women. These differences will be addressed. (Supported by grants CA 70655, 36701, 77550 & ES 07141.)

1945 REACTIVE OXYGEN AND NITROGEN SPECIES: CELL ACTIVATION, INJURY, AND APOPTOSIS.

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Reactive oxygen species (ROS; eg., $O_2^{\cdot -}$, H_2O_2, Fe-O_2) and reactive nitrogen species (RNS; eg., ·NO, ·NO_2, $ONOO^-$) represent important effectors in numerous toxicologic processes. Within the lung, exposure to ROS and RNS can occur (i) from direct inhalation of gas phase contaminants (eg., O3, NO2), (ii) as a consequence of xenobiotic metabolism or direct biomolecule reactions (eg., paraquat, oxidant-induced peroxidation), (iii) inflammation, or (iv) due to toxicant-induced modulation of cellular processes (eg., disruption of mitochondrial function, upregulation of nitric oxide synthase). The

cellular interactions with ROS and RNS are, at best, complex with either suppression or upregulation of cellular processes potentially occurring. These reactive species not only initiate direct oxidation of macromolecules but also critically mediate cellular homeostasis via a diverse spectrum of mechanisms. In addition, their actions may not be homogeneous especially with respect to microanatomic and intracellular concentrations. Cell-specific susceptibility is likely a function of the balance among toxicant-induced ROS/RNS production, the sensitivity of cell control mechanisms to changes in ROS/RNS concentrations, and the extent of detoxification processes. The goal is to present a state-of-the-art perspective of how ROS/RNS are generated during toxic insult, paradoxical actions of oxidants and antioxidants, how ROS and RNS mechanistically influence cell signalling and control, and how the distribution of lung injury is uniquely governed by both airspace surface phenomenon and specificity of cellular metabolism. Discussions will include specific biochemical interactions, mechanisms which lead to alterations in gene expression and cell cycle control, lung surface reactions, and the intact lung.

1946 OXYGEN RADICAL-NITRIC OXIDE REACTIONS IN OXIDATIVE TISSUE INJURY.

B A Freeman. *Depts. of Anesthesiology and Biochemistry and Molecular Genetics, Center for Free Radical Biology, University of Alabama at Birmingham, Birmingham AL, USA.* Sponsor: E M Postlethwait.

Oxidation-reduction reactions induced by xenobiotics, impaired respiratory metabolism during cell injury and the tissue inflammatory reactions that occur in response to exposure to toxic substances all result in increased rates of tissue free radical (oxygen radical and nitric oxide) production. Nitric oxide (.NO) exerts potent actions in the regulation of cell function and tissue viability that extend far beyond the recognized ability of .NO to mediate signal transduction via activation of guanylate cyclase. Chemical reactions, cell culture/animal models and clinical studies have all revealed an ability of .NO to potently modulate oxygen radical reactions and pathologic processes associated with exposure to redox-active xenobiotics and inflammation. The focus of this presentation is to reveal the reactions of .NO with metalloproteins, reactive oxygen species and lipid radicals (LO. and LOO.) and discuss how they mediate the tissue-protective and injurious toxicological actions of .NO. Free radicals (such as .NO) and other oxidizing species have the capacity to directly mediate toxic reactions or initiate secondary deleterious reactions that become self-sustaining by regenerating propagating radicals. The best defenses against these events are to directly scavenge the initiating radical or to terminate the radicals that sustain propagation, via an integrated network of tissue antioxidant mechanisms. The products of .NO-oxidant reactions, NO2- (nitrite), ONOO- (peroxynitrite) and both LONO/LNO2 and LOONO/LONO2 adducts, are also reactive and can serve as mediators of inflammation and tissue injury. Through the catalytic actions of these labile secondary intermediates in tissues undergoing oxidative stress, .NO can lead to the formation of more stable nitrogen-containing adducts of aromatic amino acids in proteins (S-nitrosothiols, nitrotyrosine and nitrotryptophan), carbohydrates, purines and DNA. These nitrogen-containing products, many observed to be present in high concentrations in inflammatory and vascular diseases, serve as footprints for .NO-mediated events in tissue injury and display unique biological and signal transduction properties. Recent laboratory and clinical studies are also revealing that, under some circumstances, .NO can also play a central role in tissue antioxidant and anti-inflammatory defensive networks by serving as a terminator for propagating species formed during membrane and lipoprotein oxidation. The injurious versus tissue-protective roles of .NO seem to be determined by its concentration and the underlying extent of tissue oxidative stress. In summary, new understanding will be presented regarding the molecular mechanisms of .NO action and the critical mitigating factors that manifest the toxic qualities of .NO or alternatively, mediate its tissue-protective properties during oxidative stress.

1947 OXIDANT AND ANTIOXIDANT REGULATION OF CELLULAR SIGNALING.

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Signal transduction is a regulated sequence of biochemical steps through which a stimulant conveys a message resulting in a physiological response. There is increasing recognition that the effects of oxidative stress are not all mediated through damage of cellular constituents. Rather, reactive oxygen species can alter cell function by acting as, mimicking, or affecting the intermediates (second messengers) in signal transduction. Indeed, superoxide, hydrogen peroxide, and nitric oxide possess many of the essential characteristics of second messengers. Antioxidants therefore have the potential to counter or modulate the effects of oxidants and thereby modulate signal transduction. Examples in which oxidants have been shown to participate in signaling are the conversion of protein kinase C (α and β) to calcium- and phospholipid-independent activity and the activation of arachidonic acid metabolism. In much of "oxidative signal transduction" however, the actual redox reaction involved is not known and in many cases, participation of oxidants is only implied by the effects of compounds with antioxidant properties or correlation with changes in the "redox state." Nonetheless, oxidative activation does not necessarily require an imbalance in the "redox status" of the cell as recently shown for activation of NF-$_\kappa$B. Other recent investigation has suggested that the ras, p38-MAP kinas kB. Other recent investigation has suggested that the ras, p38-MAP kinase e, and sphingomyelinase pathways are activated by oxidative stress with the evidence provided through inhibition with N-acetylcyteine or other thiols. Thus, the importance of understanding the role of oxidants and antioxidants in cell signaling underlying normal physiology and pathophysiology is apparent. Nonetheless, the potential for action of agents, such as N-acetylcysteine and other "antioxidants" to act in a manner independent of their antioxidant capacity must also be considered.

1948 INDUCTION OF CELL SIGNALLING CASCADES AND TRANSCRIPTION FACTORS IMPORTANT IN CONTROL OF PROLIFERATION OR APOPTOSIS IN PULMONARY EPITHELIAL CELLS EXPOSED TO REACTIVE OXYGEN OR NITROGEN SPECIES.

Y M W Janssen and R P Soultanakis. *University of Vermont, Department of Pathology, Burlington, VT, USA.* Sponsor: E M Postlethwait.

Epithelial cells of the lung are exposed to reactive oxygen and nitrogen species (ROS and RNS, respectively) produced by cell types of the lung or as a result of inhalation of environmental oxidants. The functional responses of rat alveolar type II epithelial cells (RLE) to ROS/RNS are complex and include apoptosis, proliferation and the induction of inflammatory mediators. Exposure of RLE cells to H_2O_2 or NO causes dosage dependent increases in apoptosis or DNA synthesis which are preceded by the activation of extracellular signal regulated kinase (ERK) and c-jun-N-terminal kinase (JNK) members of the Mitogen Activated Protein Kinases (MAPKs) proteins and the induction of c-fos/ c-jun proteins which comprize the transcription factor, activator protein-1 (AP-1). Exposure of confluent cultures of RLE cells to peroxynitrite (ONOO-), or the ONOO- generator, 3-morpholino sydnonimine (SIN-1) causes a pronounced activation of JNK, but does not result in the induction of apoptosis, despite the formation of protein nitrotyrosine residues.These data suggest that tyrosine nitration per se is not important in the apoptotic response in our model system and that different reactive species elicit distinct phenotypic outcomes. Exposure of RLE cells to gas phase NO_2 (1-5 ppm) for 4 hrs also causes formation of nitrotyrosine residues and induces both ERK and JNK MAPKs in RLE cells. Following 24 hrs of exposure to NO_2, evidence of apoptosis is apparent in NO_2-exposed RLE cells. Localization of ERK and JNK MAPK in cells reveals that active JNK and c-jun are localized in cells in G2/M phases of the cell cycle whereas expression of ERK is found in apoptotic cells, suggesting that ERK and JNK may regulate cell cycle progression and apoptotic responses, respectively. The kinetics of generation of ROS/RNS, may dictate the extent and duration of ERK vs. JNK MAPK activation and the subsequent induction of apoptotic or proliferative responses in epithelial cells of the lung.

1949 GENERATION OF LUNG SURFACE SECONDARY REACTIVE SPECIES DURING INHALED OXIDANT EXPOSURE.

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The airspace surfaces are covered by an aqueous epithelial lining fluid (ELF) that blankets all cells except airway macrophages and is the first compartment inhaled gases encounter. The ELF contains substrates (eg. antioxidants, lipids, proteins) that rapidly react with oxidants. Ozone (O_3) and nitrogen dioxide (NO_2) are ubiquitous oxidants that display high reactivity but limited aqueous solubility. Near interfacial reactions within the ELF maintain the driving force for the net uptake of both toxicants, limit diffusion of the solute gases, and produce reaction products. Thus, because most respiratory tract cells do directly contact the inspired oxidant, cellular oxidative stresses are most likely initiated by extracellular products. However, direct cellular reac-

tion with $\cdot NO_2$ may occur due to its formatain the driving force for the net uptake of both toxicants, limit diffusion of the solute gases, and produce reaction products. Thus, because most respiratory tract cells do directly contact the inspired oxidant, cellular oxidative stresses are most likely initiated by extracellular products. However, direct cellular reaction with $\cdot NO_2$ may occur due to its formation from other NOx (eg., $\cdot NO$, $ONOO^-$). Inhaled $\cdot NO_2$ preferentially reacts with ELF antioxidants to produce reactive oxygen species (ROS) which can initiate membrane oxidation. Although $\cdot NO_2$ reaction with ELF lipids is limited, unsaturated lipids may protect the underlying epithelial membranes by scavenging ROS and reducing antioxidant prooxidant activity. Conversely, reaction with ELF antioxidants appears to primarily quench inhaled O_3. Although O_3 reaction with ELF lipids is limited, lipid-derived bioactive species are produced which likely contribute to its cytotoxicity. The rates of ELF constituent turnover may not be sufficient to preclude exposure-induced concentration changes, especially in microanatomic regions of high deposition. The heterogenous nature of the ELF may account for response variations across individuals, species, and exposure time. Overall, the net extracellular production of bioactive species results from the flux of reduced substrates into the ELF, reaction with inhaled oxidants, and secondary reactions which either quench or produce other bioactive/toxic species. Consequently, ELF composition will directly influence oxidant dosimetry. Differences among various in vivo and in vitro models in terms of surface chemistry may impart substantial dissimilarities in the toxic species which cells encounter.

1950 3-D MAPPING OF OXIDANT-INDUCED TRACHEOBRONCHIAL EPITHELIAL CYTOTOXICITY.

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The cellular injury response of the respiratory system to reactive oxygen species, whether gases, such as ozone, or products of xenobiotic metabolism, such as naphthalene oxide, is focal, target phenotype-specific and microenvironment selective. To identify the mechanisms of injury within the numerous phenotypes of the respiratory system requires specific identification of the sites of cellular injury and a characterization of the individual cellular responses in relation to hallmarks of cell disintegration preceding death. A combination of airway microdissection to identify specific microenvironments within the respiratory system and cellular methods for determining loss of membrane integrity and the content of intracellular antioxidants, such as glutatione, are applied to lung samples which can then be used for high resolution histopathology and ultra-structural characterization of the status of cellular breakdown. Whole animals are exposed to toxic compounds and the lungs processed for multiple analysis using a series of fluorochromes to characterize membrane integrity (ethidium homodimer-1) and glutathione content (monochloro-bimane) followed by analysis using laser confocal scanning microscopy and quantitative epifluorescence. Density of membrane permeant cells and their distribution throughout the airway can be quantified by airway generation. Analysis of viable airway explants are used to map individual cell glutathione content on a site-selective basis. To date, this approach indicates that many markers of intracellular stress, including clearing of the cytoplasm, alterations in mitochondrial ultrastructure, rearrangement and dilation of endoplasmic reticulum, and formation of cytoplasmic blebbing, occur after intracellular glutatione has been depleted and prior to loss of plasma membrane integrity. The pattern of changes in membrane integrity varies by site and cell phenotype depending on the nature of the toxicant and the pattern of exposure.

1951 ENDOCRINE DISRUPTION AND NEUROTOXICITY: WHY TOXICOLOGISTS SHOULD BE CONCERNED ABOUT THE ACTIONS OF ESTROGENIC CHEMICALS IN THE CNS.

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Susceptibility, whether acquired or innate, can dictate the biological response to a chemical and gender is one such susceptibility factor. Many toxicological responses have been examined in the context of gender and the recent concern regarding the "hormone-like" properties of certain man-made chemicals has focused interest on how estrogen and chemicals with estrogenic properties can influence toxicological endpoints. Reproductive endpoints, however, have been of primary concern and there has been little examination of neurotoxic endpoints in this context despite evidence that estrogen can have both neuroprotective as well as neurotoxic properties in the CNS. This workshop will present a general overview of estrogen and the mechanisms by which this hormone influences biological actions including a discussion of estrogen α and β receptor actions and how these actions impact toxicity. The remainder of the session will provide a more focused examination of estrogens' influences in the CNS and will examine its putative antioxidant actions, the role of receptor-mediated actions, direct membrane effects and neurosteroid properties in its neuroprotective actions. The influence of estrogenic chemicals on CNS injury utilizing well-established models of neurotoxic insult as well as their influence on brain structure in sexually-dimorphic areas of CNS will be examined. This workshop concerns endocrine disruption, estrogenic chemicals and injury processes. (Supported by CDC/NCTR.)

1952 BIOLOGICAL ACTIONS OF ESTROGEN: GENOMIC AND NON-GENOMIC MECHANISMS.

S C Laws. *Reproductive Toxicology Division, NHEERL, U.S. EPA, Research Triangle Park, NC, USA.* Sponsor: D B Miller.

Estrogen (E) plays a key role in male and female mammalian sexual development as well as adult reproductive behavior and function. Here we review the mechanisms through which E regulates the growth, differentiation and function of sex specific target tissues as well as the CNS, bone and cardiovascular systems. The classical genomic action of estrogen is mediated through its interaction with nuclear estrogen receptors (ERα and ERβ) upon which E induces conformational changes in the ER. E-ER complexes can then bind in dimer formation to the ER- response element (ERE) of specific target genes and facilitate gene transcription. Thus, E in conjunction with the ER acts as a tanscription factor by modulating mRNA and protein synthesis, with subsequent regulation of specific cellular and physiological functions. While E-mediated genomic responses generally require several hours to occur, short-term E-mediated responses (occurring within seconds) have been observed, suggesting alternative non genomic mechanisms of action. E-binding plasma membrane receptors and second messenger pathways may be mechanisms through which these rapid responses to E occur. Numerous reports suggest that environmental chemicals can interfere with E-mediated physiological responses by mimicking the actions of this steroid hormone by direct and indirect mechanisms. For example, phytoestrogens, industrial by products (alkylphenols), and pesticides (o,p'-DDT, methoxychlor, chlordecone) can bind to the ER and induce ER-mediated responses *in vivo*. Alternatively, lindane (γ -HCH, an organochlorine pesticide) and atrazine (a triazine herbicide) can interfere with E-induced effects without a direct interaction with the ER. (This abstract does not reflect U.S. EPA policy.)

1953 THE ROLE OF ESTROGENS IN SHAPING BRAIN STRUCTURE.

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Prenatal and/or early postnatal exposures to estrogens have profound effects on the anatomy of the brain in the developing rodent fetus. Structural differences between adult male and female rats in hypothalamic cytoarchitecture as well as the functional expression of various mRNAs and their protein products have been reported. These "organizational" effects appear primarily mediated by brain estrogen receptors, which may be activated either directly by estrogenic chemicals or indirectly by androgens which are first converted to estrogens via cytochrome P450 aromatase. These "organizational" differences may be over-ridden by perinatal events, including exposure to endocrine disrupting chemicals, resulting in an adult hypothalamic structure and its associated functions that are incongruent with the original genetic sex of the animal. Sexually dimorphic hypothalamic nuclei within the medial preoptic area (MPO) and the bed nucleus of the stria terminalis (BNST), are most often considered the neuroanatomical targets of sexual differentiation in the CNS of rats. In humans it is uncertain which hypothalamic nuclei are comparable in terms of plasticity and function although the sexually dimorphic nucleus of the MPO, the BNST and the suprachiasmatic nuclei appear to be focal areas for the organizing effects of steroid hormones in both species. A scheme utilizing neuromorphometric (e.g., 3-D reconstruction methods) and neurochemical (e.g., ELISA measurement of estro-

gen receptors) approaches will be described that will be useful in assessing the impact of estrogenic compounds (e.g., phytoestrogens,) and other known or suspected endocrine disrupting chemicals on the development and function of these sexually dimorphic brain areas. (Supported by and Interagency Agreement between FDA & NIEHS.)

1954 NEUROPROTECTIVE ACTIONS OF ESTROGEN IMPLICATIONS FOR EXPOSURE TO ESTROGENIC CHEMICALS.

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The recent focus on the hormone-like effects of many xenobiotics has led to a renewed interest in both the reproductive and nonreproductive actions of estrogen. Its protective or age-retardive actions are well established for multiple body systems including skin, bone and teeth, colon, blood vessels and heart. Clinical and epidemiological studies suggest neuroprotective properties in Parkinson's and Alzheimer's disease. *In vitro* and *in vivo* experimental data indicate estrogen may prevent the neuronal cell damage and necrosis observed following excitotoxic, metabolic and oxidative insult. Conversely, protracted estrogen exposure whether due to age or exogenous administration damages certain brain areas. Mechanisms mediating these protective or pathological actions may include activation of pathways or release of substances through specific intracellular receptor-mediated actions; direct, rapid, nongenomic effects at the cell membrane, at membrane receptor sites and ion channels or at cyclic AMP response elements; altered metabolic profile of critical brain enzymes; and alteration of neurotransmitter and neuropeptide transmitter mechanisms in brain. Utilization of transgenic technology has been useful in elucidating the mechanism of estrogen's protective effects in the vasculature and bone and should be useful in examining its CNS protective and pathological actions. Whether compounds with estrogen agonist, antagonist or mixed actions (e.g., tamoxifen, phytoestrogens, triphenylethylenes) can interfere with or mimic the neuroprotective or neuropathological actions of estrogen has received little scrutiny or debate. Tamoxifen can, however, block some but not all of the neuroprotective actions of 17α or β -estradiol *in vitro* and can modulate certain aspects of nigral striatal function including release of dopamine in response to stimulation and the response to dopaminergic neurotoxic insult. Actions of other estrogenic agents in these models is unknown. (Supported by CDC/NCTR.)

1955 NEUROPROTECTIVE ROLE OF ESTROGEN IN OXIDATIVE STRESS INDUCED NEURODEGENERATION: IMPLICATIONS FOR NEUROTOXIC INSULT.

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Certain neurodegenerative diseases like Parkinson's disease, affect more men than women and women with Alzheimer's disease receiving hormone replacement therapy show a lessened severity and slowed disease progression. Estrogen may have antioxidant properties as these diseases are linked to oxidative damage. *In vitro* studies utilizing neural tissue have shown that 17β -estradiol can decrease excitotoxic damage by directly inhibiting the NMDA receptor and limit the damage caused by β -amyloid peptides, a suspected etiological agent in Alzheimer's disease. In examining the striatal dopaminergic neurotoxicity of agents such as MPTP and methamphetamine (METH) that are suspected to cause neuronal injury by oxidative stress, greater dopaminergic neurotoxicity was noted in males. Evidence linking estrogen to this gender difference was provided by the finding that ovariectomized (OVX) mice, compared to mice receiving 17β -estradiol, displayed greater striatal damage in response to MPTP suggesting that estrogen has neuroprotective properties in this model of striatal dopaminergic neurotoxicity. Protection was not linked to alterations in metabolism or generation of the proximal toxicant although estrogen is known to affect metabolic processes. Similar neuroprotective effects of estrogen were found for METH-induced dopaminergic neurotoxicity and others have reported greater striatal damage in OVX female rats receiving 6-hydroxydopamine, another dopaminergic neurotoxicant suspected to damage tissue through a free radical/oxidative stress mechanism. Whether other estrogenic compounds, including tamoxifen, the triphenylethylenes, and the phytoestrogens, can mimic or disrupt the neuroprotective actions of estrogen has received limited examination or discussion. (Supported by NCTR/CDC.)

1956 COMPLEMENT ACTIVATION IN TRIMELLITIC ANHYDRIDE (TMA)-INDUCED PULMONARY HYPERSENSITIVITY IN THE GUINEA PIG.

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TMA is a small molecular weight compound known to cause hypersensitivity reactions in the lung, particularly occupational asthma. Actively sensitized guinea pigs challenged intratracheally with TMA conjugated to guinea pig serum albumin (TMA-GPSA) respond with an immediate bronchoconstriction and delayed eosinophil, neutrophil and mononuclear cell infiltration into the lungs and lung hemorrhage. These events are usually attributed to antigen interaction with mast cell antibody. However, our previous studies using Cobra venom factor to deplete complement indicated a role for the complement system in delayed cellular infiltration into the lungs and lung hemorrhage (Journal of Pharmacology and Experimental Therapeutics 273:793-801, 1995). Thus, we hypothesized that the complement anaphylatoxins C3a and C5a were the relevant products of complement system activation mediating the cellular infiltration and lung hemorrhage. The purpose of the present study was to determine if the complement system activation product C3a was produced in the bronchoalveolar lavage (BAL) fluid of actively sensitized guinea pigs in response to challenge with TMA-GPSA. Guinea pigs were sensitized intradermally with TMA in corn oil. Three weeks later animals were challenged by intratracheal instillation of either TMA-GPSA (n=33) or GPSA (n=28). Twenty-four hours after challenge, the guinea pigs were anesthetized with pentobarbital and the lungs lavaged with phosphate buffered saline. Complement activation product C3a was measured in the BAL fluid by Western blot and immunodetection. The quantity of C3a in the BAL fluid of TMA-GPSA challenged animals was significantly greater than that found in GPSA challenged guinea pigs ($p<0.01$). Our finding of increased C3a is consistent with a potential role for complement anaphylatoxins in TMA-induced cellular infiltration and lung hemorrhage. (Supported by NIH ES 07406.)

1957 ASSESSMENT OF RESPIRATORY SENSITIZING POTENTIAL OF ACID ANHYDRIDES BY CYTOKINE FINGERPRINTING.

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We have demonstrated previously that contact allergens such as 2,4-dinitrochlorobenzene (DNCB) and respiratory allergens such as trimellitic anhydride (TMA) induce discrete type 1 and type 2 cytokine secretion patterns, respectively, following prolonged topical exposure of BALB/c strain mice. These observations have formed the basis of an approach to the identification of respiratory allergens known as 'cytokine fingerprinting'. We have now compared the patterns of cytokine production by TMA- and DNCB-activated draining lymph node cells (LNC) with those provoked by concurrent treatment with a number of additional acid anhydrides which have been implicated as potential occupational respiratory sensitizers. These comprised maleic anhydride, hexahydrophthalic anhydride and phthalic anhydride. Under immunogenic conditions of exposure, determined as a function of the induction of significant lymphocyte proliferation in draining nodes, all of the selected acid anhydrides stimulated a type 2 cytokine secretion phenotype. In each case interleukin 10 expression reached comparable levels to those provoked by the reference respiratory allergen TMA. Detectable mitogen-inducible interleukin 4 (IL-4) was also induced, although levels were generally much lower than those elicited by TMA. In contrast DNCB-activated LNC failed to produce detectable levels of these cytokines. Low expression of the type 1 cytokines interferon γ and interleukin 12 was observed in response to treatment with the acid anhydrides, compared with the substantial production of these cytokines by LNC cultured from DNCB-exposed mice. These data provide further confirmation that cytokine fingerprinting represents a robust and reliable approach for the identification of potential respiratory allergens.

1958 TRIMELLITIC ANHYDRIDE (TMA) HYPERSENSITIVITY IN MICE AFTER DERMAL EXPOSURE AND INTRATRACHEAL (IT) CHALLENGE.

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TMA causes Th2 related respiratory (RH) and Th1 related contact hypersensitivity (CH) responses. Elevated total serum IgE has been reported after

dermal exposure to TMA or other RH haptens, but not after exposure to exclusively contact sensitizing haptens, such as dinitrofluorobenzene (DNFB). Typically, Th1 and Th2 responses are thought to be mutually antagonistic. This makes the expression of both strong IgE and CH responses following TMA exposure perplexing. Our goal was to investigate the effect of additional TMA sensitizations and the IT challenge route on the Th1/Th2 responses. DNFB was used as the CH/Th1 control. We attempted to amplify the Th2 response and produce a comprehensive murine model of RH to small molecular weight chemicals. Mice were topically sensitized using a variety of exposure regimens. CH was assessed 4 days after final sensitization by the Mouse Ear Swelling Test. Results indicate that CH, following TMA sensitization, was significantly increased with repeated dermal applications, compared to a single sensitization protocol or DNFB exposure. Mice required at least 2 wks of dermal sensitization and an IT challenge to produce significant serum and bronchoalveolar lavage fluid (BALF) changes suggestive of a Th2 response (i.e. elevated total IgE, IL4). Other parameters indicated these TMA IT challenge doses cause non-specific inflammation. When challenge doses were reduced and the responses were examined, the indicators of RH were abrogated. These results may indicate that a slightly irritating IT dose of TMA is needed to induce the Th2 responses indicative of the respiratory sensitization. Of interest, was an increase in IFN-γ found in the BALF of TMA treated mice. This appears to be related to the Th1 component of the TMA response and may not be a result of lung irritation. Our aim is to evaluate alternative mouse exposure strategies and mechanisms involved in CH/RH (Th1/Th2 relationship) of low molecular weight compounds. (This abstract does not reflect EPA policy.)

1959 PHYSICOCHEMICAL CHARACTERISTICS THAT CONTRIBUTE TO THE SENSITIZING POTENCY OF ACID ANHYDRIDES.

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Acid anhydrides are small reactive industrial chemicals that have been associated with hypersensitivity disease of the lung. However, not all acid anhydrides have this biological activity. A study was undertaken to determine the relationship of anhydride structure with ability to induce immunologic lung hypersensitivity in animals. Anaphylactic antibody titer was used as a surrogate for hypersensitivity potency. A data base was constructed using physicochemical properties of the 15 acid anhydrides and the anhydride specific anaphylactic antibody titer in rats and guinea pigs. Nineteen parameters that represented transport, steric and electronic properties were evaluated. The single predictor that had the greatest statistical relationship with antibody titer was Hansen hydrogen bonding, with R^2 (adjusted) = 0.62, $p < 0.000$. The mathematical model indicates an inverse relationship, highest titers were associated with low hydrogen bonding. The best model that utilized two variables included HOMO and Hansen hydrogen bonding. Increased activity was associated with increased HOMO energy. This model had an R^2 (adjusted) of 0.72, $p < 0.05$. When tested for ability to predict the activity of 3,4,5,6 tetrahydrophthalic anhydride, an acid anhydride that had been deliberately excluded, the latter model accurately predicted the chemical to have minimal activity. These SAR model results indicate that for the pulmonary hypersensitivity of acid anhydrides, and increase in hydrophobicity and oxidation potential are important determinants of activity. (Supported by NIEHS #05651.)

1960 TOLUENE DIISOCYANATE-INDUCED SKIN HYPERSENSITIVITY REACTIONS IN GUINEA PIGS SENSITIZED BY INHALATION OF THE CHEMICAL.

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Toluene diisocyanate (TDI) is a known causative agent of occupational asthma. It is also a potent skin sensitizer in animals. It is uncertain if TDI can induce skin hypersensitivity reactions when exposure is via the inhalation route. The airways are the most likely exposure route of factory workers who are in contact with airborne TDI, and also exposed to TDI by skin contact. We attempted to develop a modified skin sensitization test. Seven week old female guinea pigs were exposed in a snout only exposure chamber to TDI mist for 4 hr at a concentration of 0.085 mg/L. TDI mist was generated using an atomizer. Two to 3 weeks later, the flank was exposed epicutaneously to 1% TDI dissolved in liquid paraffin for 6 hr using an occulusive patch. The skin sites were observed at 24 and 48 hr after removal of the patch. All of the 8 sensitized and challenged guinea pigs exhibited positive skin reactions (flare) at both time periods. By contrast, 8 non-sensitized and challenged control animals exhibited negative reactions at 24 hr. Two of the 8 control animals showed mild flare on the skin at 48 hr. These findings suggest that inhaled TDI has the potential to sensitize the skin of guinea pigs, and that skin hypersensitivity reactions may occur when TDI later contacts the skin.

1961 THE RELATIONSHIP BETWEEN TOTAL IgE AND ENVIRONMENTAL FACTORS.

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A study was undertaken of environmental factors that may influence levels of total IgE in industrial workers. Factors considered were exposure to industrial chemicals, tobacco smoke, allergy to aeroallergens, and respiratory tract infections. From routine testing of industrial workers, total serum IgE was found to be elevated for a group located in South Carolina relative to a comparable group in Pennsylvania. Forty-four Pennsylvania (PA) workers exposed to toluene diisocyanate (TDI) and 19 workers from South Carolina (SC) exposed to cyanuric chloride (CyCl) were selected for detailed study. None of the workers reported industrial chemical exposure. Smoking status was determined by cotinine levels in serum. Total IgE in smokers was not statistically different from that in non-smokers. IgG antibodies was used to indicate previous respiratory tract infection. There was no statistically significant difference in total IgE levels of workers with previous respiratory tract infection compared to those without such infection. IgE antibodies to 10 aeroallergens were measured by RAST. Total IgE levels of SC workers who had numerous positive allergen RAST (9-10 allergen types) were higher than both PA and SC workers who had fewer positive RAST (0-8 allergen types). Exposure to industrial chemicals, tobacco smoke and previous respiratory tract infection did not contribute to high total IgE. Only allergy to a high number of aeroallergens correlated with high levels of total IgE. These results indicate that finding high total IgE in industrial workers may relate more to seasonal allergies rather than to chemicals in their industrial environment.

1962 ANALYSIS OF THE SPECIFICITY OF ANTIBODY DETECTION IN RELATION TO DIISOCYANATE ASTHMA.

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Numerous etiologies for diisocyanate-induced asthma have been proposed and evaluated including both immunologic and pharmacologic mechanisms. Despite the detection of specific antibodies (IgE and IgG) in the sera of some exposed workers, the results are not always consistent and often are not well correlated with disease. The question still remains as to what role, if any, IgE and/or IgG antibodies play in this disease process. The low signal found in ELISA for specific antibodies in workers exposed to toluene diisocyanate (TDI) necessitates methods that offer greater sensitivity without the loss of specificity. The present study began as an analysis for antibody responses in control population sera. Serum from thirty individuals with no known exposure to diisocyanate compounds were screened for specific IgG and IgG4 antibodies. Diisocyanate-human serum albumin (HSA) conjugates, a trimellitic anhydride-HSA conjugate, HSA and ragweed allergen were used as test antigens. Specific and nonspecific inhibition were performed to determine the specificity of any positive antigen recognition. Responses were corrected for total immunoglobulin concentration in the original serum sample. Several high responses (5/30) were detected in this control population and all but one of the five samples showed generalized antigen recognition that could not be specifically inhibited. These results suggest that within the general population, nonspecific reactions can compromise the specificity of diisocyanate antibody testing and indicate that further scrutiny of the responses may permit reclassification and the potential for more accurate diagnosis.

1963 ALBUMIN CONJUGATES OF HEXAMETHYLENE DIISOCYANATE AND HEXAMETHYLENE DIISOCYANATE-BIURET DETECT ANTIBODIES IN CAR PAINTERS.

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Car painting has been associated with occupational asthma. Isocyanates, in particular hexamethylene diisocyanate (HDI) , and its biuret (HDI-B), are frequently employed in this industry, and have been implicated as causative agents contributing to the disease. Based on previous experimental studies, we hypothesized that antibodies may be produced as a result of exposure to these isocyanates, and investigated the production of several classes of isocyanate-specific antibodies. Using ELISA and RAST, we examined the serum from 3 HDI asthmatic patients and 27 workers from three different car body shops and examined the incidence of antibodies to HDI and HDI-B. Several sera demonstrated antibodies to albumin conjugates of HDI and HDI-B. Titers were always greater with the latter reagent. Interestingly, IgG antibodies were detected in some workers and IgE antibodies in others. We also determined the ability of peripheral blood mononuclear cells from these subjects to proliferate in response to the same HDI conjugates using a ^{3}H-thymidine based assay. HDI conjugates induced proliferation in all HDI asthmatics and in approximately 20% of the other exposed workers. There was a good correlation between patients that displayed antibodies to HDI and those that showed lymphoproliferative responses. These results indicate that albumin conjugates of HDI and HDI-B can be used for biological monitoring of exposure through antibody determinations, and cell proliferative responses to isocyanates.

1964 INFLUENCE OF CARRAGEENAN ON ANTI-PROTEIN ANTIBODY ISOTYPE DISTRIBUTION.

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The increased use of protein effect molecules has resulted in a growing interest in the evaluation of their allergenic potential. We have shown previously that intraperitoneal (ip) administration to BALB/c strain mice of the protein allergen ovalbumin (OVA) stimulates the production of both specific IgG and IgE antibody. Exposure under identical conditions to bovine serum albumin (BSA), which, by comparison, has weaker allergenic potential, elicits vigorous IgG, including IgG2a, antibody responses, but no, or only low titer, IgE antibody. We have now examined the influence of carrageenan, reportedly a specific IgE adjuvant, on the selectivity and sensitivity of antibody responses to these materials. Groups of BALB/c strain mice (n = 5) received a single ip injection of OVA or BSA (2, 0.2, or 0.02%) in the presence or absence of 250μg of carrageenan type V (iota). Serum samples were collected 28 days after exposure and analyzed for the presence of specific IgE, IgG, IgG1 and IgG2a antibody. In the absence of adjuvant there was a marked difference in the ability of OVA and BSA to provoke IgE antibody responses, with the former, but not the latter, inducing detectable IgE antibody at all doses tested. Co-administration of carrageenan resulted in more vigorous IgE responses to both materials, although exposure to OVA was associated with somewhat higher titers than was exposure to equivalent amounts of BSA. Treatment with adjuvant also enhanced IgG, IgG1 and IgG2a antibody responses to both proteins. These data suggest that carrageenan enhances both IgG and IgE antibody responses to proteins, lowering the concentration threshold at which antibody production is observed, but with a consequent loss in the selectivity of IgE antibody responses to proteins of differing allergenic potential.

1965 RESPIRATORY SENSITIZATION OF BALB/C MICE WITH THE BIOPESTICIDE.

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Metarhizium anisopliae, an entomopathogenic fungus licensed for indoor use, has previously been shown to elicit allergic responses in BALB/C mice intraperitoneally (IP) sensitized and intratracheally (IT) challenged with the soluble factors from a *M. anisopliae* crude antigen (MACA). In this study the IT route of sensitization is compared to the IP sensitization and to an IT vehicle control. The IT route consisted of 4 IT exposures of 10 μg MACA in 50 μl HBSS over a four-week period (vehicle controls received HBSS only). While the IP route consisted of IP sensitization with 25 μg MACA in 0.2 ml of 1.3% alhydrogel followed 14 days later with an IT challenge (10 μg MACA/50 μl HBSS). Mouse airway reactivity was assessed by methacholine challenge at 1 and 3 days following the last MACA IT challenge (DPIT). Additionally both serum and BAL fluid (BALF) samples were harvested at 1,3, and 8 DPIT. Significant increases in total BALF cellularity as well as macrophage, neutrophil, lymphocyte and eosinophils were seen in IT sensitized mice when compared to both IP and vehicle controls at 1 DPIT but only total cellularity and eosinophilia were still significant at 3 DPIT. BALF total protein and LDH levels for IT mice were significantly different from both IP and vehicle control mice at 1 DPIT. At 3 DPIT both IT and IP mice were elevated compared to vehicle controls but were near control levels by 8 DPIT. Only IP sensitized mice demonstrated elevated IL-4 levels. Airway reactivity (airway resistance and compliance) was significantly increased for IT mice. In summary we found that multiple IT exposures without adjuvant demonstrated elevated responses for most endpoints assessed at early time points and had resolved by 8 DPIT. (Supported by USEPA Cooperative Training Agreement (T-901915-02) with UNC-CH.) (This abstract does not reflect EPA policy.)

1966 IMMUNE RESPONSE TO ENZYMES IN THE MOUSE INTRANASAL TEST: STRAIN COMPARISON.

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The mouse intranasal test (MINT) was developed to assess the immunogenic potential of detergent enzymes. Mice receive an IN dose of enzyme protein on days 1, 3 and 10 with serology on day 15. The BDF1 mouse (H-$2^{b/d}$), a cross of C57Bl/6 (H-2^b) x DBA/2 (H-2^d) has been used for most of the development work. Preliminary data in the CB6F1(H-$2^{b/d}$) a cross of C57Bl/6 x Balb/c (H-2^d) showed that this strain was similar to the BDF1 in its response to enzymes. These data also showed that the parental strains did not respond the same way as the F1 hybrids. To better understand the influence MHC background may have on immune responses to enzymes, 3 different enzymes were tested in the 3 parental strains and 2 hybrid strains. Enzymes are ranked as more, less or equipotent to the protease Alcalase. BDF1 mice rank enzymes the same as the guinea pig which in turn correlates with sensitization in occupationally exposed humans. The ranking is based upon the dose of enzyme needed to give ½ maximal IgG1 antibody response (ED50). Termamyl is more potent than Alcalase which is equipotent to Savinase. The H-2^d strains ranked the enzymes the same as the BDF1 but generated ED50's that were one order of magnitude greater than the BDF1 ED50's. The H-2^b strain did not rank the enzymes the same as BDF1 but the ED50's were similar to or lower than the BDF1 ED50's. Initial data show that all strains will make enzyme specific IgE antibody to high doses of enzyme with DBA/2 > Balb/c > C57Bl/6 in terms of the robustness of the response. Taken together, these data suggest the H-2^d background brings the robust IgE response and the ability to rank enzymes which correlates with human data. The H-2^b background brings sensitivity or responses to much lower doses of protein allergen. Therefore, the F1 hybrid is an appropriate strain for evaluating allergic responses to enzymes.

1967 DETECTION OF ALLERGIC INFLAMMATION AND ENZYME SPECIFIC IGE ANTIBODY IN THE MOUSE INTRANASAL TEST.

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The BDF1 mouse has been used in the development of the mouse intra-nasal test (MINT) for the assessment of the immunogenic potential of enzymes. In this model, mice receive an intranasal (IN) dose of enzyme protein on days 1, 3 and 10 with serology on day 15. The IgG1 antibody (AB) response to several enzymes has been well characterized in this model. The goal of the present work was to begin characterizing allergic inflammation and IgE AB after IN exposure to enzyme allergen. Mice exposed to 1.6ug protease enzyme (Alcalase) on days 1, 3, and 10 did not produce enzyme specific IgE AB as detected by the rat passive cutaneous anaphylaxis test. However, mild subepithelial inflammatory infiltrates of eosinophils along with serocellular deposits (plugs) were seen in the nasal cavity of these animals 24 hours after

the last exposure to enzyme (day 11). The infiltrates and plugs were noted in the front, mid and pos-terior sections of the nasal cavity. Little inflammation and no plugs were observed in the nasal cavity of control animals. The eosinophilic infiltrates were less intense 5 days after the last exposure to enzyme. A 2 to 3-fold increase in blood eosinophils was also noted. Extension of the dosing protocol to exposure on days 1, 3, 10, 17 and 24 with serology on day 29 led to enzyme specific IgE production to 0.3 and 1.6ug Alcalase enzyme. In addition, IgE antibody was found in sera from mice dosed with 0.1 and 0.3ug amylase enzyme (Termamyl). Heat inactivation of these sera led to abrogation of the PCA response, confirming the presence of IgE antibody. These data show that enzyme allergen will generate a mild eosinophilic inflammatory response in the nose along with mild blood eosinophilia after 3 IN exposures and induce enzyme specific IgE antibody after 5 IN exposures to enzyme. The current MINT protocol is sufficient to induce mild allergic inflammation in the nose and a slight modification of the protocol will lead to development of allergen specific IgE antibody in the BDF1 mouse.

1968 STRAIN VARIATION IN THE IgG1 ANTIBODY RESPONSE TO PROTEINS ADMINISTERED INTRANASALLY IN THE MOUSE.

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Proteins, including enzymes, have the potential to behave as respiratory allergens. Consequently guinea pig methods were developed to permit assessment of their respiratory allergenic potential relative to that of an appropriate reference. Recently, a murine model, the mouse intranasal test (MINT) has been proposed as a potential alternative. However, to be of value, the new method should give a rank order of relative potency for a range of proteins which correlates will with that found in guinea pig models and in human experience. Using the mouse strain recommended for the MINT, BDF1, in an extensive intralaboratory assessment the relative potency of several of the 8 proteins used was at variance with that expected from the historic data. Where genetic factors are important, as in the assessment of allergenicity, the rank order for a range of proteins in a particular inbred strain may not reflect that in humans. To examine whether these observations were a strain rather than a species dependent phenomenon, four proprietary enzymes of varying allergenic potency were selected and tested using the MINT protocol in BDF1, Balb/c, CBA/Ca and CB6F1 inbred strains, as well as in the outbred Swiss S strain. Additionally ovalbumin was tested in the BDF1 and Balb/c strains. The results clearly indicated that the relative potency of the proteins (rank order) was dependent on the mouse strain used and thus haplotype. When assessed against the standard reference enzyme, Alcalase (as used in historical models to establish occupational exposure guidelines), results from none of the mouse strains would have led to similar conclusions to those derived from existing models. Based on the presently available information, it is not possible to be certain that any mouse model reliant on the responsiveness of a particular strain (including the MINT) might not lead to an incorrect estimation of respiratory allergenic potency. It is concluded therefore that the MINT requires substantial further investigation if it is to be adopted as a model for the assessment of the relative allergenic potential of proteins.

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