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Preface

The issue of the Toxicologist is devoted to the abstracts of the presentations for the symposium, platform, poster/discussion, and poster sessions of the 30th Annual Meeting of the Society of Toxicology, held at the Loews Anatole Hotel, Dallas, Texas, February 25–March 1, 1991.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), begins on page 357.

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

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Symposia

Tuesday, February 26
8:30 a.m.

Delayed Manifestations of Developmental Neurotoxicity

Chairperson: Bernard Weiss, University of Rochester Medical Center, Rochester, NY and Kenneth R. Ruehl, Rutgers University, New Brunswick, NJ.

1. Introduction, Implications of Long-Latency Neurotoxicity. Bernard Weiss, University of Rochester Medical School, Rochester, NY.

Interference with the complex processes of early brain development may not become apparent until much later in the life cycle. Even latencies extending into old age, after early exposure to neurotoxicants, have been observed. These are crucial issues for toxicology because they represent one of the ways in which developmental neurotoxicity could be expressed. Lead offers one cogent example; dentine levels measured at 7 years of age predicted high school failure and reading disability at age 18. Amyotrophic lateral sclerosis/Parkinsonism-dementia in the western Pacific, associated with cycad seed consumption, may be acquired early in life and remain clinically silent for as long as 45 years. Its chemical triggers may provide clues to other degenerative disorders. Some clues to such long-latency manifestations are offered by animal studies. Mice exposed prenatally to methylmercury and certain pesticides, although overtly normal for most of their lives, begin to show signs of age-associated dysfunction earlier than controls. Anatomical studies disclose that disrupted brain development is one source of such findings, but these are also accompanied by endocrine and immune system disorders. Some of the new techniques of molecular toxicology can help dissect the mechanisms responsible for these apparent delayed toxic consequences, which exemplify the broad impact on social and economic problems that neurotoxic processes may exert.

2. Amyotrophic Lateral Sclerosis and Parkinsonism-Dementia (ALS/P-D) Complex: A Long-Latency Neurodegenerative Disorder Associated with Cycad Exposure. Peter Spencer, Oregon Health Sciences University, Portland, OR.

ALS/P-D is a fatal neuronal disease of the brain and spinal cord that has been highly prevalent in three western Pacific population groups that have used neurotoxic cycad seed kernels (Cycas spp.) for food and/or medicine, and in others who have shared their environment. In some cases, evidence suggests disease is acquired early in life and remains clinically silent for up to 45 years. Disease manifests as ALS, P or D (with aluminum-containing neurofibrillary tangles of the Alzheimer type), or in combination, the manner of expression and age of onset perhaps relating to dose and individual susceptibility. Intensive efforts are underway to understand the chemical triggers of ALS/P-D and the relationship of this prototypical disorder to related diseases elsewhere.

3. Prenatal Neurotoxicant Exposure: Consequences in Advanced Age. Joan M. Cranmer, University of Arkansas for Medical Sciences, Little Rock, AR.

Damage induced by neurotoxicants during early brain development may be functionally invisible during much of the lifespan because of the inherent compensatory capacity of the central nervous system. With advancing age, however, and a loss of reserve capacity, the compensatory buffer erodes, the effects of the early, silent, damage may emerge. Two groups of experiments with mice support this principle. In one, later replicated, methylmercury was administered in various doses during gestation and the offspring maintained until old age. Compared to controls, exposed mice showed signs of aging, such as coordination and cognitive deficits, much earlier. Anatomical studies indicated interference with neuronal migration. Several similar studies with pesticides also support this principle. Also, they show impaired immune and endocrine function as well. The intimate connections between these three systems suggest that their deficits may be linked at the molecular level.

4. Long Term Effects of Early Asymptomatic Lead Exposure. Herbert L. Needleman, University of Pittsburgh, PA.

Lead toxicity is expressed through many mechanisms. Some, such as increased serum ALA-D, may be pharmacologic, and function may improve when the burden is lowered. Others, such as decreased synaptogenesis, may result in fixed deficits. A third outcome, perhaps due to shortened neuronal life span, could be increase or alteration of the nature of effect over time. A sample of 270 asymptomatic children, classified at age 7 by the concentration of lead in shad calcifrus teeth showed lead-related deficits in psychometric intelligence, language and classroom behavior, adjusting for covariates. To determine whether these deficits were lasting, eleven years later, we identified and evaluated 132 of these subjects as young adults. High dentine lead in 1977 was associated with a 7-fold rate of high school failure, and a 6-fold rate of reading disability. Other deficits included lower class standing, more absenteeism in the final year of high school, deficits in vocabulary and grammatical reasoning and fine motor function and reaction time. The implications of these findings for neurotoxicology and child development will be discussed.

Mechanistic Bases of Long-Latency Development Neurorotoxicity. Kenneth R. Ruehl, Rutgers University, New Brunswick, NJ.
Symposia

Transgenic Animals for Mutagenesis and Carcinogenesis Testing

Chairpersons: Louis E. Sendelbach, South Bridge, MA, and Natalie S. Rudolph, Transgenic Sciences Inc., Worcester, MA.

5. Introduction. Louis E. Sendelbach, South Bridge, MA.

This symposium was organized to introduce to SOT an important new genetic technology that is being applied to toxicology testing: transgenic science. This refers to the generation of animals that contain and transmit genes from another species, so that the foreign genes are appropriately regulated in specific tissues at defined developmental stages. The goals of such genetic manipulation may be to alter phenotypes in particular species or strains to improve the efficiency of carcinogen screening, or to provide a specific target for studying mutagenesis in vivo. For example, mice expressing the bacterial lacZ gene as a transgenic target/reporter system may be used for in vitro detection of in vivo mutagenesis, whereas mice carrying selected activated protooncogenes may be highly susceptible to chemically induced tumor formation in certain tissues at specific ages. At the same time, it is important to include strain-specific properties and other factors that have become standardized in large-scale screening studies. In general, these transgenic mice react to certain genotoxic or carcinogenic insults with a limited set of specific, well-characterized responses that are readily quantified. This should shorten many acute and chronic exposure studies, and could provide models for studying mechanisms of mutagenesis, carcinogenesis and chemoprevention. Ultimately, this technology may reduce the total number of animals required for such assays. The four speakers in this symposium will discuss general and specific aspects of transgenic models being designed for toxicology research and testing. The objectives are to summarize the current state of the art, and to stimulate thought and discussion among toxicologists working in different areas as to how transgenic technologies may be applied to their own testing needs and to the development of new mechanistic models.


Two methods for the genetic manipulation in mice, and several applications, will be discussed. Transgenic mice are produced by microinjection of DNA into the fertilized egg. By this method, virtually any gene can be introduced into the mouse genome, although not into specific genomic loci. The expression of the transgene is governed primarily by regulatory sequences included on the introduced DNA, and can result in a wide variety of phenotypic alterations. Examples will include a mutine model for sickle cell disease, and a mouse strain susceptible to human poliomyelitis.

A complementary method, which allows the specific modification of endogenous mouse genes, involves the use of embryonic stem (ES) cells. These are pluripotent embryonic cells which can be manipulated and then allowed to colonize somatic and germ cell lineages in a host embryo. "Gene targeting" techniques, permitting the specific mutagenesis of endogenous genes in ES cells, will be described. Mutant ES cells can be used to produce chimera mice, and the phenotypic effects of the mutation can thus be characterized. The use of this approach to study the developmental role of a transcription regulatory factor, involved in hemopoietic cell differentiation, will be discussed.

7. Pathology and Toxicology Associated with Chemical Carcinogenesis Treatment of Transgenic Mice Carrying Oncogenes. Ray Tennant, NIEHS, Research Triangle Park, NC.

The development of mouse strains carrying stable germ-line insertions of cloned, transcriptionally altered oncogenes provides a unique target for chemical effects. Three transgenic strains developed by Leder and collaborators (Stewart, et al., Cell 38:627, 1984; Muller, et al., Cell 54:105, 1988) with the onc genes, ras, myc, and neu driven by a mouse mammary tumor virus (MMTV) promoter have been used in chemical exposure studies. 1-Gresidine (2-methoxy-5-methyl-2-benzeneamine) was used because it is a mutagenic carcinogen that induced bladder neoplasms in a bioassay in B6C3F1 mice. Since each of the transgenic mouse strains develop specific patterns of spontaneous neoplasms, related to regulation of the MMTV LTR, it is important to determine the pattern of response at other tissue sites and to relate it to the patterns of tumors induced in the B6C3F1 strain. Reserpine, an alkaloid, induced mammary tumors and seminal vesicle carcinomas in the B6C3F1 strain. It was selected for assay in the transgenic strains because it was nonmutagenic and induced tumors at a site related to expression of the transgenes (mammary gland). The goals of these studies are to determine the relative sensitivity of the transgenic strains based upon the dose of the chemical, time to tumor induction and the frequency and patterns of induced tumors.


The identification of suspect mutagens is essential for regulating exposure to harmful agents. However, the ability to confirm or extend the findings of in vitro short-term mutagenesis assays with comparable in vivo tests is limited. The majority of in vivo assays capable of detecting point mutations are costly, time-consuming, require large numbers of animals, and are not extremely sensitive or easy to interpret. The use of shuttle vectors, which permit transfer of DNA interchangeably between prokaryote and eukaryote, has the potential to overcome many of the disadvantages of existing in vivo tests. This is made pos-
sible by the ability to monitor reporter genes, such as those controlling the expression of β-galactosidase, in which the presence of a mutation can be easily detected after transfer via the shuttle vector to a prokaryotic system. In addition to estimating the induced frequency of mutation, this technique allows direct correlation of the nucleic acid alteration to a particular mutagenic event. Mutations can be evaluated in both somatic and germ cells which will allow determination of tissue specific mutagenesis.


The willful introduction and stable integration of exogenous or foreign genes into a variety of animal species will allow the creation of transgenic systems with enormous potential and resolving power in basic and applied research. Construction and utilization of transgenic mice with the proper genetic backgrounds containing defined genetic targets to assay for the specific molecular mechanisms of action of carcinogens including mutagens, recombinogens, and deletionogens will be discussed. Such strategies must surely include the use of DNA restriction enzyme specificities, DNA probes, nucleotide sequence analysis, and the polymerase chain reaction when analyzing the molecular effects of genotoxics on both activation of specific protooncogenes and the inactivation of individual tumor suppressor genes responsible for the induction of neoplasias and metastases. Careful planning in the creation of critical strains of transgenic mice with the correct combinations of human oncogenes in the appropriate genetic background will result in the strategic genomic tailoring of such animals.

Tuesday, February 26
1:30 p.m.

Assessment of Exposure to Pulmonary Toxicants: Use of Biological Markers

Chairpersons: James A. Bond, CIIT, Research Triangle Park, NC and Rogene F. Henderson, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

211. Overview and Applications of Biological Markers of Exposure to Pulmonary Toxicology. James A. Bond, CIIT, Research Triangle Park, NC.

Biological markers can serve as tools that can provide quantitative estimates of the causal linkages between exposure, internal dose, dose to critical sites, early alterations, and late-occurring disease. A key component of understanding the potential risk of toxicants in humans is knowledge of the dosimetry of the material in question. Biological markers of exposure can be useful in helping to quantitatively relate external exposure to internal dose. The ideal biological marker of exposure should be chemical specific, detectable in traces quantities, available by non-invasive techniques, inexpensive to assay, vary consistently and quantitatively with the extent to exposure, and integrate the exposure over time.

The concept of biomarkers is not new nor unique to toxicology. For example, industrial hygienists have employed the concept of biomarkers of exposure in their analysis of urine, expired air, hair, and serum for evidence of toxicant chemical exposure. Clinicians for years have relied on clinical signs to aid in their diagnosis and therapeutic regimens. The challenges and opportunities for toxicologists in the future are to identify useful biological markers of exposure and validate exposure-dose relationships in studies of large human populations.

212. Exhaled Breath as a Measure of Exposure to Volatile Organic Compounds. Lance A. Wallace, EPA, Warrenton, VA.

EPA's TEAM study has measured about 25 VOCs in exhaled breath of 800 persons representing about 800,000 U.S. residents. Concurrent airborne exposures were also measured, allowing a relationship between exposure and body burden to be established. A simple multi-compartmental pharmacokinetic model has been developed and tested in chamber studies and in the field, establishing typical residence times in the body for about 20 VOCs. The breath sampling methods, the mathematical model, and the results of chamber and field tests involving exposure in 32 common microenvironments (hardware stores, beauty shops, etc.) will be presented.


As a basis of risk estimation and for setting occupational standards it is important to know the relation between exposure dose and dose in target tissues of toxic chemicals or, if metabolic activation is required, of their active metabolite(s). The quantification of products (adducts) of chemical reactions between electrophilically reactive chemicals/metabolites and hemoglobin or serum albumin can be used for determining individual blood doses of such agents. The relation between target-tissue dose and blood dose has to be inferred from animal experiments. Current human studies at our laboratory concern cigarette smoking, occupational exposures to ethylene oxide, propylene oxide, ethane, butadiene and styrene and a characterization of the background load of low-molecular weight adducts, particularly from ethane and certain aldehydes. For these analyses the N-alkyl Edman method is sufficiently sensitive. Data on hydroxylethyl adduct levels (originating from ethane/ethylene oxide) in smokers and in ethylene oxide-exposed workers are compatible with a linear relationship between blood dose and exposure. Also corresponding data on propylene oxide agree with linearity.

214. DNA Adducts as Molecular Dosimeters. George W. Lucier, NIEHS, Research Triangle Park, NC.

There is compelling evidence that DNA adducts play an important role in the actions of many pulmonary carcinogens. During the last ten years sensitive methods (antibodies and $^{32}$P-postlabeling) have been developed that permit detection of DNA adducts in tissues of animals or humans exposed to low levels of some genotoxic carcinogens. This capability has led to approaches designed to more reliably estimate the shape of the dose-response curve in the low dose region for a few carcinogens. Moreover, dosimetry comparisons can, in some cases, be made between animals and humans which help in judging the adequacy of animal models for human risk assessments. There are several points that need to be considered in the evaluation of DNA adducts as a molecular dosimeter. For example, DNA adduct formation is only one of many events that are needed for tumor development and some potent carcinogens do not form DNA adducts; i.e., TCDD. Other issues that need to be considered are DNA adduct heterogeneity, DNA repair, relationship of DNA adducts to somatic mutation and cell specificity in DNA adduct formation and persistence. Molecular epidemiology studies often require quantitation of adducts in cells such as lym-
phocytes which may or may not be reliable surrogates for adduct concentrations in target tissues. In summary, accurate quantification of low levels of DNA adducts may provide data useful in species to species extrapolation of risk including the development of more meaningful human monitoring programs.

Urinary Naphthalene Mercapturates: Biological Monitors for the Stereochemistry of Aromatic Hydrocarbon Epoxidation In Vivo.


The epoxidation of aromatic hydrocarbons by cytochrome P450 monooxygenase isozymes frequently occurs with a remarkable degree of stereoselectivity. The chiral epoxides resulting from this and further hydrolytic/oxygenation reactions often differ substantially in their toxicity. Therefore, techniques capable of monitoring the formation of chiral epoxides in vivo could provide useful measures of exposure of target tissues/cells to metabolites closely associated with toxicity and also may be useful in examining inter-individual differences in P450 isozyme composition.

Naphthalene produces highly selective injury to Clara cells of the mouse but not rat or hamster and to nasal olfactory epithelium in all three species. The cell-selective toxicity of the compound correlates with the stereoselective formation of 1R,2S-naphthalene oxide. Mercapturates are the primary urinary metabolites of GSH conjugates formed at the allylic carbon of both chiral epoxides (but not at the benzylic carbon of the 1S,2R epoxide). Enantioselective metabolism by epoxide hydrolase or GSH transferases does not appear to be the primary determinant in the ratio of diastereomeric mercapturates excreted in the urine. The ratio of mercapturates derived from the 1R,2S vs. 1S,2R epoxide in mice exposed to naphthalene by inhalation varied from 5:1 to 10:1, depending upon the exposure level. Similar studies in rats showed the ratios to be less than 1:1. Under appropriate conditions, naphthalene diastereomeric mercapturates may be useful in monitoring the chirality of naphthalene epoxide formation in the lung.

216. Strategies for the Use of Biological Markers of Exposure, Rogene F. Henderson, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

The relationship between breathing polluted air and the subsequent development of adverse health effects is a major risk assessment problem. Part of the problem is in being able to assess the exposure of individuals to specific pollutants. A recent approach has been to find biological markers indicative of exposure. Some markers, such as the presence of the parent compound or its metabolites in exhaled breath, are markers of recent exposure. Other markers, such as those in blood proteins, have a longer time period. Some markers, such as urinary metabolites, may represent a large fraction of the internal dose received by the individual and can serve as sensitive indicators of the continuous exposure to low levels of chemicals near a waste site. Other markers, such as DNA adducts, will contain only a minute fraction of the internal dose, but indicate dose to a sensitive site. Determination of the rate of formation and breakdown of the various types of markers will allow development of mathematical models that can predict steady state levels of the markers under exposure conditions. Thus, each type of marker can be useful, and a battery of markers will be more useful than any single marker in defining past exposures.

Mouse Liver Carcinogenesis: Mechanisms and Relevance

Chairperson: Jay I. Goodman, Michigan State University, East Lansing, MI.

Introduction. Jay I. Goodman, Michigan State University, East Lansing, MI.

The overall objective behind this symposium is to provide a forum for the presentation of current concepts in carcinogenesis. The liver of the B6C3F1 Mouse is uniquely prone towards development of both spontaneous and chemically-induced hepatomas. Therefore, it provides a good model system which is being employed successfully to discern mechanisms underlying the multistep/multistage process by which a normal cell is transformed into a cancer cell and progresses to a frank malignancy. This research enhances our understanding of fundamental aspects of biology. In addition, the practical significance here is that this inquiry is providing the type of information which is required in order to take a rational approach toward assessment of the carcinogenic potential which a chemical might pose. The presentations which comprise this symposium will illustrate how some of the most important questions faced in toxicology (e.g., high dose to low dose extrapolations, species to species extrapolations, the existence of thresholds, etc.) are being addressed in a rational manner through a mechanism of action based approach. Indeed, the linking of the "overall objective" and "practical significance," as noted above, will be the central theme of this symposium.
217. **Mouse Liver Carcinogenesis: Initiation/Promotion/Progression.** Jerrold M. Ward, National Cancer Institute, Frederick, MD.

The mouse liver is an important target organ of genotoxic and nongenotoxic chemical carcinogens. It can serve as an important model for studying mechanisms of carcinogenesis by these agents. The mouse hepatocellular carcinoma develops in a well-recognized sequence from initiated hepatocytes, hepatocellular foci and adenomas. Carcinogenesis can be initiated by a single exposure to a genotoxic initiating agent. Nongenotoxic tumor promoting agents can hasten the carcinogenic process by targeting initiated cells, cells in foci or adenomas. A unique malignant tumor of mouse liver, the hepatoblastoma, can be induced and promoted by specific chemical regimens with genetic factors playing a role. The mechanisms of action of promoting agents may involve direct or indirect mitogenesis, chronic toxicity, enzyme induction and other unknown effects. Nongenotoxic agents can also promote spontaneous hepatocarcinogenesis by targeting spontaneously-initiated hepatocytes, foci or adenomas. Progression of adenomas into carcinomas may be encouraged by genotoxic agents or endogenous factors. Oncogene activation, other genetic events, mitogenesis and chronic toxicity may all play various roles in hepatocarcinogenesis of mouse liver.

218. **The Role of Cytotoxicity and Cell Proliferation in Mouse Liver Carcinogenesis.** James A. Popp, ClIT, Research Triangle Park, NC.

Despite the frequent occurrence of mouse liver tumors in bioassays, the relevance of this endpoint for assessing human cancer risk is unknown. In an attempt to explain the mechanism of mouse liver tumor induction, chemically-enhanced cell proliferation secondary to hepatocytosis has been invoked as a potential causative factor. However, the level of discussion on the relationship of cytotoxicity, secondary enhancement of cell proliferation and the induction of mouse liver tumors has surpassed the actual experimental data available. Additional understanding of the links between cell proliferation and tumor formation is becoming available through the development of experimental data and the use of the 2-stage cell growth and mutation model of Mooiogavk and co-workers. Additional research is also beginning to distinguish the association of enhanced hepatocyte replication and liver tumors in mice secondary to cytotoxic induction of cell proliferation from that induced by direct mitogenic chemicals. The lack of this distinction in the past has clouded discussions of the role of cytotoxicity in tumor induction. New information on the mechanistic linkage between chemically-induced cytotoxicity and an enhancement of hepatocyte proliferation may allow the use of both of these factors in evaluating the relevance of mouse liver tumors for assessing risk.

219. **The Role of Cell Proliferation and Altered Gap Junction Expression in Mouse Liver Carcinogenesis.** James E. Klaunig, ClIT, Research Triangle Park, NC.

The B6C3F1 mouse is particularly sensitive to the hepatocarcinogenic effects of a number of chemicals that fail to induce liver cancer in other species. Many of these compounds do not appear to directly damage cellular DNA and may function through "nongenotoxic" mechanisms. Why this mouse strain is highly sensitive to these hepatocarcinogens remains unresolved. One possible explanation may be that "nongenotoxic" carcinogens allow the selective expansion of spontaneously initiated neoplastic cells already present in the B6C3F1 mouse liver. Our group has examined the effects of several "nongenotoxic" carcinogens on two biological endpoints: induction of cell proliferation and modification of membrane proteins (specifically the gap junction protein) in neoplastic murine liver loci. Hepatocytes in foci, in contrast to the normal surrounding hepatocytes, display a selective sustained increase in cell proliferation during carcinogen treatment. Similarly, the focal lesions exhibited a decreased expression of gap junction protein from that observed in the normal liver. These observations support the possibility that "nongenotoxic" murine hepatocarcinogens may selectively target the initiated cells, thus allowing the clonal expansion of a neoplastic hepatocyte population.

220. **The Role of H-ras Proto-Oncogene Activation in Chemically Induced Mouse Liver Carcinogenesis.** Tony R. Fox, Dow Chemical Company, Midland, MI.

The male B6C3F1 mouse has a high spontaneous liver tumor incidence (approx. 30%). Increases in this tumor incidence are frequently observed in many bioassays after exposure to a variety of chemical agents. The relevance of this increase to potential human risk is presently a controversial issue and one which complicates the interpretation of the bioassay results. A mechanistic understanding of how the spontaneous tumors arise and how certain classes of chemicals cause an increase in this tumor incidence will play an essential role in resolving this problem. Recently several laboratories have studied the role that cellular oncogenes play in the development of these tumors. Results from these efforts have revealed that the H-ras oncogene is activated in a large percentage of spontaneous tumors and tumors induced with many genotoxic agents but not in tumors induced with several nongenotoxic hepatocarcinogens. The significance of these results within the context of interpreting the bioassay results will be presented. In addition, the utility of using mutational analysis of the H-ras oncogene in B6C3F1 mouse liver tumors as an indicator of in vivo genotoxic activity will be discussed.
Hypomethylation of Proto-Oncogenes: A Possible Mechanism Involved in the Promotion Stage of Carcinogenesis. Jay I. Goodman, Michigan State University, East Lansing, MI.

The B6C3F1 mouse exhibits a high propensity towards development of both spontaneous and chemically-induced hepatomas. Therefore, it provides an attractive model system in which to investigate potential mechanisms involved in carcinogenesis. Our research addresses the issue of aberrant expression of proto-oncogenes associated with carcinogenesis. While it is clear that mutation appears to play a role in the transformation process, it is axiomatic that (with the possible exception of tumor suppressor genes) the mutated gene must be expressed in order to affect phenotype. The hypothesis which underlies our research is: decreased DNA 5-methylcytosine content of proto-oncogenes (i.e., hypomethylation) is a mechanism involved in tumor promotion. The methylation state of a gene is one of the mechanisms involved in the regulation of gene expression. Hypomethylation is necessary, but not sufficient, for transcription. Indeed, a hypomethylated gene can be considered to possess an increased potential for expression as compared to a hypermethylated gene. During hyperplasia the pattern of DNA methylation is maintained by the activity of DNA maintenance methylase. Therefore, during periods of sustained cell proliferation, inhibition of maintenance methylation can result in hypomethylation. This is a heritable epigenetic event which may be brought about by threshold mechanisms. The presentation will focus on a consideration of hypomethylation as a possible mechanism involved in tumor promotion and the role it might play as a determinant of the high susceptibility of the B6C3F1 mouse to development of hepatomas. Emphasis will be placed on testing the working hypothesis within the context of the need to take a more rational approach towards evaluation of carcinogen bioassay data.
Symposia

Wednesday, February 27
8:30 a.m.

Risk Assessment and Immunotoxicology

Chairpersons: Michael I. Luster, NIEHS, Research Triangle Park, NC, and Edwin V. Buehler, Hill Top Biolabs Inc., Cincinnati, OH.


Although immunotoxicology, as a specialty in toxicology, has been recognized for a short period of time, immunotoxicologists have utilized well established models to evaluate xenobiotics for their potential to produce hypersensitivity responses or immunosuppression. These studies have provided a large data base of potential immunotoxic chemicals, information on their mechanisms and evidence that the immune system may be very sensitive to chemical injury. The first two speakers will focus on immunosuppression produced by xenobiotics. While sensitive models exist for quantitating immunosuppression, as of yet animal data have not been used to a significant extent in human risk assessment. This is probably due to the uncertainties of extrapolating high-dose animal studies to potential low-dose human exposure, as well as relating the significance of altered immune function tests to clinical outcome (i.e., neoplastic or infectious disease). The last two speakers will discuss hypersensitivity responses to chemicals using beryllium, proteins and low molecular weight compounds as examples. Discussions will focus on the development of specific animal models for studying these responses. Comparative human data will be provided. All speakers will also address the following questions: 1) Do immunotoxicology data lend themselves to risk estimation or quantification? 2) Which types of immunotoxicology data are particularly suited to risk estimation/assessment activities? 3) Can qualitative comparisons be made across species? If so, can quantitative comparisons also be made? 4) Can immunotoxic effects be readily interpreted in terms of human disease or impairment?

395. Biological Considerations Derived for Animal Studies from Risk Quantification in Immunotoxicology. Michael I. Luster, NIEHS, Research Triangle Park, NC.

One of the objectives of the National Toxicology Program is to provide data on the potential adverse effects of chemicals and therapeutics on the immune system. Due to the complexity of the immune system, a tier screening approach has been routinely used to assess the potential of compounds to produce immunosuppression. This testing configuration includes assays that measure immunopathological changes (e.g., lymphoid organ weight, and leukocyte cell numbers), alterations in immune functions, and decreases in resistance following challenge with infectious agents or transplanted tumor cells. A large data base consisting of over 50 compounds has been collected from these studies and statistically analyzed for various parameters. Among these include: determining whether the various tests follow linear or threshold responses; establishing the relationship between the pathologic, immunologic and host resistance assays, particularly at the low end of the dose response curves; providing quantitative values for specificity, sensitivity and concordance for each of the tests, individually as well as in combination and; examining the potential relationships between immunosuppression and mutagenic, reproductive and carcinogenic effects for the chemicals studied. Such information should be useful for developing appropriate risk assessment models for immunotoxicological evaluation.

396. Interspecies and Dose-Response Considerations for Extrapolation of Immunotoxicologic Data. Gerry M. Henningsen, NIOSH, Cincinnati, OH.

The proper acquisition and application of immunotoxicology data for risk assessments requires an understanding of basic principles in immunology, toxicology, and the risk assessment process. Extrapolation of immunotoxicologic effects should be based upon considerations of unique dose-responsiveness of the immune system as well as interspecies differences in immune structures and functions. This is a timely subject because of rapidly expanding knowledge of immunotoxicity in animals and the growth in capabilities to construct mathematical models for predicting and regulating similar types of effects in humans. The aims of this talk are to: 1) present an overview of important definitions and principles for immunotoxicologic risk assessment, 2) provide meaningful considerations in comparative immunotoxicology with examples of studies and data to demonstrate salient points, and 3) conclude with some unifying concepts and discuss future directions for risk assessment of immunotoxicity data.

Immunologic dose-responses and terminology such as hazard, risk, and exposure assessment will be initially explained. Comparative medical and toxicological aspects of immune responses that could be critical for accurate extrapolation will be described next, along with examples of differing interspecies responses (including human) to immunotoxicants such as cyclosporin, glycol ethers, and TCDD. Finally, current and future uses of immunotoxicity data in risk assessment models, especially within proposed paradigms, will be summarized.
397. **Risk Assessment and Respiratory Allergy: Immediate Hypersensitivity to Proteins and Low Molecular Weight Chemicals (LMWC).** Katherine Sarlo, The Procter & Gamble Company, Cincinnati, OH.

We have developed models for evaluating the respiratory allergenicity of proteins and LMWC. Information gained from these models is used as part of an overall process for setting safe exposure guidelines. A guinea pig intratracheal (IT) test has been used to evaluate the allergenicity of detergent enzymes and a tier approach incorporating a guinea pig inhalation model has been used to evaluate the allergenicity of LMWC. Human experience with Alcalase allowed us to set a dust level well within the guidelines that would result in no or minimal sensitization. In the IT test, new enzymes are compared to the benchmark enzyme Alcalase in their ability to induce allergic antibody (Ab). The test demonstrates if a new enzyme is more, less or equal to Alcalase as a respiratory allergien and the data are used to help set exposure levels in the workplace. Comparisons of new enzymes to Alcalase in the IT model will be presented along with corroborating human data. Phthalic anhydride (PA), a LMWC occupational allergen, was tested in a tier approach and shown to be immunogenic. Following injection into guinea pigs and allergenic in our inhalation model at levels as low as 0.5 mg/m³ dust. These dust levels are comparable to exposure levels of humans sensitized to PA. For humans and guinea pigs, the presence of allergic Ab correlates with exposure. These data suggest that the guinea pig is a good model for evaluating proteins and LMWC as respiratory allergens.

398. **Cell-Mediated Immunity in Chronic Beryllium Disease: Implications of Animal and Human Data for Risk Assessment.** Lee S. Newman, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO.

Chronic beryllium disease is a granulomatous process which is closely associated with the development of a T cell immune response directed to beryllium. Both animal and human data suggest a central role for a beryllium specific T cell mediated immune response in the pathogenesis of this disease process. As such, it allows us to examine issues in risk assessment in a hypersensitivity condition in which cell-mediated immunity is critically involved. Recent human studies have shown that individuals exposed to beryllium may develop: a) no immunological response, b) beryllium specific T cells in blood but without lung disease, or c) beryllium specific T cells in both blood and bronchoalveolar lavage (BAL) accompanied by granuloma formation in the lungs. Recent animal studies, using both rat and dog models, have confirmed findings in both clinical and epidemiologic studies regarding the significance of cellular immunity to beryllium in disease. Interestingly, the response to this antigen (or hapten-protein complex) is not directly related to dose of exposure, in fact, some individuals with “trivial” exposure develop beryllium sensitization and disease. The implications of these findings for risk assessment will be discussed.

**Molecular Approaches to Understanding Retinoid Teratogenicity**

**Chairperson:** Arthur A. Levin, Hoffmann-La Roche, Inc., Nutley, NJ.

399. **Introduction.** Joseph F. Grippa, Hoffmann-La Roche, Nutley, NJ.

Recent advancements in molecular and developmental biology have led to the identification of critical molecular events in embryonic development. This information is providing a framework for understanding abnormal development at the molecular level. Normal embryonic development is dependent on a delicate balance of spatially and temporally regulated gene expression. Retinoids control processes of normal development and can alter the expression of a family of developmentally significant genes: the homeobox genes. Recently discovered nuclear receptors for retinoic acid are now known to modulate gene expression through ligand-dependent transcriptional regulation. This symposium will explore the hypothesis that retinoid-induced teratogenesis results from the altered expression of developmentally-important genes in the embryo and will identify approaches to define cellular and molecular mechanisms of retinoid-induced teratogenesis. The speakers in this symposium will focus on: 1) cellular and molecular events occurring during teratogenesis, 2) the biochemistry and molecular biology of the nuclear retinoic acid receptors, 3) retinoid-responsive gene expression in embryos and embryocarcinoma cell lines, 4) the role of retinoids as mediators of normal development, and 5) the use of transgenic mice as a model system to study retinoid-responsive genes in teratogenesis.

400. **Experimental Retinoid Teratogenesis: Selective Vulnerability of Regions of Physiological Cell Death to Retinoid Insect.** Ajit J. Alix and Kathleen K. Sulik, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Physiological cell death is an important component of normal embryonic development. Probably the best known example is the shaping of the digits by cell death in the interdigital tissues. In addition, PCD helps shape the nervous system, face, urogenital system and gut, among others. Both supravital staining with Nile blue sulphate and routine histological techniques show that retinoids cause an increased amount of cell death in sites of PCD. For example, retinoids administered on gestational day 8...
(GD8, GD0 being the day of mating) to pregnant C57Bl/6J mice, cause spina bifida in the exposed fetuses. Twelve hours after exposure, treated embryos have excessive cell death in the tailbud, just ventral to the posterior neuropore. In contrast, control embryos have PCD at this site. We postulate that this excessive death causes a growth disparity between the ventral and dorsal regions of the tailbud, preventing the closure of the neuropore and causing spina bifida. Another example is the induction of limb-reduction defects. Retinoids administered on GD11 to pregnant C57Bl/6J mice cause misshapen and shortened long bones in the resulting progeny at term. Twelve hours after exposure, there is an increased amount of cell death in the core of the limb, a site that contains PCD in control animals. We have proposed that this interferes with the condensation and shaping of the precartilaginous mesenchyme and causes defects in long bone formation. Experiments in our laboratory have shown that the induced cell death has similar characteristics to PCD. We have localized retinoic acid to sites of PCD. We are presently studying the mechanisms that mediate the vulnerability of sites of PCD. On the basis of our experiments, we propose that the selective vulnerability of sites of physiological cell death is a common denominator that underlies retinoid teratogenesis.


The biologic effects of retinoids are mediated by nuclear receptors for retinoic acid (RARs) that belong to the superfamily of steroid hormone receptors. Like the steroid hormone receptors, binding of retinoids to the RARs results in a ligand-receptor complex which binds to DNA and acts as a transcription factor thus modulating gene expression. Three receptor subtypes: alpha, beta, and gamma have been described for retinoic acid (RA) each with a distinct pattern of expression in adult and embryonic tissues. The purpose of these studies is to relate receptor binding affinities and receptor specificity with teratogenic potential. Binding of RA to RARs is high affinity, specific and saturable. Highly teratogenic retinoids displaced 3H-RA bound to RARs with EC50s similar to those for RA. Although the specific receptor subtype responsible for the teratogenic effects of retinoids has not been described, the alpha and beta receptors have been implicated. One compound RO-40-6055, a very potent teragen, showed a markedly greater affinity for RAR alpha compared to beta or gamma, and a retinoid that shows specificity for the beta receptor is also highly teratogenic. The role of the gamma receptor in teratogenesis and the teratogenic potential of gamma specific ligands remains to be elucidated. The data obtained from this assay system may allow us to relate the toxicologic or pharmacologic activities of these compounds with binding to specific nuclear receptors.


In support of our hypothesis that high doses of retinoids may induce teratogenic events by causing the aberrant expression of certain developmentally-important genes including homeobox genes, we have begun to examine the retinoid-induced changes in expression of homeobox genes in embryocarcinoma cell (EC) lines. EC lines differentiate along particular pathways in response to retinoic acid (RA). Thus when F9 cells are treated with retinoic acid, large increases in the accumulation of mRNA species for the basement membrane proteins Laminin and Collagen type IV is seen as these cells become parietal endoderm. Additionally, RA dramatically induces the expression of a number of the homeobox genes, including Hox 1.3 and Hox 1.6. Curiously, different retinoids appear to have distinct effects on gene expression in the F9 cells. For example, the highly teratogenic RO-40-6055, activates the expression of Hox 1.3 and Hox 1.6 to a level five to ten times higher than that achieved by RA. RO-40-6055 is, in fact, considered to be more teratogenic than RA. However, RA is more potent than RO-40-6055 in activating the expression of other genes, including Laminin and Collagen type IV, suggesting that the activation of select genes pathways, perhaps those of the homeobox genes, by retinoids may determine the teratogenic potential of a series of retinoids. These observations provide a framework for a model system utilizing F9 cells that would indicate whether various synthetic retinoids alter the expression of genes, like the homeobox genes, that may control both normal and abnormal developmental events.

403. Abstract for Molecular Mechanisms of Retinoid Induced Teratogenesis. Olof Sundin, Harvard Medical School, Boston, MA.

The chick embryo is highly accessible to experimental manipulation and has yielded key insights into the nature of retinoid induced teratogenesis. Localized application of retinoic acid to the anterior margin of the limb bud has been shown to induce the formation of additional digits and to orient the anteroposterior axis of these digits. Of special interest is the fact that this applied retinoic acid mimics the function of the ZPA, an embryonic organizer tissue which governs the anteroposterior polarity of the limb. In addition, during normal development of the limb endogenous retinoic acid is found at higher concentrations in the region of ZPA organizer tissue. This further suggests that retinoids play an essential role as signalling molecules during the process of pattern formation. More recently, it has been shown that when frogs or chick embryos are treated with retinoic acid during the late gastrula stage, they later exhibit a striking absence of anterior structures, including most of the head. Interestingly, the tissues fated to become forebrain, retina and midbrain are not destroyed by retinoic acid, but are instead re-programmed to develop as more posterior neural structures. This reveals elements of similarity to the limb system, where retinoic acid can also respectively...
the developmental fate of tissues, inducing an anterior tissue to assume a more posterior fate. In order to observe this respecification process in precursors of the central nervous system, we are using immunohistochemistry to detect a protein encoded by the chick homeobox gene Ghox-lab. The protein is expressed at high levels within a well-defined domain which covers the posterior two-thirds of the gastrula stage embryo. Shortly after retinoic acid treatment this domain of Ghox-lab expression shifts to a more anterior position, a result consistent with the respecification of anterior tissue. In addition, the sensitive period during which retinoic acid can effect this gene induction appears to coincide with the sensitive period for "lost head" teratogenesis. Homeobox genes such as Ghox-lab are the vertebrate counterparts of a class of genes known to regulate pattern formation in insects. It is tempting to speculate that the induction of Ghox-lab expression contributes to the teratogenic effects of retinoic acid in the early embryo.

404. Retinoic Acid, Homeobox Genes and the Definition of the Anteroposterior Axis in Mice. Michael Kessel and P. Gruss, Max-Planck Institute of Biophysical Chemistry, Gottingen, FRG.

We have generated murine gain of function mutants by introduction of a ubiquitously expressed homeobox transgene into the mouse genome. The Hox-1.1 transgene interferes with cranial neural crest cells and with the somitic mesoderm at the craniocervical transition. The pathology of resulting craniofacial and vertebral abnormalities will be discussed with regard to a proposed developmental control function of Hox-1.1. Exposure of murine embryos to high doses of RA leads to severe malformation, some resembling the phenotype observed in the transgenic animals. We describe the generation of homeotic transformations along the complete vertebral column after RA exposure at different embryonic stages. A developmental model will be presented postulating the definition of the anteroposterior body axis by a "Hox code", i.e., the combination of Hox gene products specifying a body segment. The morphogen RA seems to be involved in the initial specification of this code during gastrulation. We demonstrate also the capacity of retinoic acid to respecify the Hox code in a later phase. The Hox code appears not to be limited to the vertebral column but seems to be applicable to the neuroectoderm and to the limb axes as well.
Assessment of Reproductive Potential in the Non-Pregnant Female

Chairpersons: Ralph L. Cooper, EPA, Research Triangle Park, NC, and Betsy Carlton, Rhone Poulenc, Research Triangle Park, NC.

627. Introduction. Ralph L. Cooper, EPA, Research Triangle Park, NC, and Betsy Carlton, Rhone Poulenc, Research Triangle Park, NC.

Typical assessment of reproductive toxicity is based on paradigms that incorporate long-term exposure and rely primarily on measures of fertility as the main quantifiable endpoints. This approach does not always identify the sex affected and rarely can the target organ(s) and mechanism(s) of the compound's action be determined. Thus, development of alternative techniques to identify the target organ and mechanism associated with the loss of reproductive function following chemical exposure would greatly enhance the risk assessment process. In this regard, a great deal of recent research has focused on the effects of xenobiotics on sperm and other testicular endpoints. In contrast, there have been few systematic studies of the effect of toxicants on the reproductive system of the non-pregnant female. This is surprising in light of the fact that effects on reproductive success can be readily identified apart from any effects on pregnancy itself. Such studies could provide more sensitive measures of a toxicant's action and insight into the physiological mechanisms involved. This symposium will focus on methods to identify the effects of toxicants on the non-pregnant female, including the strengths and weaknesses of various procedures for identifying normal and aberrant ovarian function in rodents and humans, as well as the effect of toxicants on the processes of folliculogenesis, ovulation, oocyte maturation and fertilization.

628. Monitoring Ovarian Cycles for Assessing Reproductive Toxicity: Comparison of Human & Rodent Cycles. Claude L. Hughes, Jr., Duke University Medical Center, Durham, NC.

Since female reproduction is life-cycle dependent, reproductive toxicants that reversibly compromise adult ovarian function can decrease lifetime fertility. This adverse outcome would result from reduction in the number of normal ovulatory cycles by blockade of ovulation or increased occurrence of abnormal cycles with abnormal fecundity. While ovulation per se and fertilizability of released ova can be directly assessed in rodents, similar data for humans can only be derived from constrained clinical circumstances such as in vitro fertilization in infertile couples. Thus in the general case, assessment of ovulation in humans depends upon monitoring to detect and define normally of cycles. Characterization of rodent or human ovarian cycles as normal or "dysfunctional" depends upon direct and indirect measures of hormonal patterns and functional morphology of the ovary. Definitive assessment is approximated by a "cycle profile" in which the monitoring protocol incorporates the essential attributes of within-cycle correlation among parameters and assessment of all three portions (follicular phase, peri-ovulatory interval and luteal phase) of the ovarian cycle. Since ovarian cycle phenomena are comparable in rodents and humans, expectations for adverse effects on ovulation can be anticipated if aberrant parameters of monitored cycles are detected.

629. The Effect of Toxicants on the Neuroendocrine Control of Ovulation. Ralph L. Cooper, EPA, Research Triangle Park, NC and Jerome M. Goldman, NSI Technology Services, Research Triangle Park, NC.

The purpose of this presentation will be to examine the endocrine events that serve as triggers for the process of ovulation and the potential consequences of disrupting this complex regulation by short-term exposure to xenobiotics. The key endocrine event responsible for the release of the oocyte from the preovulatory follicle is the mid-cycle surge of luteinizing hormone (LH). The LH surge occurs in response to ovarian hormonal feedback in concert with appropriately timed signals from within the brain. Thus, hormonal events inducing ovulation require the normal interaction of a number of organs comprising the female's reproductive system. As a result, the LH surge is subject to disruption by toxicant action at several different levels of the brain-pituitary-ovarian axis. Studies will be described evaluating changes in the appearance of the LH surge in females treated with pesticides known to affect brain activity. The consequence of delaying expression of the LH surge on the oocyte, pregnancy maintenance and fertility will be discussed. In addition, the effect on LH release of acute vs. subchronic exposure to certain compounds will be considered within the context of the development of tolerance to the compound, periods of increased sensitivity during the ovarian cycle and current protocols used to assess the female reproductive toxicants.

630. Folliculogenesis and Ovarian Resilience. Anne N. Hirshfield, University of Maryland, Baltimore, MD.

Folliculogenesis is a lengthy process that transforms primordial follicles into large, preovulatory follicles. Close regulation of this process results in the production of a species-specific, highly consistent number of follicles which ripen each reproductive cycle at precisely the appropriate time for ovulation. This presentation will describe features of folliculogenesis that may account for
the remarkable ability of the ovary to continue to function normally after major insults. Such features include: a great excess of developing follicles (most of which will degenerate rather than ovulate), the exponential nature of follicular growth, the acceleration of cell proliferation as follicular size increases, and the location of the principal feedback regulatory step at the penultimate stage of the developmental process. These aspects of folliculogenesis have important implications for toxicological studies. Because the ovary can respond quickly and completely to loss of homeostasis over the short term, damage from toxic insult may not be readily apparent. However, long-range fertility may nevertheless be impaired. The finite size of the follicular pool and absence of feedback regulatory steps during the early stages of follicular growth render the ovary incapable of restoring the status quo among small and medium sized follicles. This will eventually result in loss of fine control over the number of follicles that ripen and the regularity of the reproductive cycles, and could reduce the overall duration of the fertile lifespan.

631. Assessment of Toxicant Insult on Oocyte Maturation and Fertilization. Sally D. Perreault, EPA, Research Triangle Park, NC.

The final phase of oocyte maturation, characterized by the resumption of meiosis, is triggered by the preovulatory surge of luteinizing hormone. This phase is critical for subsequent oocyte function (fertilization). While difficult to evaluate in humans, oocyte maturation and fertilization are amendable to study in animal models using both in vivo and in vitro approaches. This presentation will focus on direct assessments of oocyte maturation and fertilization in rodents following in vivo toxicant exposures during critical peri-fertilization periods. Examples of methods for evaluation of oocyte morphology, chromosomes, meiotic competence and ability to support pre-implantation development will be provided. In addition, application of in vitro fertilization and sperm micro-injection methods to assess specific aspects of oocyte function will be discussed. These examples will serve to emphasize the utility of simple, direct, cellular measures in the assessment of sites and mechanisms of toxicant action in the non-pregnant female.

Cell Membranes as Targets for Chemical Toxicants

Chairperson: William D. Atchison, Michigan State University, East Lansing, MI.

632. Introduction. William D. Atchison, Michigan State University, East Lansing, MI.

Cell membranes are the first target of exposure to toxicants. Thus interactions with the cell membrane represent some of the earliest signs of toxicity. Moreover, cell membranes maintain the ability to regulate a number of crucial processes associated with signalling within the cell, signalling other cells, controlling transport and secretory phenomena, and regulating and initiating intracellular processes. Cell membranes at the same time contain components unique to individual cells, as well as those which are ubiquitous to all cells. Thus study of interactions of toxicants with cell membranes provides some potentially exciting insights into mechanisms of cellular toxicity in general, as well as specific target-directed toxicity. Recently, major advances have been made in our understanding of the physiological and biochemical processes underlying the role of membranes in cell signalling. Despite this, the study of effects of toxicants on cell membrane function remains underdeveloped. This symposium is directed at examining mechanisms by which a variety of dissimilar toxicants including heavy metals, insecticides and antibiotics interact with membranes, particularly with processes associated with cell signalling. The four presentations will describe state-of-the-art methodology used to examine alteration of membrane function. The talks will focus on two areas, disruption of ion channel function, and alteration of membrane-associated second messenger systems.

633. Nerve Membrane Ion Channels as the Target of Insecticides. Tosho Narahashi, Northwestern University Medical School, Chicago, IL.

Chlorinated hydrocarbon and pyrethroid insecticides have long been known to act on the nervous system thereby causing toxic effects. Progress in understanding the mechanisms of action of these insecticides depended largely on the advancement in electrophysiology. It was not until mid-1960's that DDT and pyrethroids were clearly demonstrated by voltage clamp experiments to alter the sodium channel function. A prolonged sodium current flows across the nerve membrane which in turn elevated and prolong the depolarizing after-potential leading to repetitive after-discharges. Patch clamp experiments have shown that the gating kinetics of individual sodium channels are markedly slowed causing them to be kept open for extremely long periods of time, often lasting for as long as several seconds as compared with the normal open time of a few milliseconds. There are some quantitative differences between the non-cyano (type I) pyrethroids and the alpha-cyano (type II) pyrethroids, yet the basic mechanism of action on the sodium channels is the same. It has been suspected since the early 1980's that alphacyano pyrethroids block the GABA receptor-channel system, but recent patch clamp experiments have unequivocally excluded the possibility. On the other hand, lindane and cyclodiene insecticides such as diekdrin and endrin block the GABA system thereby causing hyperexcitation.
634. **Nerve Terminal Membranes as a Target Site of Action of Inorganic and Organic Mercurials.** William D. Atchison, Michigan State University, East Lansing, MI.

Nerve terminals have the crucial role of synthesis and release of chemical neurotransmitters. This latter process is critically dependent upon regulation of the movement of calcium ions across the membrane. Organic and inorganic mercurials, both of which are highly neurotoxic, disrupt nerve terminal membrane excitability in both specific, and nonspecific manners. The former effects appear to be related directly at voltage-gated Ca channels which regulate neurotransmitter secretion. Uncharged mercurials such as dimethylmercury and PCMB, do not affect depolarization-dependent influx of Ca$^{2+}$ into isolated nerve terminals, whereas ethylmercury, and methylmercury which are charged, do. The nonspecific effects are characterized as an overall increase in membrane permeability, resulting in, among other things, depolarization of the membrane. Here, inorganic divalent mercury differs in its effects from methymercury, a charged, lipophilic metal. Hg$^{2+}$ seems to have a more prominent depolarizing action on the plasma membrane whereas methylmercury preferentially depolarizes the mitochondrial membrane in addition to depolarizing the plasma membrane. These effects are not dependent upon extracellular Na$^+$ or Ca$^{2+}$ and apparently do not involve cationic channels. Each of these disparate actions could contribute to diminished entry of trigger calcium into the nerve terminal, and depressed release of chemical transmitters.

635. **Interaction of Aminoglycoside Antibiotics with Calcium Channels and Membrane Phosphoinositides.** Jochen Schacht, University of Michigan, Ann Arbor, MI.

Aminoglycoside antibiotics cause both acute and chronic toxicity to the inner ear. The acute mechanisms affect the excitability of the sensory cells and are related to extracellular block of transduction and voltage-gated calcium channels. Chronic mechanisms involve binding to phosphatidylinositol 4,5-bisphosphate (PtdInsP$_2$) on the cytoplasmic site of the plasma membrane resulting in inhibition of second messenger actions. While drug toxicity is rather tissue-specific *in vivo*, the aminoglycosides have become valuable tools to probe calcium channels and phosphoinositide metabolism in a variety of tissues *in vitro*.

Isolated outer hair cells in short-term culture have recently been established as models for the study of hair cell physiology and pathology. We used these cells to evaluate the acute effects of gentamicin. Intracellular calcium levels were monitored with the fluorescent dye fluo-3. In response to cell depolarization, calcium increased from 218 ± 102 nM to 2018 ± 1077 nM. Both gentamicin and neomycin blocked the voltage-gated calcium channel at an IC$_{50}$ of 50 μM. This action may explain the acute block *in vivo* of the cochlear microphonic potential.

Chronic toxicity appears to require entry of the drug into the cell. Aminoglycosides have a high affinity for PtdInsP$_2$ and can inhibit its hydrolysis. Since this hydrolysis is the first step in the second messenger signal transduction cascade the drug/lipid interaction would block this vital cellular process. In addition, the complex formation between aminoglycoside and PtdInsP$_2$ leads to non-specific permeability changes of the membrane contributing to the lethal actions of these drugs.

636. **Lead Modulation of B-T Cell Interaction.** Michael McCabe and David Lawrence, Albany Medical College, Albany, NY.

B and T lymphocytes interact via B cell membrane glycoproteins, termed la (m1a). B-T cell contact is necessary for B cell differentiation (BCD) into antibody forming cells, and the level of m1a regulates this process. Lead (Pb), which augments BCD, has been shown by flow cytopometric analysis to increase the B cell's m1a by 2-fold. By western blot analysis Pb increases (2-fold) the total cellular pool of la β-chains, ruling out a simple redistribution of a constant amount of la. The increase in m1a caused by Pb is comparable to that caused by other B cell activators but is mechanistically unclear. Calcium (Ca) flux has been implicated to increase m1a; however, Pb does not facilitate $^{45}$Ca uptake or generate IP3, an intracellular Ca releasing factor. Hence, if the mechanism of Pb-increased B cell m1a relates to modulation of cellular Ca, then Pb, itself, must cross the plasma membrane to mobilize/displace Ca intracellularly. Pb does not increase the steady-state levels of la mRNA, but it does increase the incorporation of $[^{35}S]$-methionine into la-associated chains, which by pulse-chase analysis persists in Pb-treated B cells. Hence, degradation of la-associated chains appears decreased in Pb-treated B cells. It has been hypothesized that m1a recycling is important for la function. Since Pb alters the phospholipids, lipids and protein constituents of the lymphocyte plasma membrane, resulting in aberrant regulation of B cell M1a expression, B-T cell interaction and subsequent immunoregulation of self/non-self discrimination is affected. Effects of Pb on lymphocyte membrane fluidity and capping of selected surface proteins are currently being studied. These studies may substantiate alteration of the lymphocyte plasma membrane by Pb, which could explain many of the diverse functional immunomodulatory aspects of Pb.
Symposia

Thursday, February 28
8:30 a.m.

Indirect Mechanisms of Immune Modulation

Chairpersons: Nancy Kerckvliet, Oregon State University, Corvallis, OR and Virginia M. Sanders, NIEHS, Research Triangle Park, NC.

829. Introduction. Virginia M. Sanders, NIEHS, Research Triangle Park, NC.

Xenobiotic-induced modulation of immune function occurs via direct and/or indirect mechanisms. Direct mechanisms of immune modulation involve interaction of the xenobiotic or its metabolite with an immune cell-associated receptor whose activation induces a modulatory signal. Direct mechanisms of immune modulation can be measured in vitro. Indirect mechanisms of immune modulation involve interaction of the xenobiotic or its metabolite with a non-immune cell-associated receptor whose activation induces the release of a biological messenger possessing immunomodulatory activity. Cells of the neuroendocrine system are both the primary target for the xenobiotic and the source of these messengers, although some messengers are released as a consequence of xenobiotic-induced tissue damage. These mechanisms of xenobiotic-induced immune modulation can only be measured in vivo. In some instances, xenobiotic-induced immune modulation occurs via a combination of direct and indirect mechanisms: An understanding of both mechanisms shall allow for: 1) a more definitive characterization of the seemingly contradictory immunomodulatory results produced by xenobiotics in vitro as opposed to in vivo, and 2) the design of pharmacologic interventions capable of modulating the intensity of the xenobiotic-induced effect. This symposium shall address four indirect mechanisms of immune modulation.


Immunologists have long believed the regulation of the immune system to be autonomous. That is, the cells participating in and controlling the kinetics, intensity, and type of an immune response were thought to be limited to those cells recognized as members of the immune system. The belief that immune regulation occurs in the absence of any modulatory influence from the central nervous system is (or was until very recently) accepted by all but a minority in the field of immunology. The first part of this talk will briefly review the current information which suggests that the immune system is actually responsive to modulatory signals which arise in the brain. The immunomodulatory roles of the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) will be discussed. Special emphasis will be placed on the involvement of these systems after certain forms of toxic insult.

The second part of this talk will focus on the role of the SNS in the modulation of humoral immune responses in the spleen. Mice treated with the neurotoxic drug 6-hydroxydopamine have been reported by a number of labs to have subnormal humoral responses. Work in this lab has focused on using the density of lymphocyte cell surface β-adrenergic receptors, as well as use of in vivo and in vitro experiments with the non-neurotoxic 5-hydroxydopamine to demonstrate that the SNS is involved in the observed immunomodulation.

831. Indirect Immunological Effects of Morphine. Stephen B. Pruett, Mississippi State University, Miss. State, MS.; B.A. Fuchs, Y.C. Han, and A.E. Munson, Medical College of Virginia/VCU, Richmond, VA.

Morphine suppresses humoral immune responses and decreases the weight and cellularity of the thymus. The present investigation was conducted to assess the role of direct and indirect mechanisms in these morphine-induced immune alterations. Programmed cell death (apoptosis) was examined as a possible mechanism for morphine-induced thymic atrophy. Splenocytes obtained from B6C3F1 mice 24 hours after implantation of a 75 mg slow release morphine pellet produced only 25-35% as many antibody-forming cells (AFC) in Mishell-Dutton cultures as splenocytes from naïve mice or mice implanted with placebo pellets. Evidence for direct action of morphine was sought by adding morphine sulfate to primary Mishell-Dutton cultures containing splenocytes from B6C3F1 or C57Bl/6 mice and sheep erythrocytes as immunogen. Morphine at 10^-12 to 10^-4 M did not significantly alter the number of AFC detected on day 5. The role of apoptosis in morphine-induced depletion of thymocytes was examined by measuring DNA fragmentation, a distinguishing feature of this type of cell death. DNA fragmentation was 2-4 fold greater (p<0.05) in morphine treated mice than in naïve or placebo pellet groups. In a time course study, DNA fragmentation peaked at 12 hours and preceded significant decreases in thymocyte numbers (24 hours). In contrast, exposure of thymocytes to morphine in vitro did not cause DNA fragmentation or decrease viability in 24 hour cultures. These results suggest that morphine induces splenocyte dysfunction and thymocyte apoptosis in vivo by an indirect mechanism.
832. Immunomodulatory Action of a Polychloronated Biphenyl via Endocrine Dysregulation? Nancy Kerkvliet, Oregon State University, Corvallis, OR.

Halogenated aromatic hydrocarbons (HAH) have received much attention from researchers and the public for their potential immunotoxicity, but a uniform hypothesis for their mechanism of immune suppression has yet to be accepted. Suppression of the allospecific cytotoxic T lymphocyte (CTL) response in C57B1/6 mice by 3,3',4,4',5,5'-hexachlorobiphenyl (HxCB) occurs only at doses which also result in coincident and time dependent thymic atrophy and elevation of plasma corticosterone (CS), the major glucocorticoid (GC) in mice. When added directly to cultures, HxCB has no effect on in vitro generated CTL suggesting that the interaction of endogenous factors is necessary for the expression of immune suppression. Since both thymic involution and CTL suppression can be induced by therapeutic GC administration, the role of elevated CS in this model has been investigated. Adrenalectomized (ADX) HxCB treated mice suffer an increased mortality rate (>75%) following alloimmunization (Al). In survivors, ADX does not eliminate CTL suppression, thymic involution or CS elevation. CS elevation is not observed following Al alone, but Al enhances the elevation observed following HxCB treatment. Noninoculated HxCB treated ADX mice also have elevated CS levels. These data suggest that extra-adrenal steroidogenic tissue is the likely source of CS in HxCB treated ADX animals. More recently, pharmacologic methods are being used to antagonize CS elevation. Preliminary results using the antiglucocorticoid RU-486, a glucocorticoid receptor antagonist, will be presented.

833. Immunomodulation by Acute Phase Reactant, Serum Amyloid A (SAA) Protein. Norbert E. Kaminski, Medical College of Virginia/VCU, Richmond, VA.

Acute phase reactive proteins (APRP), are a family of peptides which are synthesized and released into circulation very rapidly in response to tissue damage and inflammation. The primary biologic function of APRP, including SAA, and their functional relevance with respect to tissue damage is poorly understood. Interestingly, many APRP are capable of modulating immune responses. It has been proposed that the release of immunomodulatory APRP following tissue damage, especially those which down regulate immune responses, may serve as a defense mechanism by tempering the immune response against intracellular antigens released following cell lysis. SAA is one of several APRP which dramatically increases following tissue damage and is also an immunomodulator. Marked increases in SAA levels have been reported following a broad range of injuries including various types of hepatic damage, renal injury and rejection of renal allografts, etc. The present studies were undertaken to further characterize the mechanisms by which SAA regulates immune responses and to develop a model system in the B6C3F1 mouse to study whether immunosuppression following chemical injury can occur indirectly through the release of SAA. SAA induction in the B6C3F1 mouse is paralleled by marked suppression of immune responses, specifically those requiring macrophages as accessory cells. Cell separation-reconstitution studies indicate that the primary cell-type responsible for decreased immunocompetence associated with elevated SAA levels is in fact the macrophage.

Involvement of Cytoskeleton in The Mechanisms of Chemically Induced Neurotoxicities

Chairperson: Mohamed B. Abou-Donia, Duke University Medical Center, Durham, NC.

834. Introduction. Mohamed B. Abou-Donia, Duke University Medical Center, Durham, NC.

A number of neurotoxic chemicals interact selectively with cytoskeleton components. Early studies have used these chemicals as probes to investigate the function of the cytoskeleton. Recently, the action of some neurotoxins on cytoskeletal proteins have been studied. The cytoskeletal is a structure that supports eukaryotic cells internally. It is formed of three main fibrillar proteins: microtubules, intermediate filaments, and actin filaments. Intracellular organelles are embedded in the cytoskeletal matrix. Our present knowledge of the mechanisms by which these cytoskeletal structures are involved in neurotoxicities is very fragmentary. We know very little about the effect of neurotoxins on cytoskeleton dynamics in cells. Questions regarding the action of neurotoxins on the functions of cytoskeleton and its regulatory role of cell metabolism, growth, and differentiation are yet to be answered. In this symposium we present studies on the involvement of the cytoskeleton in chemically induced neurotoxicities. First, we will briefly discuss the cytoskeleton: morphology, distribution, molecular composition, assembly, and disassembly. Then, we will present the role of these proteins in the development of chemically induced neurotoxicities.


The exposure of neurons and their processes to any of a large variety of insults results in a reorganization of the neuronal cytoskeleton. These insults include physical crushing and cutting of the nerve and chemical agents of great diversity including
aluminum, acrylamide, IDPN, the vinca alkaloids, n-hexane and many others. The most common response to these agents is a bundling and apparent increase in the number of 10 nM intermediate filaments in either the cell body or the neuronal processes. In some cases, such as vinca alkaloids toxicity, these changes reflect a direct interaction between the toxin and a cytoskeletal element. In others the changes are in reaction to a more general insult to the cell and can be thought of as an attempt to repair the injury. In this presentation, the reaction of the nerve cell to physical crushing of its axon will be used as a model to discuss the role of tubulin, actin and two distinct neuronal-specific intermediate filaments - the neurofilament proteins and peripherin - in neurofibriillary pathology and in nerve regeneration. The reciprocal regulation of these two filament species in response to injury will be explored and the results extended to chemical intoxication and the role of molecules which bridge cytoskeletal elements in homotypic and heterotypic fashion.

836. Molecular Mechanism of Neurofilamentous Neuropathies Induced by Hexane and Carbon Disulfide. Doyle G. Graham, D.C. Anthony, V. Amarnath, S. Pyle, and W. Valentine, Duke University Medical Center, Durham, NC.

2,5-Hexanediol (HD), the γ-diketone metabolite of α-hexane, and carbon disulfide result in an identical distal neurofilamentous axonopathy in humans and experimental animals. With both toxicants, the neurofilament-filled swellings of the axon appear to result from covalent crosslinking of neurofilaments. HD reacts with protein amino groups to yield pyrrollyl derivatives. The lack of neurotoxicity of 3-acyethyl-2,5-hexanediol (AChD) shows that pyrrole formation must be followed by ring oxidation for neurofilamentous swellings to develop. The resulting electrophillic then reacts with a second protein nucleophile, the sulfhydryl group, to result in crosslinking. It is postulated that the great stability of the neurofilament makes crosslinking of this protein the toxicologically relevant event in γ-diketone toxicity. Other stable proteins, such as erythrocyte spectrin are similarly crosslinked and provide a convenient biomarker of significant exposure. Carbon disulfide reacts with protein amino groups to yield a thioisocarbamate, which then undergoes elimination to the electrophilic isothiocyanate. Again, the protein nucleophile which results in crosslinking is the sulfhydryl group, which yields in an N,S-dialkyl disulfocarbamate. It is remarkable that these two dissimilar toxicants result in the same molecular sequence; reaction with protein amino groups, an activation step, then reaction with protein sulfhydryl groups to yield neurofilament crosslinking.

837. Changes in the Economy of Neurofilaments In Chemical Neurotoxicity. John W. Griffin, Johns Hopkins University, Baltimore, MD.

A variety of neurotoxic agents alter the economy of the neurofilaments, either by altering synthesis or by affecting their distribution and axonal transport. For example, distal axon degeneration (dying back) due to agents such as acrylamide induces changes in synthesis of neurofilaments in a fashion identical to those induced by mechanical axotomy. Other agents produce changes in the axonal transport of neurofilament proteins. Different and distinctive abnormalities are produced by β, β-iminodipropionitrile and 2,5-hexanediol.

These models will be considered in the context of two recent changes in the understanding of the axonal cytoskeleton. First, there is increasing evidence that much of the axonal cytoskeleton is stationary and not undergoing continuous transport. Second, new evidence that complete interruption of axonal transport (even by axonal transaction) for long periods does not necessarily induce axonal degeneration; genetic models of prolonged axonal survival indicate that factors other than simple blockade of axonal transport are required to initiate prompt axonal breakdown. These issues promise to be central to our future understanding of the pathogenesis of neurotoxic injuries.

838. The Cytoskeleton: A Target for Organophosphorus Ester-Induced Delayed Neurotoxicity. Mohamad B. Abou-Donia and D.M. Lapadula, Duke University Medical Center, Durham, NC.

Organophosphorus ester-induced delayed neurotoxicity (OPIDN) is characterized by ataxia and paralysis accompanied by a Wallerian-type degeneration of the central and peripheral nervous systems. Recent studies from our laboratory showed an increase in the activity of Ca²⁺/calmodulin kinase II in animals treated with delayed neurotoxic organophosphorus compounds such as tri-o-cresyl phosphate (TOCP). This led to increased autophosphorylation of this enzyme as well as of a- and b-tubulin, MAP-2, and neurofilament triplet proteins. These results suggest the involvement of CaM kinase II in the pathogenesis of OPIDN in the light of studies from other laboratories. The phosphorylation of MAP-2 by CaM kinase II resulted in the depolymerization of microtubules. Furthermore, the phosphorylation of brain synaptosomal tubulin by this kinase transformed it into twisted filamentous polymers distinct from microtubules. Thus, TOCP-induced enhanced activity of CaM kinase II results in increased phosphorylation of cytoskeletal proteins, leading to the formation of twisted, condensed, and aggregated neurofilaments and microtubules seen by electron microscopic studies in nerve tissues of animals paralyzed by TOCP.
Thursday, February 28
1:30 p.m.

Health Effects of Atmospheric Acid Aerosols: A Model Problem in Inhalation Toxicology and Air Pollution Risk Assessment

Chairpersons: Richard B. Schlesinger, NYU Medical Center, New York, NY, and Judith Graham, EPA, Research Triangle Park, NC.


Air pollution risk assessment has focused traditionally on individual chemicals. Such assessments are based on: 1) animal and human clinical inhalation toxicology studies, which most often involve only one pollutant, and 2) epidemiology studies, which allow the ential complex mixture exposures, frequently focus on impacts of a few pollutants within the mixture. This traditional approach has enabled great strides in knowledge and regulations protective of the public health, but has inherent limitations in assessing "real world" risks of complex mixtures. The emerging data base on acid aerosols presents an opportunity for a case study of how complex mixture research programs can be developed and resulant data applied in risk assessments.

Acid aerosols are of significant health concern, although much remains to be learned about their effects and exposures. They are a heterogeneous mixture of several classes of pollutants. They contain both particles and gases; strong and weak acids; and fine and coarse mode sized particles. Their chemical composition and ambient air levels vary geographically and temporally. Their heterogeneous nature presents significant research challenges in both exposure assessment and health assessment. As with any complex mixture, all possible exposure scenarios cannot be studied. The research performed with acid aerosols provides excellent examples of how best to approach such a problem. Animal inhalation toxicology, human clinical, field, and epidemiological studies have creatively elucidated health effects of concern and have resulted in provocative findings and hypotheses about both acute and chronic health effects that can be used in a risk assessment. All of these issues will be discussed in the symposium.


Toxicologic studies using laboratory animals are essential for the development of an adequate database which will allow reliable estimation of risk to humans from exposure to acidic aerosols. Such studies are described to identify the nature of responses and the range of concentrations and exposure durations over which such responses may occur, as well as to provide information on the underlying mechanisms of potential human toxicity. They are especially important for protocols that relate to chronic or repeated exposures, scenarios which may result in delayed or irreversible changes. The database indicates that acid aerosols are both upper and lower airway irritants, producing such diverse effects as increased airway hyperactivity, changes in tracheobronchial mucous transport rate, alterations in pulmonary macrophage function, and modulation of pulmonary arachidonic acid metabolism. Some of these effects are consistent with those found in controlled clinical studies and epidemiologic surveys. Repeated exposures have been associated with airway morphologic changes characteristic of mucous hypersecretion and chronic obstructive lung disease. Although the toxicologically active portion of acid aerosols is likely the hydrogen ion, what remains uncertain is the relationship between hydrogen ion content and response. There is evidence that different acid aerosols are not equipotent even when the hydrogen ion content of each compound is taken into account. In any case, the response to acid aerosols is due to some combination form of exposure concentration and duration. Thus, data obtained from toxicologic studies likely have relevance to effects experienced by the general population.


Controlled clinical studies have linked acid aerosol inhalation acute respiratory effects. A series of inhalation studies have revealed that: 1) The more acidic sulfates, H₂SO₄ and NH₄HSO₄, provoke the greatest changes in lung function in normal and asthmatic subjects, 2) exercising allergic, asthmatic, asthmatics demonstrate bronchoconstriction at sulfuric acid aerosol concentrations of 50-100 μg/m³ (30 minutes at rest followed by 10 minutes of exercise), 3) elevated respiratory ammonia levels mitigate responses to acid aerosols in exercising asthmatics, and 4) tracheobronchial clearance rates are altered by sulfuric acid aerosol concentrations of 100-1,000 μg/m³ in healthy volunteers. In addition, field studies have shown an apparent potentiation of ozone-induced functional decrements by acid aerosols. Although adolescent asthmatics and children appear most sensitive to ambient levels of acid aerosols, not all clinical or field studies have found decrements in lung function. Most prolonged, low-level studies in susceptible populations are needed to further characterize responses. However, the findings from available clinical studies together with results from animal toxicology and epidemiologic studies suggest a significant impact of ambient levels of acid aerosols on human health.

Although epidemiologists, toxicologists and atmospheric chemists have postulated for many decades that atmospheric acid aerosols may be important components of the air pollution mix producing acute and chronic health effects ranging from reversible changes in lung function to mortality, the direct evidence for such an association is only now being developed. Epidemiologic investigations are one component of the multidisciplinary evidence necessary to support the definition of acid aerosols as a criteria air pollutant. In studying free-living, naturally exposed population samples, the epidemiologist does not have control over exposure to specific air pollutants or other determinants of response. Defining an association depends on study, design and statistical analysis. For acid aerosol studies, definition of exposure is particularly difficult, as these pollutants are always accompanied by other potentially toxic, chemical species – sulfur oxides and particles in the historical acid aerosol episodes, and particles and photochemical pollutants in acid aerosol events currently being studied. Nevertheless, epidemiologic evidence from historical air pollution episodes and from current studies in progress are consistent with hypothesized acute and chronic health effects of acid aerosols. While these epidemiologic associations do not alone show causation, when put in the context of controlled human exposure and animal studies, a consistent picture begins to emerge.

1007. Integration of the Evidence: Risk Assessment for Ambient Acid Aerosols. Roger O. McClellan, CIIT, Research Triangle Park, NC.

This presentation will emphasize the use of a risk assessment orientation incorporating hazard identification, exposure-dose-response assessment and exposure assessment components to integrate and assess the current status of our knowledge on the human health risks of exposure to acid aerosols. This integrative approach uses complementary data obtained from multiple sources; epidemiological studies, controlled human exposures and investigations with laboratory animals. The epidemiological studies demonstrate an association between pulmonary alterations and exposure to ambient concentrations of acid aerosols. Interpretation of these studies is complicated by the complex exposure environment (mixtures of acidic compounds and other pollutants). Controlled exposure studies with specific acidic compounds conducted in humans provide information on pulmonary responses to acute exposures while those conducted with laboratory animals provide information on responses to both acute and subchronic exposures. These results must be interpreted in the context of environmental exposures to complex mixtures of acid aerosols and other pollutants. The results obtained from the complementary approaches will be discussed from the viewpoint of their implications for setting ambient air quality standards for acid aerosols and to aid in identifying the need for additional research. Consideration of the acid aerosol experience provides valuable insights into how the risks of other complex mixtures may be assessed.

An Update on Exposure and Effects of Lead

Chairperson: Barbara D. Beck, Gradient Corporation, Cambridge, MA.


Despite knowledge of the toxicity of this metal since Roman times, lead remains a persistent public health concern. Lead is pervasive in the environment, found in a variety of media such as urban soils, house paint, and ambient air near smelters. In addition, concern has increased for low level effects of lead including neurobehavioral deficits in children and for possible carcinogenicity. This symposium will present current topics in the toxicology of lead as related to risk assessment, beginning with a multimedia exposure model aimed at predicting population distributions of blood lead. Next there will be a discussion of prospective studies, focused on neurobehavioral effects, and their relationship to definition of acceptable blood levels for both the fetus and the child. The third presentation will describe studies of lead carcinogenicity in animals, their implications for lead as a possible human carcinogen and the possible need to consider carcinogenicity in lead regulation. The last presenter will describe recent work on lead binding proteins and the importance of such proteins in differential susceptibility to lead. The symposium will link fundamental issues of lead exposure and toxicology to lead risk assessment. The symposium will be multidisciplinary in nature and therefore of interest to basic researchers, regulatory agency scientists, and industry scientists.

1009. The Use of Site-Specific Data In Models for Lead Risk Assessment and Risk Management. Allan H. Marcus, Battelle-Columbus, Research Triangle Park, NC.

The concentration of lead in whole blood is presently accepted as the best index of body lead burden, and thus the best indicator of the reduction of population exposures to lead from abatement programs. Computer models for predicting changes in the blood lead distribution over time for children of various ages have been developed by the U.S. EPA and the U.S. CDC. These
models can be adapted to site-specific risk assessments for lead, if the following data are available: 1) characterization of the distribution of lead levels in diverse media to which children have been exposed, since conception including air, water, soil, house dust, paint, and food; 2) characterization of factors that affect the biological availability of lead, including age of the child, chemical speciation, particle size, and dietary cofactors; 3) demographic and socioeconomic factors that affect the amount of childhood lead exposure, including age of children, parental involvement, and supervision, and frequency of outdoor play; 4) environmental factors that may alter lead biokinetics, such as inadequacies in calcium or iron nutrition; 5) characterization of the changing time patterns of environmental lead following the proposed abatement. The sensitivity of the model parameters will be demonstrated for three scenarios of current regulatory interest: a) soil lead abatement for lead smelter and mining communities; b) water lead abatement by control of the corrosivity of water; c) paint lead abatement in occupied dwelling units.

1010. Lead Neurotoxicity: Recent Finding and Their Implications. David Bellinger, Children's Hospital, Boston, MA.

Knowledge about lead's neurotoxicity at low dose increased dramatically during the 1980's. Meta-analyses of cross-sectional studies suggest that the overall finding of an inverse association between exposure and children's IQ scores over the range of 0 to 40 ug/dL is unlikely to be the result of chance. A threshold for this apparent effect is not readily discernable. Serious long-term academic deficits have been linked to childhood blood lead levels greater than 30 ug/dL. A series of ongoing prospective studies provide the opportunity to evaluate the relative impact of prenatal and postnatal exposure on development, the reversibility of early effects, and correlates of vulnerability. Despite some inconsistencies in the findings of these studies, especially with respect to the impact of prenatal lead exposure on fetal growth and maturity, the U.S. EPA has cited 10 ug/dL as the maximum permissible blood lead level for the fetus and young child.

1011. Nephrotoxicity and Carcinogenicity of Lead. Robert A. Goyer, University of Western Ontario, London, Ontario, Canada

The carcinogenic potential for lead is of concern because of the occurrence of renal adenocarcinoma in rats fed lead for long periods of time. Risk to humans, however, is uncertain. All people have some level of lead in the kidney. Yet renal adenocarcinoma is a relatively uncommon tumor. Only two case reports of renal adenocarcinoma in lead workers are in workers with severe lead nephropathy. Lead induced tumors in experimental animals have not been observed at a level of exposure that does not produce nephrotoxicity. This observation has led to the suggestion that renal carcinogenicity from lead may be a threshold event dependent on prior existence of nephrotoxicity. The only multiple dose response study of lead induced tumors did not find any tumors or toxicity at the two lowest doses tested (Azur et al., 1972). These results, although not inconsistent with a linear dose response, are also consistent with a threshold model.

To date a mechanistic threshold has not been established for any known human carcinogen. Three mechanistic factors are proposed that may relate to the tumorigenic effect of lead. These are the occurrence of nuclear inclusion bodies which are lead protein complexes involving a non-histone acidic protein. Lead also activates protein kinase at pico Molar levels. This enzyme in turn activates growth factor receptors and proto-oncogenes. And, finally, lead nephrotoxicity is characterized by tubular cell hyperplasia - a known risk factor for renal cell carcinogenicity.

1012. Role(s) of Lead-Binding Proteins (PbBP) in the Renal and Neurotoxic Effects of Lead in the Rat. Bruce A. Fowler, University of Maryland, Baltimore, MD.

The kidney and brain are major target organs for lead toxicity but the molecular factors which mediate the low-dose effects of this metal in these tissues are not currently understood. Recent studies on the PbBP have demonstrated that this molecule which is a cleavage product of ~2 U globulin acts as a "receptor" for lead in the kidney by facilitating Pb movement into renal tubule cell nuclei and subsequent changes in renal gene expression. The brain PbBP which has a similar amino acid composition but an apparent molecular mass of 29,000 daltons and is immunologically distinct from ~2 U globulin also appears to mediate the effects of Pb on ~-aminolevulinic acid dehydratase in brain. Further studies are in progress with regard to the purification and regulatory roles played by PbBP in human and non-human primate brain and kidney tissues to determine if analogous PbBP's exist and play similar functional roles. The current hypothesis is that since ~2 U globulin is a member of the retinol binding protein family which is expressed in humans and since both kidney and brain show similar biological responses in non-human primates, that analogous but not identical PbBP's will be isolated.

Development of radioimmunoassays for such molecules may permit identification of persons at special risk for low dose Pb toxicity.
Friday, March 1

8:30 a.m.

Quinone Chemistry and Toxicity

Chairperson: Terrence J. Monks, University of Texas, Austin, TX.

1238. Introduction. Terrence J. Monks, University of Texas, Austin, TX.

The human use of quinones dates back to antiquity. Crude plant preparations from senna and rhubarb were used as drugs and contained a variety of anthraquinones as the active constituents. Pigments prepared from henna and madder were used as cosmetics and dyes and contained derivatives of 1,4-naphthoquinone and anthraquinone respectively. Documented use of the Cinchona bark to control fever dates back to at least the 17th century. The quinones of polycyclic aromatic hydrocarbons are prevalent as environmental contaminants and provide an additional current source of human exposure to quinones. This symposium will focus on the chemistry of quinones, their role in the development of a variety of toxicities and potential mechanisms of action. Aspects of quinone chemistry essential for their biochemical (re)activity will be reviewed. The relative importance of redox cycling and arylation to the cytotoxicity of quinones will be illustrated by studies of structurally related quinones which differ in their relative ability to arylate and/or redox cycle. Several recent studies suggest that both these functions of the reaction of glutathione with quinones exhibit a variety of toxicological activity. Specific examples of these functions are presented. The role of DT-diaphorase and the two electron reduction of quinones in the bioreductive activation of certain antitumor quinones will also be discussed. Thus, dependent upon the structure of the quinone, DT-diaphorase may serve either a toxifying or detoxifying function. Finally, the potential toxicity of endogenous quinones will be illustrated by studies in which the neurotoxicity of catecholamines has been investigated. Such neuronal degeneration may contribute to the pathology of aging and Parkinson's disease.

1239. Bio-Organic Chemistry of Quinones. Robert P. Hanzlik, University of Kansas, Lawrence, KS.

The formation of quinones by living organisms and their potential for toxicity to living organisms, have been appreciated since the mid-19th century. Outwardly the most conspicuous property of quinones is their color, but of greater biological significance is their reactivity as oxidants and/or electrophiles. Important endogenous quinones include vitamin K, coenzyme Q and methoxanthin (PQQ, coenzyme M), a newly discovered component of serotonin oxidase and adrenal dopamine β-hydroxylase. Critical to their function in vivo is the controlled two-electron redox interconversion of their quinone and hydroquinone forms. Uncontrolled one-electron processes facilitated by seemingly analogous quinones (e.g. medamidine, adriamycin) leads to redox cycling and production of reactive oxygen radicals which can be deleterious. Other xenobiotic quinone metabolites (e.g. bromobenzoquinones, DES-quinone) and cogeners (e.g. BHT-quinone methide, acetaminophen quinominime) are electrophilic Michael acceptors and alkylate cellular macromolecules; aspects of these have been employed in the design of antitumor agents (i.e. bioreductive alkylators). This lecture will review key features of quinone chemistry and SAR in a biochemical context, and relate the underlying chemical principles to those which govern the behavior of other classes of chemically-reactive toxic agents.


Quinones may be toxic by a number of mechanisms, including redox cycling, arylation and interference with mitochondrial respiration. Cellular reductases may reduce quinones by either one or two electron addition to the corresponding semiquinone or hydroquinone respectively. The semiquinones may be toxic per se or they may react with molecular oxygen forming superoxide anion radical with regeneration of the parent quinone, which may again be reduced, so undergoing a futile redox cycle. This redox cycle results in a disproportionate consumption of cellular reducing equivalents and generation of active oxygen species, causing an oxidative stress. Quinones may also react directly with cellular macromolecules particularly by nucleophilic Michael addition to thiol and amino groups.

In this presentation, the mechanisms of cytotoxicity to isolated hepatocytes of a number of simple, structurally related quinones, with differing abilities to arylate and/or redox cycle, will be discussed. Non-toxic concentrations of 2-methyl-1,4-naphthoquinone (menadione; both redox cycles and arylates), 2,3-dimethoxy-1,4-naphthoquinone (a pure redox cycle) and 2-hydroxy-1,4-naphthoquinone (neither arylates nor redox cycle) by a one electron reduction) all caused markedly similar concentration dependent changes in pyridine nucleotides, including an initial decrease in NADP*, accompanied by a small transient rise in NADP*, followed by a more prolonged increase in NADPH and total NADPH & NADP*. These changes were not observed following exposure to quinones, such as p-benzazinquinone, which act primarily by arylation. Finally, the relative importance of redox cycling and arylation in the toxicology of these quinones will be discussed.
1241. Toxicology of Quinone-Thioethers. Terrence J. Monks and Serrine S. Lau, University of Texas at Austin, Austin, TX.

Quinones constitute an important class of naturally occurring compounds that are found in plants, fungi and bacteria and which function primarily as components of the electron transport chains involved in cellular respiration and photosynthesis. Human exposure to quinones occurs clinically, via the diet and via airborne pollutants. The reactivity of quinones resides in their ability to either generate aggressive oxygen species via redox cycling and/or to react directly with cellular nucleophiles, such as protein and non-protein sulphydryls. Glutathione (GSH) is the major non-protein sulphydryl present in cells. The conjugation of reactive electrophiles, such as quinones, with GSH, usually results in thiether formation and excretion in the urine of the corresponding mercapturic acids. The reactions normally result in the formation of biologically benign metabolites. However, several studies indicate that quinone-thioethers possess significant biological (re)activity. For example, certain quinone-thioethers retain the ability to redox cycle with the concomitant generation of reactive oxygen species, and the formation of a stable 2,6-dimethoxy-quinone-GSH free radical has been implicated in the pathogenesis of diseases caused by dietary quinones. In addition, the activity of several enzymes, such as NAPD-linked 15-hydroxyprostaglandin dehydrogenase, GSH S-transferase isoenzymes and &gamma;glutamyl transeptidase can be inhibited by a variety of quinone-thioethers. Halobenzene nephrotoxicity may be mediated via quinone-thioether formation. Quinone-thioethers also exhibit anti-mitotic and anti-bacterial activity and have been shown to bind to DNA. There is, therefore, ample evidence attesting to the biological activity of quinone-thioethers.

1242. Bioreductive Activation of Quinones. Activation and Deactivation of Quinones by DT-diaphorase. David Ross, University of Colorado, Boulder, CO.

Reactions of electrophilic quinones with nucleophiles and one electron reduction of quinones to semiquinones followed by oxygen radical generation, are accepted as potential mechanisms underlying quinone toxicity. Two electron reduction of quinones to hydroquinones catalyzed by DT-diaphorase, on the other hand, is most often categorized as a detoxification mechanism. For example, DT-diaphorase plays a role in cell specific protection against 1,4-benzoquinone, derived from the benzene metabolite hydroquinone, in different bone marrow stromal cell types. Activation of quinones via DT-diaphorase is also a possibility, however, resulting in hydroquinones which can either autoxidize and generate oxygen radicals or lead to production of alkylating species. The role of DT-diaphorase in the bioreductive activation of antitumor quinones in human tumor cells has recently been examined. Using cell lines and DT-diaphorases purified from rat liver and human tumor cells, we have implicated DT-diaphorase in the bioactivation of AQZ and mitomycin C. These data suggest that following two electron reduction of either AQZ or mitomycin C, reactive alkylating species are generated which induce DNA crosslinking and cytotoxicity. Thus, DT-diaphorase mediated metabolism of quinones can result in either activation or deactivation and this is dependent on quinone structure.

1243. The Toxicity of Endogenous Quinones. Doyle G. Graham, Duke University Medical Center, Durham, NC.

Catecholaminergic neurons of the central and peripheral nervous systems autoxidizable substrates not present in other cells. Continuous autoxidation of catecholamines over the lifespan is evident in the progressive cytoplasmic deposition of neuromelanin, which results from polymerization of quinone oxidation products. It has been postulated that progressive injury to catecholamine neurons from quinone species and from reduced oxygen species may result in the loss of these neurons in aging and contribute to the degeneration of neurons in Parkinson's disease. This hypothesis suggests that L-dopa therapy may provide additional autoxidizable substrate while providing the brain with needed dopamine. The oxidation potentials for catecholamines and related polyphenols vary widely, as does the reactivity of resulting quinone species with cellular nucleophiles. The neurotoxicant 6-hydroxypseudopine undergoes rapid autoxidation and is cytotoxic in vivo and in vitro through synthesis of the products of partial reduction of oxygen. On the other hand, dopamine is the most cytotoxic of the naturally-occurring catecholamines because of both higher rate of autoxidation and greater availability of its orthoquinone to react with cellular nucleophiles. The chelation of manganese ions (Mn(II) and Mn(III)) by catecholamines results in acceleration of catecholamine oxidation and may account for the neurotoxicity of manganese.

Neurotoxicity Risk Assessment: State of the Art


Neurotoxicity guidelines exist to collect data that can be used by the risk assessment process to reduce the risks to human health associated with handling or exposure to neurotoxic chemicals. This presentation will explore issues of importance to the neuroscientist for understanding what data need to be collected and evaluated for developing a risk assessment. It will also cover issues important to the risk assessor for understanding what caveats are associated with neurotoxicity data so that limits are put around how the data are used.


The current practice for qualitative neurotoxicity risk assessment is to determine the no-observed-adverse-effect level and the lowest-observed-adverse-effect levels (LOAEL) in studies conducted by relevant guidelines. This presentation will deal with how experimental neurotoxicity data are evaluated in the assessment process to reach conclusions about the possible risks to human health.

1247. Quantitative Risk Assessments: The Reference Dose (RFD) and Research to Improve This Model Including The Use of Average Uncertainty Factors. Michael Dourson, EPA, Cincinnati, OH and Steven Lewis, Exxon Biomedical Sciences, East Millstone, NJ.

The Reference Dose (RFD) is a model similar to the Acceptable Daily Intake (ADI). Both have been used extensively for estimating exposures that are unlikely to be without any significant risk, for human populations. The RFD can be improved through a critical analysis of its subcomponents, the NOAEL or LOAEL and the uncertainty factors used in its calculation. This presentation will briefly summarize the RFD model and then highlight current research to improve it, including the use of average uncertainty factors.


The assessment of the risk associated with exposure to neurotoxins at environmentally relevant exposure levels which approach the NOAELs determined in animal studies can be very difficult because the uncertainties inherent in the current risk assessment methods are large and poorly defined. Under such conditions, an understanding of the basic biological principles underlying neurotoxicology may help to uncover where significant uncertainty exists and to put limits around the uncertainty.

Exogenous Modulation of In Vitro Hematopoiesis

Chairpersons: Oliver P. Flint and Michael Kowolenko, Bristol-Myers Squibb Company, Syracuse, NY.


The bone marrow often serves as the target organ for a wide variety of toxicants. In vitro hematopoietic culture systems serve an important role in determining mechanisms of toxicity within this system. The ability to control differentiation and proliferation within bone marrow cell systems enables the investigator to identify both cellular and biochemical targets of select toxicants. The objectives of this symposium are to: 1) provide an overview of hematopoiesis which includes the various autocrine loops involved in hematopoiesis and shows how modification of one autocrine can alter hematopoiesis, 2) demonstrate how stromal cell cultures can be used to provide data on the effects of anti-neoplastics on hematopoiesis, 3) display how antivirals alter bone marrow cell responsiveness to distinct colony stimulation factors, and 4) demonstrate how hematopoietic cell lines can be used to evaluate the molecular toxicity of antiviral compounds. As more information is gained concerning the interaction between the cells and factors involved in hematopoiesis through these in vitro systems, a greater understanding of the mechanisms of toxicity can be expected.

1250. Overview: Regulatory Factors Involved In Hematopoiesis. Louis M. Pelus, SmithKline Beecham Pharmaceuticals, King of Prussia, PA.

Hematopoietic cell differentiation and proliferation is controlled by specific growth stimulatory cytokines, the colony stimulating factors (CSFs), and regulated by a number of synergistic, stimulatory, and inhibitory cytokines. The CSFs have been cloned and are in clinical trials. Specific CSFs, i.e., granulocyte-CSF (G-CSF), granulocyte-macrophage (GM-CSF), interleukin-3 (IL3), macrophage-CSF(MCSF, CSF) and erythropoietin (Epo) control differentiation within specific cell lineages or across cell lineages. Thus G-CSF and M-CSF modulate the proliferation of lineage restricted granulocyte and macrophage progenitor cells (CFU-
GM, CFU-G, CFU-M) and many functions of the mature cells within these lineages. In contrast IL3 and to a degree GM-CSF modulate the proliferation of progenitor cells not yet committed to specific lineages (CFU-GEMM) and which have the capacity to differentiate down several lineages simultaneously (i.e., from granulocytes, macrophages, erythroid precursors and megakaryocytes). The proliferation of precursor cells for all of these lineages are tightly controlled but are capable of responding to an increased demand due to infectious disease or hematopoietic insult. One physiological regulatory which participates in homeostasis is Prostaglandin E2 (PGE2). This factor participates in a negative regulatory loop in controlling myelopoiesis, whereby elevated CSF levels induce PGE2 production which dampens myelopoiesis in vitro and in vivo mechanisms whereby PGE2 functions in controlling hematopoiesis have been delineated. A novel PGE2 induced myelopoietic inhibitor (MW 6-8 KD) and its potential mechanisms will be discussed.

1251. Long Term Bone Marrow Cultures (LTBMC) as an In Vitro Method for Detecting Toxicity to Stromal Cells. Daniel Wierda, Eli Lilly and Company, Greenfield, IN.

Clinical chemotherapy for cancer treatment frequently results in impaired hemopoiesis due to myelotoxicity. Ideally, new anticancer drugs should be active toward neoplastic cells but spares primitive stem cells and bone marrow stromal cells. The goal of the present study was to determine the feasibility of utilizing LTBMC as a model for predicting drug toxicity to stromal cells. Two to three month old LTBMC were established from mouse bone marrow cells under conditions conducive for lymphoid cell growth. Conditioned media removed from LTBMC induced the growth and differentiation of early progenitor B cells and pre-B cells as measured by immunofluorescence. No growth was observed in the absence of conditioned media. In separate studies, it was determined that LTBMC would support the growth of an IL-7 dependent B lineage cell line, designated SCID-7. IL-7 has previously been shown to stimulate proliferation of early B precursors and is likely involved in the stimulation of early B lineage development in LTBMC. Overall, these results indicate that assessment of soluble IL-7 production, and potentially, IL-7 mRNA expression in LTBMC will provide a method for evaluating the activity of antineoplastic drugs on bone marrow stromal cell function.

1252. Inhibitory Effects of Nucleoside Analogues on In Vitro Hematopoiesis. Michael Kowolenko, Bristol-Myers Squibb Pharmaceutical Research Institute, Syraucuse, NY.

The most effective treatments for inhibiting replication of HIV have been the nucleoside analogs, the prototypical compound being 3'-Azido-3'-deoxynucleosine (AZT). The limiting toxicity of AZT has been bone marrow suppression. The objective of these studies was to determine if in vitro treatment (24 hr or chronic) of bone marrow cells (BMC) with the nucleoside analogs 2',3'-dideoxynucleosine (ddI) or 2',3'-dideoxy-2',3'-dideoxynucleosine (ddC) resulted in similar effects on responsiveness to the growth factors Interleukin 3 (IL-3), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF), or Macrophage Colony Stimulating Factor (CSF-1), when compared to AZT. Stromal cells were also treated with AZT, ddI and ddC then assessed for their ability to support proliferation. The data obtained indicates that AZT (10g/ml) and ddC (10g/ml) inhibit colony formation responsiveness to all 3 growth factors upon chronic exposure. Acute exposure (24 hr) resulted in a reversal of the effects noted with ddC while AZT treated cells continued to support growth of soft agar overlays; however, liquid cultures treated with AZT produced fewer numbers of cells and displayed a shift in the granulocyte:monocyte ratio indicating a specific effect on granulocyte lineages of BFC.

1253. 3'-Azido-3'-Deoxythymidine (AZT) and its metabolite, 3'-Amino-3'-Deoxythymidine (AMT), Selectively Inhibit Globin Gene Transcription in Human K-562 Leukemia Cells. Jean Pierre Sommadossi, E. Creton, E.G. Bridges, and D.A. Weidner, University of Alabama, Birmingham, AL.

The major limitation to AZT treatment in AIDS patients is bone marrow toxicity, manifested as anemia and neutropenia. The present study examines potential mechanism(s) of AZT-induced anemia by studying the effect of AZT exposure on hemoglobin (Hb) synthesis machinery in by acute acid-induced K-562 human leukemia cells. As assessed by benzidine assay, exposure to AZT (25-250 µM) for 72 hrs resulted in an inhibition of Hb synthesis by as much as 60%. In contrast, other 2',3'-dideoxynucleotides which have been shown to be active against HIV in vitro were demonstrated to have minimal or no effect on Hb synthesis under similar conditions. These included 2',3'-dideoxyctydine, 3'-Azido-2',3'-dideoxyctydine, 3'-methyl-3'-Azido-2',3'-dideoxyctydine, 2'-deoxy-3'-thiacytidine (BCH-189), 2',3'-dideoxythymidine (dTm), and 3'-fluoro-3'-deoxythymidine (Fdt). For AZT treated cells, globin messenger RNA steady state level was inhibited from 48-77% (as measured by Northern blot analysis) at AZT concentrations between 25-250 µM with no measurable effects on actin mRNA level. The inhibition of globin mRNA level was associated with a decreased rate of globin transcription as determined by a nuclear run-on assay which demonstrated 20 and 50% decrease in globin specific in vitro synthesized transcripts at 25 and 100 µM AZT, respectively. AMT was recently identified in our lab as an AMT metabolite, both in vitro and in vivo. Studies of the effect of AMT on Hb synthesis machinery and globin gene expression demonstrated that AMT had a similar degree of inhibition to AZT. In conclusion, these data demonstrate that the parent drug, AZT, and its metabolite, AMT, specifically inhibit globin gene expression, which may play an important role in AZT-induced cytotoxicity to erythroid cells in patients.

Tritiated 2,3,7,8-tetrachlorodibenzo-p-dioxin (TrCDD), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TrCDF), 2,3,7,8-tetrachlorodibenzo-p-dioxin (PCDD), 2,3,7,8-tetrachlorodibenzo-p-dioxin (PCDF), and 2,3,7,8-tetrachlorodibenzo-dioxin (PCDD/F) were synthesized and the kinetics of their association with the rat cytosolic Ah receptor were determined. The affinities of these congeners for the Ah receptor as determined by conventional Scatchard analysis varied by less than two-fold (range of Kd: 5.0 - 9.3 nM). The temperature-dependent rate of association of these ligands with the Ah receptor was studied and the competing thermal inactivation of the unbound receptor was determined. The equilibrium constants, Keq, were also obtained as the ratio of the rate constants for dissociation and formation, respectively, of the receptor-ligand complex. The results of the kinetic studies showed that (1) activation energies for ligand binding were not temperature-dependent; (2) 2,3,7,8-TCDD and 2,3,7,8-TCDF bound significantly more slowly to the Ah receptor than the other radioligands at all temperatures (15-37°C) and this paralleled the lower biochemical potencies of the congeners; and (3) the Keq values obtained kinetically were in the sub-nM range, with the smallest Keq values observed for those ligands which bound most rapidly to the receptor. (Supported by N.I.H., ES03354.)


The photofinity labeling of the nuclear aryl hydrocarbon (Ah) receptor from mouse Hepa 1O17, rat hepatoma H-4-II-E and human Hep G2 cells using [3H]-TCDD as the photoligand was investigated. The cells were incubated with [3H]-TCDD in the presence or absence of actinomycin D and a 200-fold excess of 2,3,7,8-TCDF and after 3 hours the nuclear extracts were isolated and irradiated for 5 minutes (> 300 nm). The yields of specifically-bound photocovalent adducts from the Hep G2, H-4-II-E and Hepa 1O17 cells were 56 ± 10.6, 48 ± 12 and 36 ± 1%, respectively. Comparable yields were observed for the photolysis of nuclear extracts from cells treated with actinomycin D. SDS-PAGE analysis of the photocovalent nuclear Ah receptor adducts from Hep G2, H-4-II-E and Hepa 1O17 cells gave a single specifically-bound band with apparent molecular weights of 115-, 100- and 95 kDa, respectively, and these results correspond with photoscintuated cytosolic Ah receptor adducts. The in situ photolabeling of the nuclear Ah receptor in Hepa 1O17 cells was also investigated. The results showed that after a 3-minute irradiation of cell suspensions treated with [3H]-TCDD for 3 hours, the yield of nuclear Ah receptor photocovalts was 85 ± 2%. The results and applications of these photofinity labeling studies will be discussed. (Supported by N.I.H., ES03843.)

12 CHEMICAL CROSS-LINKING OF THE CYTOSOLIC AND NUCLEAR FORMS OF THE Ah RECEPTOR: DETERMINATION OF PROTEIN COMPOSITION. C. H. Furley, Department of Foods and Nutrition, Purdue University, West Lafayette, IN.

Upon binding a ligand such as 2,3,7,8-tetrachlorodibenzo-p-dioxin, the Ah receptor (AhR) apparently translocates into the nucleus and binds to specific DNA sequences. The addition of the photoaffinity ligand 2-azido-3-[125I]iodo-7,8-dibromodibenzo-p-dioxin to Hepa 1 cells in culture results in the specific labeling of the AhR. Both the cytosolic fraction and nuclear extract were isolated and subjected to sucrose density gradient analysis. The cytosolic fraction contained a single 95 peak of photoaffinity-labeled AhR. The nuclear extract contained both a 65 and a 95 peak. Both the nuclear and cytosolic forms of the photoaffinity-labeled AhR were subjected to chemical cross-linking with dimethyl pimelidimate and analyzed by SDS-PAGE. Cross-linking of the cytosolic form of the AhR revealed monomeric (97 kDa), dimeric (190 kDa), trimeric (280 kDa), and tetrameric (330 kDa) complexes. Cross-linking of the photoaffinity-labeled nuclear form of the AhR resulted in monomeric (97 kDa), and dimeric (184 kDa) complexes. In a time course of exposure to the cross-linking reagent, the largest forms given above become the predominant AhR forms in each extract. The 330 kDa cytosolic species apparently consists of a 97 kDa AhR, a dimer of 90 kDa heat shock protein and a 50 kDa protein. Previous work has shown that AhR is bound to HSF90 in cytosolic extracts. The 184 kDa nuclear AhR consists of a 97 kDa AhR, and an 87 kDa protein. (Supported by NIH ES04869.)


The dioxin receptor regulates transcription of the cytochrome P-450A1 gene in response to 2,3,7,8-tetrachlorodibenzo-p-dioxin or structurally related environmental contaminants. Upon ligand binding the dioxin-receptor complex becomes activated to a form which apparently translocates from the cytoplasm to the nucleus in vivo. More recently, we and others have shown that the in vivo activated dioxin receptor recognizes xenobiotic response elements (XREs) which modulate the activity of linked promoters, thereby serving as dioxin-inducible enhancers. We have used the dioxin receptor system to investigate the role of ligand in modulating functional activities of the receptor. To this end, we have developed an in vitro system in which addition of ligand to a partially purified latent (and cytosolic) form of the receptor leads to the activation of the XRE-specific DNA-binding activity. Our results indicate that the latent form of the receptor contains the 90 kD heat shock protein (hsp 90), that hsp 90 is required for efficient binding of ligand and that dissociation of hsp 90 is sufficient to unmask the cryptic XRE-specific DNA binding activity of the receptor. Importantly, in vitro activation of the receptor was mimicked by dioxin or other receptor ligands in a manner that directly reflected the rank order of their relative affinities for the receptor in vivo, and their relative potencies to induce gene transcription in vivo. In vivo, dioxin treatment does not alter the chromatin structure at the XREs at about -1,000 upstream of the rat P-450A1 gene during gene activation. In vivo we can detect a constitutive, nuclear XRE-specific factor which is distinct from the dioxin receptor. These data indicate that a specific DNA-protein architecture may be maintained at the XREs, regardless of the transcriptional state of the gene, and that such a structure could be important in strong and rapid transcriptional responses to extracellular signals.
ALTERATIONS IN THE STRUCTURE AND DNA BINDING OF Ah RECEPTOR IN THE PRESENCE OF POLYAMINES. C C Chao, M A Gallo, and T Thomas. Division of Toxicology, Department of Environmental and Community Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Polyamines, putrescine, spermidine, and spermine are organic cations present in all cells. They have multiple regulatory roles in cell growth and differentiation. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), and related polycyclic aromatic hydrocarbons exert their adverse effects by binding to an intracellular protein (Ah receptor). Interaction of Ah receptor with specific DNA sequences triggers gene regulatory effects of TCDD. We examined the effect of polyamines on the structure and DNA binding of Ah receptor isolated from a human squamous carcinoma cell line, A431. [3H]TCDD-labeled Ah receptor was sedimented in the 9S region of sucrose gradients in hypotonic buffer. Polyamines caused a concentration dependent condensation and precipitation of Ah receptor. In the presence of 1 mM spermine the receptor was completely precipitated which could be recovered from the bottom of the sucrose gradients. This precipitation did not occur with RNase-treated Ah receptor. Incubation of RNase-treated Ah receptor with 1 mM spermidine increased its DNA binding 10-fold compared to controls having equivalent ionic strength. These results suggest that endogenous polyamines may influence the structural organization and gene regulatory effects of TCDD. NIH grant CA 42439 (T.T.)

EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON T47-D HUMAN BREAST CANCER CELL LINES. P Fernandez, M Harris and S Safe, Dept. of Vet. Phys. & Pharm., Texas A&M Univ., College Station, TX.

The T47-D human breast cancer cell line contains both the aryl hydrocarbon (Ah) and estrogen receptor (ER) but exhibits only limited estrogen responsiveness. TCDD induces monoxygenase enzyme activities in these cells and causes a downregulation of the nuclear ER receptor. However, progesterone receptor levels in this cell line were not modulated by TCDD, 17β-oestradiol or TCDD plus 17β-oestradiol. 17β-Estradiol (10-6 M) did not significantly increase T47-D cell proliferation whereas treatment of the cells with transforming growth factor-α (TGF-α) did cause a concentration-dependent increase in cell proliferation with the maximum response (ca. 2-fold) observed at a concentration of 10 ng TGF-α/ml.

It was also evident from the cotreatment studies that TCDD caused a concentration-dependent decrease in the TGF-α-induced proliferation of T47-D cells. Parallel experiments on the effects of TGF-α, TCDD and TCGD plus TGF-α on [3H]-thymidine uptake in these cells will also be discussed along with the proposed mechanism of TCDD-induced growth inhibition. (Supported by N.I.H., ESO4176.)

EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON GROWTH FACTOR-INDUCED PROLIFERATION OF HUMAN BREAST CANCER CELLS. L Biegl, D Dennis and S Safe, Dept. of Vet. Phys. & Pharm., Texas A&M Univ., College Station, TX.

TCDD exhibits a broad spectrum of antiestrogenic activities in Ah-responsive MCF-7 cells but not in Ah non-responsive MDA-MB-231 cells. For example, in MCF-7 cells, TCDD significantly inhibits 17β-oestradiol-induced cell proliferation. However, in this cell line, estrogens also induce the secretion of diverse growth factors and mitogens which can stimulate cell growth in an autocrine and paracrine fashion. Both insulin-like growth factor-I (IGF-I) (0.01 - 10 nm) and epidermal growth factor (EGF) (0.01 - 10 ng/ml) caused a concentration-dependent increase in growth of MCF-7 cells using DME-F12-depleted media, 3% synthetic fetal calf serum and 1% fetal calf serum treated with DCC. Cotreatment of the cells with 10 nm TCDD and the different concentrations of the growth factors resulted in significant inhibition of EGF- and IGF-I-induced cell proliferation. In contrast, TCDD did not modulate growth-factor-induced responses in MDA-MB-231 cells. These data suggest that the TCDD inhibits the growth of MCF-7 cells by modulating the actions of both 17β-oestradiol, EGF and IGF-I. (Supported by N.I.H., ESO4176.)

INDUCTION OF CYPIA1 GENE TRANSCRIPTION BY TCDD IN MDA-MB-231 HUMAN BREAST CANCER CELLS. L Arrellano, M Harris and S Safe, Dept. of Vet. Phys. & Pharm., Texas A&M Univ., College Station, TX.

MDA-MB-231 human breast cancer cell lines express the aryl hydrocarbon (Ah) receptor which accumulates in the nucleus of these cells. However, treatment of MDA-MB-231 cells with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) did not induce P-450IA1 mRNA or the associated monooxygenases, aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin O-deethylase (EROD) activities. In a parallel study the cells were treated with solvent (DMSO; 6 hr), TCDD (10-4 M; 5 hr), and TCDD (10-4 M; 5 hr) plus 1 hr pretreatment with 10-5 M cycloheximide. After 6 hr the P-450IA1 mRNA was measured by Northern analysis and the levels (normalized to β-tubulin) were 0, 0 and 0.569 ± 0.125 RNA units, respectively. The results suggest that the repression of TCDD-induced CYPIA1 gene expression in MDA-MB-231 cells may be due to protein factor(s) which are inhibited by the cycloheximide treatment. (Supported by N.I.H., ESO4176 and ESO3843.)
The effects of TCDD on glycolysis in MCF-7 human breast cancer cells were investigated by $^{13}$C-NMR spectroscopy using a Bruker WM-300WB instrument. 1,6-$^{12}$C2-Glucose was added to MCF-7 cells which had been pretreated with either 17β-estradiol (10^{-8} M), TCDD (10^{-8} M) or TCDD plus 17β-estradiol, and the time-course metabolism of glucose was determined. In control cells, 1580 nmol of lactate/4x10^{3} cells was formed over a 7-hour time period whereas the corresponding values in the 17β-estradiol, TCDD and 17β-estradiol plus TCDD-treated cells were 2235, 1860 and 1059 nmol lactate/4x10^{3} cells, respectively. The results showed that 17β-estradiol significantly increased lactate formation in these cells, TCDD alone did not alter glycolysis whereas TCDD significantly inhibited 17β-estradiol-induced glucose metabolism. The significance of these and other effects on glycolysis will be discussed. (Supported by N.I.H., ESO4176.)

Treatment of immature female Sprague-Dawley rats with 6-NCDF caused a dose-response increase in rat uterine weight 48 hours after treatment. At a dose of 25 μmol/kg the uterotropic effects of 6-NCDF and 17β-estradiol (5 μg/rat x 2) were comparable. 17β-Estradiol also caused a significant increase in rat uterine cytosolic and nuclear estrogen and progesterone receptor levels whereas 6-NCDF significantly increased only the cytosolic progesterone receptors in the rats. In contrast, 17β-estradiol caused approximately a 100% increase in rat uterine peroxidase activity whereas 6-NCDF, like 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) caused a > 50% decrease in this response compared to (vehicle) control-treated animals. 6-NCDF competitively displaced [3H]-TCDD from the uterine cytosolic Ah receptor but did not exhibit affinity for either the uterine estrogen or progesterone receptor. The data and other results suggest that the estrogenic effects of 6-NCDF may be mediated through the Ah receptor. (Supported by N.I.H., ESO4176.)

Treatement of ary hydrocarbons (Ah)-responsive MCF-7 cells with 1 μM 17β-estradiol results in increased cell proliferation and the secretion of the 34-, 52- and 160-kDa proteins. Treatment of the cells with 1 μM TCDD alone causes a slight suppression of cell growth and the induction of CYPIA1 gene expression and cotreatment of the cells with TCDD plus 17β-estradiol results in significant antagonism of 17β-estradiol-induced cell growth and the secretion of the 34-, 52- and 160-kDa proteins. Previous studies have shown that αNF antagonizes TCDD-mediated monooxygenase induction in transformed rodent cells and it was apparent from this study that αNF also antagonized the TCDD-mediated induction responses in MCF-7. Since it is hypothesized that TCDD acts as an antiestrogen through the Ah receptor, the effects of αNF as an anti-antiestrogen were investigated. In MCF-7 cells treated with TCDD (1 μM) plus 17β-estradiol (1 μM) or TCDD (1 μM) plus 17β-estradiol (1 μM) plus αNF (1, 0.1 and 0.01 μM), αNF inhibited the anti-estrogenic effects of TCDD in a concentration-dependent manner and the results confirm the role of the Ah receptor in the antiestrogenic responses elicited by TCDD. (Supported by N.I.H., ESO4176 and ESO3845.)

MC7 human breast cancer cells contain the ary hydrocarbon (Ah) receptor and have been characterized as a TCDD-responsive cell line. Treatment of the cells with 17β-estradiol (E, 10^{-7} M) causes a marked increase in cell proliferation (1.5- to 2.5-fold) and TCDD significantly inhibits 17β-E-induced cell growth. The interactive effects of 17β-E and TCDD on the expression of c-erbB-2 (HER-2/neu), IGF-I receptor and c-myc protooncogene mRNA levels were investigated over a 24-hour time period after treatment. The results from these studies were somewhat variable and time-dependent. Within 0.5 - 6 hours after treatment, 17β-E caused a 2- to 3-fold increase in IGF-I receptor mRNA levels whereas TCDD did not effect these induced mRNA levels. In contrast, TCDD suppressed the 17β-E-induced c-myc protooncogene mRNA levels which were observed only within short time periods after treatment (0.5 - 3 hours; the time and extent of these interactions were variable). In contrast, TCDD, 17β-E and TCDD plus 17β-E did not alter the HER-2/neu mRNA levels during the first 12 hours after treatment. However, 17β-E increased message levels after 24 hours and TCDD inhibited the 17β-E-induced response. The significance of these results will be discussed. (Supported by N.I.H., ESO4176.)
The mechanism by which fibrogenic particulates cause pulmonary fibrosis in humans is not understood, but it is likely to involve the alveolar macrophage, the resident phagocytic cell of the lung. The alveolar macrophage is capable of secreting a wide variety of products, many of which have effects on other lung cell types. We have used human alveolar macrophages together with known fibrogenic and non-fibrogenic particulates as components of an in vitro model system. We have quantitated the particulate-stimulated release of several macromolecule products, including superoxide anion (O2−), tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and prostaglandin E2 (PGE2). The fibrogenic particulates asbestos and silica generally stimulate the release of these products while two non-fibrogenic particulates, namely titanium dioxide and iron carbonyl, generally did not. Interestingly, the spectrum of products stimulated by silica and asbestos were quite different. While asbestos stimulated the release of O2− exclusively, silica did not cause O2− release, but rather stimulated the release of TNF-α, IL-1β, and PGE2. Thus, these data suggest that asbestos may stimulate the human alveolar macrophage through a pathway that involves activation of phospholipase C, while silica may stimulate a pathway that involves activation of phospholipase A2. This work was supported in part by NIH grant ES-04804 and NIH grant M01-RR-02558.

**Comparison of Pulmonary Responses to Mineral Dust Administered by Intratracheal Instillation or Inhalation in Rats**

This study compared the lung's response to similar lung burdens of silica (SiO2) or titanium dioxide (TiO2) administered to rats by a single intratracheal instillation (IT) exposure (10 mg/kg) or 5 consecutive inhalation exposures (50mg/m3 x 6/h/d). Responses were assessed by determining bronchoalveolar lavage fluid (BALF) LDH, total protein (TP), cell number and type, release of fibronecin by alveolar macrophages (AM) and lung burden. Lung burdens determined 24 hr after IT or inhalation ranged from 1.8-2.1 mg dust/lung. Significant differences in SiO2 vs TiO2-induced responses were observed after both IT and inhalation exposure. SiO2 significantly increased BALF LDH, TP, neutrophils and lymphocytes relative to TiO2 or control groups. SiO2, but not TiO2, stimulated AM release of fibronecin. Differences in response associated with the method of exposure were observed. Responses were from greater magnitude after IT vs inhalation exposure. Additionally, increases in BALF parameters and AM fibronecin release occurred earlier (day 3) after IT than inhalation (day 14 BALF parameters; day 63 fibronecin). Lastly, TiO2 elicited transient elevations in BALF LDH, total protein and neutrophils after IT but not after inhalation. These data demonstrate qualitative similarities exist in respiratory tract responses to dust administered IT vs inhalation. However, the method of dust exposure can markedly effect the magnitude and temporal pattern of response.

**The In Vitro Modification of Asbestos Bioactivity Towards Human Alveolar Macrophages by the Major Alveolar Lining Fluid Components**

Asbestos is thought to induce damage to the lung and initiate fibrosis by several means. One of the mechanisms proposed for this damage involves the production of superoxide anion (O2−) by the alveolar macrophage in response to its incubation with asbestos. In this study we examine the effect of IgG, and the major protein and lipid constituents of alveolar lining fluid, namely, albumin and dipalmitoylphosphatidylcholine (DPPC), on asbestos-stimulated O2− production by human alveolar macrophages. We show that both chrysotile and crocidolite asbestos, but not silica and aluminum beads, stimulate the human alveolar macrophage to produce O2−. Preincubation chrysotile and crocidolite asbestos with IgG resulted in an enhancement of their ability to stimulate O2− production. After preincubation with IgG, both silica and aluminum also stimulated O2− production to levels similar to the IgG-preincubated asbestos. The subclasses of IgG were studied to examine the subclass specificity of this enhancing effect; on a molar basis IgG1 was most potent. Further, we show that the inclusion of albumin and DPPC in the preincubation mixture does not negate this enhancing effect. In summary, these results show that IgG can significantly enhance the bioactivity of chrysotile and crocidolite asbestos for the human alveolar macrophage in vitro. This work was supported in part by NIH grant ES-04804 and NIH grant M01-RR-02558.
We have shown previously that CHR stimulates the production of superoxide anion (O2-) by guinea pig (gp) alveolar macrophages (AM). Prolonged production of O2- in vitro by AM may cause lung injury. It is likely that CHR fibers deposited in the lower respiratory tract are coated by the components of LLF. Therefore, we have used gp LLF as part of an in vitro model system to examine the effects of LLF on CHR-stimulated O2- production by gpAM. CHR was preincubated with LLF and washed 3X with modified Hank's balanced salt solution. Compared to untreated and washed CHR, LLF-treated CHR was only 40% as active in stimulating O2- production. Fractionated LLF components and pure phospholipids were tested individually for their effects on CHR bioactivity. Isolated lung surfactant (LS) decreased CHR-stimulated O2- production by 95%. This inhibitory activity was shown to reside in a chloroform extract of LS containing lipids and hydrophobic proteins. Inhibition of O2- production could be duplicated with phosphatidylinolein (PI) and phosphatidylglycerol (PG) at concentrations equivalent to that found in LS; phosphatidylcholine, phosphatidylethanolamine, and phosphatidisyamine did not affect O2- production. These results strongly suggest that the PI and PG found in LLF can modify CHR bioactivity for the alveolar macrophage. This work was supported in part by NIEHS grant ES-04804.

BLEOMYCIN INDUCTION OF CYTOKINE RELEASE BY THE HUMAN ALVEOLAR MACROPHAGE. R. K Scheule, R Hamilton, R C Perkins, and A Holian. Departments of Internal Medicine and Pharmacology, The University of Texas Medical School at Houston, Houston, TX.

Bleomycin is an antineoplastic drug whose use is limited by the pulmonary fibrosis that it causes. The mechanism by which bleomycin causes fibrosis is not understood, but is proposed to involve the alveolar macrophage. The alveolar macrophage is a central cell in the cytokine network of the lung, and as such may be involved in the fibrotic process. We have treated human alveolar macrophages (isolated from normal subjects by bronchoalveolar lavage) with bleomycin in vitro and examined the resultant macrophage secretory products. A 24 h treatment with bleomycin (0.5-100 ml/ml) was found to result in a dose dependent (i) decrease in the ability of the macrophage to produce superoxide anion in response to phorbol dibutyrate, (ii) increase in secreted tumor necrosis factor-α (TNF-α), (iii) increase in interleukin 1β (IL-1β), and (iv) decrease in intracellular cyclic AMP (cAMP) levels. Exogenous cAMP partially blocked bleomycin stimulated cytokine production suggesting a potential regulatory role for cAMP in this process. Kinetic studies indicate that the secretion of TNF-α precedes the release of IL-1β, a finding that is consistent with a potential autoregulatory role for TNF-α in the release of other cytokines. Thus, these data support a role for the pulmonary macrophage in the development of bleomycin-induced lung fibrosis. This work was supported in part by NIEHS grant ES-04804 and NIH grant GM-01-RR-02556.

RESPONSE OF ALVEOLAR MACROPHAGES TO FRESHLY FRACTURED VS AGED SILICA: PROTECTIVE ACTION OF COATING WITH ORGANO-SILANES. V. Castranova, V Velleythan, J H Kang, N S Dalal, and K Van Dyke. NIOSH and West Virginia Univ., Morgantown, WV.

The object of this investigation was to determine whether freshly cleaved silica particles were more bioactive than aged silica and to evaluate the ability of an organo-silane material to coat silica and reduce its toxicity. Crushing crystalline silica with a ball mill grinder results in the generation of silicate-based radicals on the cleavage planes which are measurable by electron spin resonance spectroscopy and that decay with time after grinding. In vitro, both fresh and aged silica exhibit bioactivity toward alveolar macrophages. However, compared aged dust, fresh silica is 4.2 fold more cytotoxic, induces 50% more hydrogen peroxide secretion, and stimulates 4.6 fold more cellular chemiluminescence. Prossil 28, an organo-silane material, is an effective coating agent for freshly ground silica. It abolishes surface radicals associated with silica cleavage; decreases cytotoxicity of fresh silica by as much as 78%; and decreases the ability of fresh silica to induce chemiluminescence from alveolar macrophages by 58%. These data suggest that surface radicals associated with freshly cleaved silica may be an important factor in the induction of pulmonary disease. Furthermore, treating dust with coating agents may substantially decrease its pathogenicity.

BINDING OF RADIOLABELLED ASBESTOS FIBERS TO GUINEA PIG (gp) ALVEOLAR MACROPHAGES (AM). M A Giannotti, T J Tewson, M P Franchinetti, R K Scheule, and A Holian. Departments of Internal Medicine and Pharmacology, The University of Texas Medical School at Houston, Houston, TX.

The mechanism by which fibrogenic particulates cause pulmonary fibrosis in humans is not understood, but is likely to involve the AM. Using two fibrogenic particulates, namely, chrysotile (CHR) and crocidolite (CRO) asbestos and gpAM as components of an in vitro model system, we have shown that CHR stimulates the gpAM to release superoxide anion, but CRO does not. To examine whether this difference in stimulatory abilities is a result of differences in cell-asbestos binding, we have developed an efficient procedure that radioisables asbestos fibers while retaining their bioactivity. The fibers are labeled with 68Ga. The 68Ga decays into 68Ga, which can then be detected by its characteristic positron emission. Both CHR and CRO asbestos were radio labeled successfully. Mild reaction conditions and short reaction times were found under which 30% of the added 68Ga and 68Ga bound to the fibers. The radio label was retained even after washing the fibers extensively with physiologic buffers. A density gradient procedure was developed to quantitate the binding of asbestos to gpAM in suspension. The binding of both fibers increased with time over one hr. Thus, these results indicate that although both CHR and CRO interact with the gpAM, only CHR interacts productively to stimulate superoxide anion release. This work was supported in part by NIEHS grant ES-04804.
ROLE OF THE NEUTROPHIL IN THE SILICA-INDUCED INCREASE IN Lavaged Enzymes and Protein in the Lung.
SH Gouveia, EA Freund, LA Stanickiy, MA Hartschy, MC Karakostas, and DE Wareheit. E. I. du Pont de Nemours and Co., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE.

Polymorphonuclear neutrophils (PMNs) which are recruited into lung airways during inflammation may injure the lung by releasing cytotoxic oxygen radicals and proteases. In order to evaluate the role of PMNs in silica-induced lung inflammatory responses (100 mg/kg 6 hr/day, 3 days), rats were depleted of PMNs by i.p. injection of an anti-rat PMN antiserum prior to exposure (group IV), and inflammatory responses were compared to normal serum-injected exposed rats (group II) and sham-exposed normal or PMN-depleted rats (groups I and III, respectively). Lavaged fluids from group II rats contained 50% PMNs immediately after exposure compared to less than 10% for both groups I and III. Successfully depleted group IV lavage contained < 1% PMNs. Lavaged protein, indicative of alveolar epithelial permeability, was not significantly different between groups II and IV, but was significantly greater in both groups than in control groups I and III. Likewise, other lavage parameters of lung injury, such as N-acetylglucosaminidase, fibrinogen, and lactate dehydrogenase, though elevated above control levels, were not significantly different between groups II and IV. The results indicate that PMNs do not contribute to the secretion of enzymes and protein in the lung airways following acute pulmonary exposure to silica.

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ROLE OF NEUTROPHILS IN ACETAMINOPHEN-INDUCED LIVER INJURY.
H Jaeschke, CW Smith and A Farhood, Dept. Medicine, Pediatrics and Pathology, Baylor College of Medicine, Houston, Tx. Sponsor: C V Smith.

Recent studies from our laboratories showed that neutrophils (PMNs) contribute to liver injury in endotoxin shock and during reperfusion after hepatic ischemia (FASEB J. 1990, in press). To test if PMNs are also involved in acetaminophen (AAP)-induced liver damage, fasted male ICR mice received a non-lethal dose of 500 mg AAP/kg. Parenchymal cell injury started at 3 h and progressed up to 9 h after AAP administration (plasma alanine aminotransferase activity: 3800 ± 500 U/l). Histological assessment of PMNs accumulated in the liver indicated an initial increase at 3 h and maximal values of 85 ± 25 PMNs per 50 high power fields 6 h after AAP (basal values: ± 3 PMNs/50 HPF). However, in the endotoxin shock or ischemia/ reperfusion injury models 600-1000 PMNs/50 HPF were observed in the liver. Microvascular injury and hemorrhage (assessed as hepatic hemoglobin content) was found at 3 h after AAP.

Pretreatment of mice with an anti-CD18 monoclonal antibody (M18) (2 mg/kg) to block adhesion molecules on the surface of the neutrophil did not affect parenchymal cell injury, hemorrhage or PMN accumulation in the liver after AAP but significantly reduced hepatic PMN infiltration and cell injury in the endotoxin shock model. Our data support the conclusion that hepatic PMN infiltration during AAP-induced hepatotoxicity is a consequence of the injury; PMNs do not seem to contribute to parenchymal cell damage in this model. (Supported by NIH grants GM 42957 and HL 42550).

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ENHANCEMENT OF ENDOTOXIN (LPS) AND TUMOR NECROSIS FACTOR (TNF) BY LEAD (Pb); POSSIBLE MECHANISMS. R Honchel, S Shedlofsky, D Cohen, C McClain. Departments of Medicine and Immunology and Graduate School of Toxicology, University of Kentucky Medical Center and VAMC.

Previous studies have shown that lead given simultaneously with endotoxin markedly enhances LPS lethality. Tumor necrosis factor (TNF) mediates much of the cellular injury of LPS. Therefore we evaluated whether or not Pb enhances TNF toxicity and lethality in a fashion similar to LPS. We demonstrated that in Pb treated rats injected with either TNF or LPS, the major toxicity was to the liver (serum SGOT levels: LPS=34126; TNF=35852; LPS+Pb=263362; TNF+Pb=1637215). Pb given with TNF markedly increased the liver sensitivity to TNF toxicity ("superinduction"). Furthermore, Pb plus LPS produced significantly higher peak serum TNF levels (1296±216 U/ml vs. 297±45) than LPS alone, suggesting that lead also might increase TNF production ("priming"). Downregulation of cytokine production by pretreatment with either dexamethasone or prostaglandins markedly attenuates Pb plus LPS liver injury and mortality. Because many of the effects of prostaglandins and glucocorticoids are mediated via cAMP, we evaluated whether dibutyryl cAMP treatment would protect against Pb plus LPS liver injury and mortality, and we observed no protective effect. Pretreatment with anti-TNF antibody administered at a dose that neutralized peak serum TNF levels in vivo was not able to attenuate LPS plus Pb mortality. Further, studies will be performed using much higher doses of anti-TNF antibody. These findings suggest a therapeutic role for agents that regulate cytokine production in certain types of TNF toxicity.
A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR A SIMULANT FOR TOXIC GASES IN PRIMATES.
W D Crank, J R McDonalz, A Vinegar, LNSI, Technology Services Corp., Dayton, OH and
Wright-Patterson AFB, Dayton, OH.

An innocuous simulant for the uptake of toxic gases could be used in training exercises to test the effectiveness and proper use of protective equipment. Exposure to a simulant in a training scenario would be inferred afterwards by measuring the concentration of simulant in expired breath. Chloropentafluorobenzene (CPFB) has been proposed as such a simulant. To understand the relationship between exposure and CPFB concentration in expired breath, we have developed a physiologically based pharmacokinetic (PB-PK) model for CPFB in primates. To test the model, inhalation exposures were conducted on anesthetized rhesus monkeys. Simulations of CPFB uptake and clearance agreed well with experimental measurements in seven of eight monkeys. A human version of the model was used to simulate exposures consisting of a single breath or a few breaths of air containing 1, 10, or 100 ppm CPFB. By showing a measurable CPFB concentration (greater than 1 ppb) in expired breath after several hours of clearance, simulations with the human model indicated the suitability of CPFB as a simulant for toxic gases. (Supported by DOD Contract No. F33615-85-C-0532.)

A PHYSIOLOGICALLY-BASED DESCRIPTION OF ETHYLENE OXIDE DOSIMETRY IN THE RAT. K
Krishnan, ML Gargos, TR Fennell and MF Andersen, CIIT, Research Triangle Park, NC.

Ethylene oxide (EO), a sterilant and direct-acting alkylating agent, is a suspect human carcinogen. EO is metabolized by conjugation with glutathione (GSH) and hydrolysis. EO forms hydroxyethyl adducts in both DNA and hemoglobin (HB). To provide quantitative information on the disposition of EO, a physiologically-based pharmacokinetic (PB-PK) description was developed by integrating information on rat physiology, tissue:blood partitioning and metabolism of EO. This model consisted of the following tissue groups: lung, liver, brain, testes, arterial and venous blood, fat, richly perfused and poorly perfused tissues. The fate of EO in individual tissues was described by accounting for GSH conjugation, hydrolysis and binding to DNA. The partition coefficients of EO (blood:air, 159; liver:air, 311; tests:air, 134; lung:air, 86; brain:air, 61; muscle:air, 80; fat:air, 27) were determined by vial equilibration methods. The rate constants for metabolism and binding (DNA, HB) were estimated from the literature. This model simulated the levels of HB and DNA adducts, GSH depletion and urinary metabolites resulting from inhalation exposures of 1 to 1,200 ppm of EO in the rat. This PBPK description will be used in determining the relationship between EO dose, and DNA- and HB-adducts in animals and people.

PHYSIOLOGICALLY BASED PHARMACOKINETIC (PB-PK) MODEL FOR UPPER RESPIRATORY
TRACT (URT) UPTAKE AND METABOLISM OF VAPORS. J B Morris and J J Clay.
Toxicology Program, School of Pharmacy, University of Connecticut, Storrs, CT.

Uptake of several vapors including xylene, isomyl alcohol and ethyl acetate has been measured in the surgically isolated URT of the F344 rat and Syrian hamster. In vivo metabolic inhibitor studies indicated that 1-6% of inspired xylene or isomyl alcohol was metabolized in the URT via mixed function oxide or alcohol dehydrogenase, respectively. Roughly 10-30% of inspired ethyl acetate, a carboxylesterase substrate, was metabolized in that site. Uptake data were modeled via a "well-stirred" venous equilibration model or parallel tube model, with the former providing a better fit. Estimated in vivo metabolism rates were never greater and were often several-fold less than the rates determined in vitro in nasal tissue homogenates. Inclusion of a nasal compartment may further refine PB-PK models of rodent solvent vapor uptake. (Supported by NTH-E303676.)

PHYSIOLOGICALLY-BASED MODEL PARAMETER ESTIMATION FROM PERCHLOROETHYLENE TISSUE
PHARMACOKINETICS. CE Dallas, JM Gallo, XM Chen, S Maralidhara, K O'Barr and NV Bruch..
Dept. of Pharmacology and Toxicology and Pharmaceutics, College of Pharmacy, University of Georgia, Athens, GA.

Physiologically-based pharmacokinetic (PBPK) models are a logical approach to improving the scientific basis for interspecies extrapolations in risk assessments of halocarbons and other volatile organic chemicals. In order to improve the accuracy of the model predictions, tissue-blood partition coefficients were estimated using direct measurements of a halocarbon in rat tissues during and following exposure. Male Sprague-Dawley rats inhale 500 or 2500 ppn perchloroethylene (PER) for up to 2 hr in dynamic inhalation exposure chambers. Serial samples of brain, liver, kidney, lung, heart, skeletal muscle, adipose tissue, and blood were taken during exposure and up to 72 hr post-exposure. Blood and tissue samples were analyzed for PER content using a GC-ECD headspace technique. Maximum tissue concentrations (Cmax) were achieved for most tissues within 15 min, with adipose tissue achieving a Cmax up to 2 hr post-exposure. There were similar terminal elimination half-lives (t1/2) for each of the tissues, except for the fat which exhibited a longer t1/2 value. As comparable tissue Cmax are consistent with a blood-flow limited model, tissue-blood partition coefficients can be calculated for non-eliminating compartments by division of the tissue area-under-the-tissue-concentration-time curve (AUC) by the blood AUC. For the liver, the first-order metabolic rate constant and tissue PER concentrations are also employed in the calculation. Tissue concentration-time data can thus provide valuable input for halocarbon PBPK model parameter estimates (Supported by U.S. Air ForceAFOSR 870248).
A physiologically-based toxicokinetic model for fish was used to simulate the uptake and disposition of three waterborne chloroethanes in channel catfish (Ictalurus punctatus). Channel catfish were exposed to 1,1,2,2-tetrachloroethane (TCE), perichloroethane (PCE), and hexachloroethane (HCE) in fish respirometer-metabolism chambers to assess the kinetics of chemical accumulation in arterial blood and chemical extraction efficiency from inspired water. Chemical residues in tissues were measured at the end of each experiment. Extraction efficiency for TCE was close to 0% at 48 h, suggesting that fish were at or near steady-state and that systemic elimination was small. Extraction efficiencies for PCE and HCE at 48 h were approximately 15% and 35%, respectively. Parameterized with chemical partitioning data obtained in vitro and with metabolic rate and capacity parameters set equal to zero, the model accurately simulated the uptake of all three chloroethanes in blood and tissues and their extraction from inspired water. When compared with the results of earlier studies in which rainbow trout were exposed to the same chloroethanes, these data suggest that catfish approached steady-state more rapidly than trout. These results provide support for the basic model structure and the accuracy of physiologically input parameters.

ACN causes brain tumors in rats but has not been tested in other species. Hepatic oxidation of ACN to the mutagenic CE0 is probably an activation step which glutathione conjugation represents detoxification. Tissue PC3s for ACN and CE0 were determined by a modified single equilibration method to refine and expand the physiologically-based pharmacokinetic model for ACN (Gargas et al., Toxicologist, 10, 216). Rat tissues were equilibrated with ACN or CE0 in vibrating capped vials at 37°C. Headspace was analyzed by gas chromatography and tissue:air PC3s were calculated for brain, muscle, fat, and liver. Dithyl maleate (DEM) abolished the active uptake of ACN by blood, consistent with the known reactivity of ACN with sulfhydryl groups. DEM had no effect on the active uptake of CE0 by blood, so its PC3 was estimated by zero extrapolation of the time-dependent change in uptake. The fat: blood PC3s for ACN and CE0 were similar, but those in other tissues were 10- to 30-fold greater for CE0. The high tissue solubility of this mutagenic metabolite of ACN may enhance its uptake by extrahepatic target organs.

Physiologically-based toxicokinetic (PB-TK) models for fish require biologically relevant estimates for the enzyme kinetic parameters, $K_a$ and $V_{max}$. However, incubation temperatures used during in vitro biotransformation studies are often selected to maximize reaction rate, without regard for physiological temperature. The enzyme kinetics for N-hydroxylation (N-OH) of 4-chloroaniline (4-Cl) by rainbow trout acclimated to 11°C were investigated in vitro at incubation temperatures of 11°C and 25°C. The $V_{max}$ values for N-OH activity were 6.4 ± 0.6 and 22.0 ± 1.0 pmole/min/mg at 11°C and 25°C, respectively. The $K_a$ values were 0.5 ± 0.2 and 0.8 ± 0.1 mM at 11°C and 25°C, respectively. A PB-TK model for trout was then used to simulate the impact of the choice of $K_a$ and $V_{max}$ values on the uptake and disposition of inhaled 4-Cl. These simulations indicate that model sensitivity to kinetic rate and capacity parameters varies as a function of exposure concentration; at low exposure levels, metabolic pathways are saturated and the choice of kinetic constants has little effect on model outputs. At low exposure levels, however, the choice of kinetic constants has a significant impact on model predictions. These studies suggest that in vitro incubation temperature must be considered when kinetic parameter estimates are incorporated into PB-TK models for fish or other poikilothermic organisms.

Determination of cardiac output, tissue blood flow, volume and lipid content in sprague-dawley rats. RO Manning, MK Delp, RB Armstrong, and JY Bruckert, Dept. Pharmacol. & Toxicol., and Dept. Physical Education, Univ. of Georgia, Athens, GA.

One critical aspect of physiologically-based pharmacokinetic (PBPK) model development is the choice of values for organ blood flows, cardiac output and tissue volumes for input into models. These values vary depending upon the strain, size, age, and sex of animal for which a PBPK model is being developed. Tissue blood flows, cardiac output, tissue volumes, and lipid content were determined in male S-D rats, (350-375 g, N=8). A radiolabeled microsphere method utilizing Scandium ($^{46}$Sc), Tin ($^{113}$Sn), and Gdoluminium ($^{155}$Gd) was used to determine blood flow. Each rat received 3 radiolabeled injections. After the third injection, animals were sacrificed, and radioactivity in each tissue was determined in a 3-channel gamma counter. Tissues sampled include brain, heart, kidneys, liver, lungs, spleen, pancreas, adrenals, stomach, intestines, colon, testis, bone and skeletal muscle. Cardiac output was 142 ml/min. Blood flow values for eliminating organs were 0.49 (liver), 15.52 (kidney), and 1.77 (lung) ml/min/g tissue. Tissues which had significantly increased blood flow during the dark cycle included femur, abdominal fat, triceps brachii and abdominal muscles, stomach, spleen and lung. Dissociable fat, organ volume, and organ lipid content were determined in separate groups of rats (N=8). Volume and lipid content were determined for the same tissues as blood flow. Body fat was 7.35 % of bw and extractable lipid content of eliminating organs was 42.3 (liver), 45.4 (kidney), and 35.9 (lung) mg/g tissue. Precise measurements should improve the accuracy of PBPK model predictions, and therefore help in reducing uncertainties in risk assessment of volatile organics and other pollutants. (Supported by U.S. EPA CR-816238)

We have developed a generic physiological pharmacokinetic model capable of tracking the concentrations of a parent compound and 3 metabolites in blood and many tissues, including liver, kidney, fat, skin, muscle, and components of the gastrointestinal tract. This model is an extension of a model previously developed for lipophilic chemicals, which allowed for oral and/or intravenous dosing and gave a complete simulation of gastrointestinal transport, including enterohepatic recirculation. The metabolite-tracking model includes organ growth and enzyme induction submodels, plus tissue-specific metabolism submodels that utilize uptake rates, partition coefficients, and metabolism rates that can be obtained from in vivo or in vitro experiments.

THE DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL FOR 2-BUTOXYETHANOL METABOLISM IN RATS FOLLOWING DIFFERENT ROUTES OF EXPOSURE. L J Shin, P J Sabourin, M A McCormick, L J Birnbaum, and R E Henderson. Inhalation Toxicology Research Institute, Albuquerque, NM. CIIT, RTP, NC, HERLI USEPA, RTP, NC.

2-Butoxyethanol (BE) is widely used as a solvent in coatings and other consumer products, and has produced hematotoxicity in laboratory animals. We exposed F344/N rats to BE through drinking water, dermal absorption, and inhalation to study the toxicokinetics of this compound. Butoxyacetic acid was identified as the major urinary metabolite, and represented 45 to 60% of the absorbed dose by each route of exposure. Other identified urinary metabolites included ethylene glycol and BE-glucuronide. In order to provide physiological bases for extrapolating the toxicokinetic data observed in rats to humans, a blood flow rate limited physiologically based pharmacokinetic model was developed to describe the disposition of BE in rats following different routes of exposure. The total amount of urinary excretion for each metabolite were used to validate the model. Metabolic constants for each individual metabolite were estimated by fitting the data within the constraints of values derived from perfused rat liver experiments. Sensitivity analysis showed that metabolic constants and blood flow rate to liver have a relatively larger influence on the production of urinary metabolites than the organ volumes and the partition coefficient for BE. (Supported by NIEHS through Interagency Agreement ES-20092 with U.S. DOE/HER by Contract No. DE-AC04-76EV01013.)

PHYSIOLOGICALLY-BASED TOXICOGENETIC MODELING OF THE DERMAL UPTAKE OF THREE WATERBORNE CHLOROETHANES IN RAINBOW TROUT (Oncorhynchus mykiss). J M McKim and J W Nichols. U.S. EPA, Environmental Research Laboratory, Duluth, MN.

Sponsor: K Wallace

A physiologically-based toxicokinetic model for dermal uptake of organic chemicals by fish was adapted from an existing model for the rat and used to describe the uptake and disposition of three waterborne chloroethanes in rainbow trout (Oncorhynchus mykiss). Dermal uptake was described by treating the skin as a discrete compartment into which chemicals move in accordance with their permeability and the driving force for diffusion. The model was validated by exposing the trunk portion of rainbow trout for 48 h to 1,1,2,2-tetrachloroethane (TCE), pentachloroethene (PCE), and hexachloroethene (HCE) in fish respirometer-metabolism chambers. TCE was near steady-state at 48 h, while PCE and HCE continued to accumulate in blood and tissues. Parameterized with chemical partitioning data obtained in vitro, the model accurately simulated chemical concentrations in arterial and venous blood, and elimination across the gills. The kinetics of these chloroethanes in arterial blood were qualitatively similar to those previously observed during inhalation exposures, although the total amount of chemical absorbed was substantially less. These results provide support for the basic model structure and the accuracy of physiological input parameters.

INFLUENCE OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON THE PRODUCTION OF INFLAMMATORY CYTOKINE mRNA BY C57Bl/6 MACROPHAGES. L B Steppan and N L Kerkvliet. College of Vet. Med., Oregon State University, Corvallis, OR.

TCDD induces an enhanced peritoneal inflammatory response in C57Bl/6 mice sensitized to sheep erythrocytes (SRBC). Since tumor necrosis factor (TNF) and interleukin-1 (IL-1) are important mediators of inflammation, the influence of TCDD on TNF and IL-1 mRNA production was measured in vitro and in vivo. C57Bl/6 derived IC-21 macrophages were pretreated for 24 hrs with 1 x 10⁻¹ M TCDD prior to stimulation with LPS for 3–9 hrs. Results indicated that there were no differences in either the level or duration of TNF mRNA production. In contrast, 1 µM dexamethasone reduced the level of mRNA to TNF by 80% after 3 hours of LPS stimulation. Studies in progress will measure mRNA to IL-1 in this model. Peritoneal macrophages taken at 4 hrs post SRBC injection showed no difference in TNF or IL-1 mRNA levels between vehicle and TCDD treated mice. Studies are in progress to measure mRNA levels to TNF and IL-1 at various times after SRBC sensitization. Supported by NIEHS grants ES03966 and ES00040.
FLOW CYTOMETRIC DETERMINATION OF NATURAL KILLER CELL ACTIVITY ISOLATED FROM FISCHER 344 RATS EXPOSED TO PERFLUORODECANOIC ACID (PFDA). D E Frazier Jr, D L Nelson and M J Tarr. Department of Veterinary Pathobiology, The Ohio State University, Columbus, OH.

Perfluorodecanoic Acid (PFDA) is a perfluorinated carboxylic acid compound that induces hepatocellular peroxisome proliferation and thymic atrophy in exposed animals. The ability to cause thymic atrophy is also a hallmark of 2,3,7,8-tetrachlorodibenzo-p-dioxin immunotoxicity and led to our evaluation of the immunotoxic potential of PFDA. PFDA-treated, pair-fed controls, and normal Fischer 344 rats splenocytes were isolated and adherent cells removed after incubation in plastic petri dishes. Nonadherent cells were recovered and mixed in effector:target cell ratios (50:1, 25:1, and 12.5:1) with the human tumor cell line, K562. Cell mixtures were then analyzed for propidium iodide incorporation into killed tumor cells. Effector and target cell populations were discriminated by size and fluorescence and the percent of killed target cells determined by flow cytometry. Preliminary evidence shows that PFDA does not enhance or impair NK cell killing activity in exposed animals. (Supported by AFOSR-88-0216)

MODULATION OF PERINATAL THYMCYTOPE CELL SURFACE ANTIGEN EXPRESSION AND INHIBITION OF THYMCYTOPE MATURATION BY PRENATAL EXPOSURE TO TETRACHLORODIBENZO-P-DIOXIN (TCDD). S D Holladay, B L Blaylock C E Compton, J L Heidt and M J Ludwig. Immunotoxicology Group and Developmental and Reproductive Toxicology Group, National Institute for Environmental Health Sciences, Research Triangle Park, NC.

We previously reported prenatal exposure to TCDD induces thymic atrophy, reduced thymic cellularity and altered expression of CD4 and CD8 thymocyte surface antigens. In the present study, we have extended these observations to include analysis of CD4 and CD8 by cell size populations, CD and y6 T cell receptor (TCR) and J71 marker analysis in TCDD-exposed C57Bl6 mice at gestational day (gd) 18. Pregnant mice were administered vehicle, 1.5 or 3.0 μg/kg body weight TCDD by gavage on gd 6-14. Fetuses were removed on gd 18 and all thymus lobes from an entire conceptus pooled. Flow cytometry analysis showed that, when cells were gated on forward scatter versus CD4 and CD8 antigen presentation, a 44% and 64% reduction in the small CD4+CD8 double positive (DP) cells occurred in the 1.5 and 3.0 TCDD-exposed groups, respectively. In the large cell population, the 1.5 and 3.0 μg/kg TCDD-exposed thymocytes were reduced to 85% and 71% of control, respectively. There was also a significant alteration in the TCR of thymocytes from TCDD-exposed fetuses. A decrease in CD and y6 TCR of 24% and 30% was observed in the 1.5 and 3.0 μg/kg TCDD-exposed thymocytes. An increase of 20% in the 1.5 dose group and 57% in the 3.0 μg/kg dose group was observed in y6 TCR expression. J71 marker was used to distinguish between mature and immature CD8 single positive (SP) thymocytes. There was an increase in CD8 SP J71+ thymocytes in both TCDD dose groups while there was no difference in CD8 single positive (SP) thymocytes and the CD8+ SP, J11+ phenotype.


Female C57Bl/6 mice were injected with 25 μg anti-CD3 antibody in both rear footpads to assess T cell activation in the popliteal and inguinal lymph nodes (PLN). Spontaneous proliferation of PLN cells, as determined by thymidine incorporation, and the number of CD4+ and CD8 T cells in S/G2M phase were significantly increased 24 hr after anti-CD3 treatment. Expression of IL-2 receptors (IL2R) and proliferation of PLN cells to exogenous IL-2 or IL-4 were also increased. Cyclosporin A (50 mg/kg) suppressed spontaneous proliferation, IL-2/IL-4 responsiveness, and IL2R expression. In contrast, exposure to 2 or 20 μg/kg TCDD increased spontaneous proliferation and the number of T cells in S/G2M phase. IL-2 responsiveness was also enhanced following TCDD exposure with a trend towards increased IL2R expression on CD4+ and CD8+ cells. Studies are in progress to assess the effect of TCDD on the kinetics of T cell activation. Supported by Grant PO1-ES00040-26.


Two subtypes of murine T-helper cells have been characterized based on function and spectrum of lymphokines produced. D10.G4 represents a T2 subtype which secretes IL-4, an important mediator of B cell growth and differentiation. Since TCDD has been shown to suppress T cell dependent antibody responses, we examined D10.G4 cells. D10.G4 was preincubated for 0, 4, or 24 hr with 1 x 10^-7 M TCDD prior to stimulation with Con A (0-2 mg/Ml) or immobilized anti-CD3 (0-40 μg). After 24 hrs, IL-4 content was determined in the supernatants using the IL-4 growth dependent cell line, CT4.S. [H]-Thymidine incorporation was used as an index of IL-4 driven cell proliferation. TCDD pretreatment did not significantly alter IL-4 production in Con A or anti-CD3 treated cells except for occasional enhancement. In contrast, dexamethasone (1x10^-6 M) completely inhibited Con A stimulated IL-4 production by D10.G4 without being cytotoxic.

Since D10.G4 was derived from an Ah-low responsive mouse strain (AKR/J), future studies will involve looking at murine T-helper cell clones derived from Ah-high responsive mouse strains. Supported by NIEHS ES07060 and ES00040.

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50  EFFECTS OF 3,3',4,4',5,5'-HEXACHLOROBIPHENYL (HxCB) ON PRODUCTION OF INTERLEUKIN-2 (IL-2) AND INTERLEUKIN-4 (IL-4) IN ALLOIMMUNIZED MICE.  G K Dekrey, CM Neuberger, NJ Kerbel.  T Toxicology Program, Environmental Health Science Center, Oregon State University, Corvallis, OR.

Two T cell secretory products, IL-2 and IL-4, have been shown to have prominent influence on CIL activation and proliferation events. The influence of HxCB on IL-2 and IL-4 production by P815 stimulated spleen cells was investigated. C57Bl/6 mice were inoculated with 1 x 10^6 P815 cells one day after oral treatment with 10 mg/kg HxCB. Ten days later spleen cells were harvested and cultured for 4 hours with P815 cells at 37°C in a humidified 5% CO, atmosphere. Supernatant (SN) was collected for the determination of IL-2 content using the IL-2/IL-4 growth-dependent HIT2 T cell line (in the presence and absence of anti-IL-2 antibody). In addition, IL-4 content in SN was determined (in the presence and absence of anti-IL-4) using the IL-4 growth-dependent CT4.5 cell line. SN from HxCB-treated spleen cell cultures contained 50% less IL-2 and 5-fold more IL-4, when compared with control cultures, using ^3H-thymidine uptake as an index of the IL-4 driven cell proliferation. Lower IL-2 was expected because of fewer CD8+ cells in the population. However, since CD4+ numbers were not higher than in controls, IL-4 elevation could not be explained on this basis. The role that these lymphone changes play in the suppression of CIL activity by HxCB remains to be determined.


The immune system has been demonstrated to be a direct target for toxic insult by a number of chemicals and drugs. Many of these compounds require activation to toxic metabolites. While only limited studies have been conducted, immune cells have shown little capacity for metabolizing xenobiotics in vitro culture systems. These studies examined the endogenous and inducible levels of immunoreactive cytochrome P450 IA1, IA2, XVIIA1 and aldehyde dehydrogenase in varying populations of rodent macrophages and lymphocytes. Alveolar macrophages exhibited elevated levels of constitutive P450 IA1 as compared to other macrophage populations, lymphoid cells and hepatocytes. After inducing by exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) both alveolar macrophages and Kupffer cells showed significant increases in P450 IA1 and aldehyde dehydrogenase levels, while no immunoreactive metabolite was detected in resident peritoneal macrophages. Splenic lymphocytes and thymocytes exhibited increased in aldehyde dehydrogenase levels, with little detectable P450. These studies indicate that immune cells, particularly those from portals of entry may be induced to form highly specific enzymes allowing these cells to metabolize xenobiotics to toxic intermediates.

51  IN VIVO AND IN VITRO SUPPRESSION OF C3 PRODUCTION BY TCDD.  W Q Lin and K K White.  Jr, Dept. of Pharmacology and Toxicology, Medical College of Virginia/VCU, Richmond, VA.

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is an environmental contaminant capable of suppressing the complement system, a major component of innate immunity. In female B6C3F1 mice, oral exposure to TCDD results in decreased serum levels of C3, the most abundant complement component in the serum. Animals exposed daily for 14 days at 2 µg/kg had decreased C3 levels (66% control) which remained significantly decreased until 4 weeks after exposure. Since hepatocytes are the predominant producers of serum C3, studies were undertaken to determine if the suppression observed in vivo resulted from a direct effect on the hepatocytes. The effects of TCDD on two hepatoma lines and primary hepatocytes from B6C3F1 mice were studied. Mouse hepatoma (Hepa 1c1c7) cells were observed to secrete C3 and were responsive to hrIL-1β, a known inducer of acute phase proteins. However, at concentrations up to 10-5 M, TCDD failed to alter C3 production and failed to inhibit hrIL-1β stimulation of C3 production. Similarly, C3 production by the human hepatoma line (HepG2) was unaffected following exposure to TCDD. In contrast, mouse primary hepatocytes exposed to TCDD at concentrations of 10-8 M and greater had a significant decrease in C3 production 1 and 3 hours after exposure. SDS gel analysis of intracellular C3 from animals exposed to TCDD in vivo or primary hepatocytes exposed in vitro suggest that TCDD may affect processing of pro-C3 to native C3. An alteration in the intracellular processing of C3 may affect the cells' ability to secret native C3 which could be responsible for or contribute to the observed decrease in serum C3 levels. Supported by a University Grant-in-Aid.

53  TUMOR NECROSIS FACTOR (TNF) ANTIBODIES AND DEXAMETHASONE (DEX) TREATMENT REVERSE THE ACUTE TOXICITY OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD).  G Clark, G Lucier, M Luster, M Thompson, J Mahler and M Taylor.  NIEHS, Research Triangle Park, NC.

The acute toxicity of TCDD is characterized by a wasting syndrome in which animals lose body weight due to a reduction in food intake and depletion of adipose stores. TNF is a lymphoid hormone that induces a similar response during chronic infection. We have previously demonstrated that TCDD dramatically increases the serum TNF levels following endotoxin treatment. Due to the similarities in the pathologic manifestations of TCDD and TNF, we investigated the role of TNF in the acute toxicity of TCDD in C57BL/6J mice. Following exposure to a LD50 dose of 300 µg/kg TCDD, DEX (transcrip- tional inhibitor of TNF synthesis) was administered every other day until the termination of the experiment. Secondly, antibodies specific for TNF (blocks TNF action) were administered by ip injection every other day following exposure to 300 µg/kg TCDD. DEX therapy resulted in a 92% reduction in TCDD-mediated mortality while passive immunization with TNF antibodies resulted in a 54% reduction in TCDD-mediated mortality. TNF antibodies and DEX also blocked TCDD-mediated weight loss. Histological examination of liver tissue in surviving animals, 30 days after TCDD treatment demonstrated that DEX inhibited the characteristic hepatocellular hypertrophy and vacuolization associated with TCDD exposure. Clinical chemistry analysis for serum enzymes demonstrated that DEX treatment prevented TCDD-mediated increases in serum enzymes associated with liver damage. Since therapies designed to inhibit TNF action blocked TCDD's toxic effects, increases in TNF production may be the mechanism through which TCDD expresses its acute toxic effects.
Eleven chelating agents were examined to determine their relative effectiveness on the removal of strontium in mice following s.c. injection of strontium nitrate (113 mg/kg). Chelators were administered i.p. at doses equal to one-fourth of their respective \( \text{LD}_{50} \). Kryptoffix \( \text{K}_{222} \) and diethylthlenediaminetetraacetic acid \( (\text{DTPA}) \) were the most effective agents in increasing the urinary excretion of strontium and reducing the concentrations of strontium in various tissues. In a second series of experiments, tartaric acid, \( 1,4,7,10,13,16 \)-hexaazacyclohexadecane \( (18\text{-crown-6}) \), ethyleneglycol-bis(beta-amino-ethyl ether)-N,N,N',N'-tetraacetic acid \( (\text{EGTA}) \), DTPA and Kryptoffix \( \text{K}_{222} \) were administered i.p. ten minutes after s.c. injection of strontium nitrate \( (95 \text{ mg/kg}) \). At these levels of strontium, none of the chelators significantly enhanced the excretion of strontium or lowered the strontium tissue concentrations. Due to the lack of effectiveness in removing strontium from the body by the chelators tested, it was concluded that further investigations should be carried out to identify other chelating agents and/or conditions of administration which may be able to enhance the removal of strontium.

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The monomethyl ester of meso-dimercaptopusuccinic acid \( (\text{MoMeDMSA}) \) and its chelates with \( \text{Pb}^{+2}, \text{Cd}^{+2} \) and \( \text{Hg}^{+2} \) have been synthesized. The coordination sites of this new ligand were found to be similar to the sites in its precursor meso-dimercaptopusuccinic acid \( (\text{DMSA}) \). The \( \text{Hg}^{+2} \) chelate is formed by the coordination of two sulfur atoms, whereas the \( \text{Pb}^{+2} \) and \( \text{Cd}^{+2} \) chelates are formed by the coordination of one sulfur and one oxygen atom. The \( \text{Cd}^{+2} \) chelate of MoMeDMSA is dimeric whereas the \( \text{Hg}^{+2} \) and \( \text{Pb}^{+2} \) chelates are monomeric. The \( \text{pK}_{a} \)'s of MoMeDMSA and the uncoordinated groups in its metal chelates have been determined. The results are compared to the \( \text{pK}_{a} \)'s obtained for the uncoordinated groups in the metal chelates of meso-DMSA with the same metal ions. These acid-base properties in addition to the polarity of the chelating agent contribute to the effectiveness in the in vivo mobilization of intracellular deposits of \( \text{Cd}^{+2} \).

The chelates of the dimethyl ester of meso-DMSA \( (\text{DiMeDMSA}) \) with \( \text{Zn}^{+2} \) were studied by potentiometry. \( \text{DiMeDMSA} \) forms polynuclear complexes of the type \( [\text{Zn}_{2}\text{L}_{6}]^{2+} \) and \( [\text{Zn}_{3}\text{L}_{6}]^{4+} \) at pH values 3 - 6.5 and a mononuclear complex \( [\text{ZnL}]^{3+} \) that predominates at physiological pH. The chelate \( [\text{ZnL}]^{3+} \) was isolated and purified. Low temperature \( ^{1}H \) and \( ^{12}C \) NMR spectroscopy showed that \( \text{Zn}^{+2} \) is present in a tetrahedral environment surrounded by four thiolate groups from two \( \text{DiMeDMSA} \) molecules.

DMSA and DMPS are orally effective for the treatment of chronic lead or mercury intoxication in humans. DMSA \( (600-800 \text{ mg}) \) or DMPS \( (300 \text{ mg}) \) was given po to 5 fasted, normal young men. Urine and blood samples were collected over a 15 hr period. The samples were analyzed for total and unaltered DMSA or DMPS by previously published methods (unaltered DMSA or DMPS is the unbound, parent compound; total DMSA or DMPS consists of unaltered plus disulfide form). By 15 hr after administration, 24.7% ± 3.3 SE of the administered DMSA was excreted as altered (the difference between total and unaltered) DMSA (87% of the total DMSA found in the urine) and only 3.7% ± 0.67 SE was excreted as unaltered DMSA (13% of the total DMSA found). Administered DMPS was excreted as 26.7% ± 4.8 SE altered DMPS (91% of total DMPS) and 2.7% ± 0.48 SE unaltered DMPS (9% of total DMPS). Altered DMPS peaked in the urine at 4 hr while altered DMPS peaked between 4 and 9 hr. Both DMSA and DMPS were found to be confined to the plasma portion of blood. Like DMSA, very little DMPS was found in blood in its unaltered form. Altered DMSA has been found to consist of mixed disulfides of albumin in the plasma and cytokine in the urine.

Dimercoapsuccinic acid \( (\text{DMPS}) \) is an effective chelating agent for lead. However, its high carbon content is toxic. The modified compound \( (\text{DMPS}) \) is free of carbon, and the remaining sulfur is excreted more readily than the sulfur in the DMPS. The analogues were found to increase the biliary excretion of metals such as platinum \( (\text{Pt}) \) and cadmium \( (\text{Cd}) \). Lipophilic analogs of DMSA were studied for their ability to increase biliary excretion of these two toxic metals. The analogues used were the monomethyl ester \( (\text{MoMeDMSA}) \), the dimethyl ester \( (\text{DiMeDMSA}) \), and the zinc chelate of the dimethyl ester \( (\text{DMSA}) \). Rats were pretreated with either \( ^{109}\text{Cd} \) (as \( \text{CdCl}_{2} \), 1 mg Cd/kg, ip., 3 days prior) or \( \text{cis-diammine-dichloroplatinum II} \ (8 \text{ mg/kg}, i.v., 1 \text{ day prior}) \). Bile cannulas were installed and flow was established. One hour later, equimolar doses of the dithiols were administered \( (0.15 \text{ mmol/kg}, i.v.) \). Bile was collected in 30 min intervals for 8 hrs and was analyzed for either \( \text{Cd} \) by gamma-spectroscopy or \( \text{Pt} \) by atomic absorption spectroscopy. The zinc chelate showed the greatest increase of both biliary \( \text{Cd} \) and \( \text{Pt} \) levels, 350- and 5-fold, respectively. \( \text{DiMeDMSA} \) and \( \text{MoMeDMSA} \) increased biliary \( \text{Cd} \) excretion 175- and 63-fold, respectively. \( \text{DiMeDMSA} \) increased biliary Pt excretion by 2.5-fold. Under our experimental conditions, the superior chelating properties of these compounds, as far as biliary excretion is concerned, may be due to their lipophilic nature which enables them to cross the cell membrane.
Some dithiols are effective antidotes against poisoning with arsenic and several other heavy metals. In the present paper, the relative effectiveness of British Anti-Lewisite (BAL), dimercapto-propanesulfonic acid (DMPS), dimercaptosuccinic acid (DMSA) and a new metal binding agent, 2,3-bis-(acetylthio)-propanesulfonamide (BAPSA) have been evaluated by determining their effect on the biliary excretion of arsenic in perfused livers of guinea pigs after As$_2$O$_3$ injections. Guinea pigs received As$_2$O$_3$, 2.5 mg/kg sc twice a day on 5 consecutive days. Sixteen hrs after the last dose, the livers were perfused (35 ml/min) with Krebs-Henseleit buffer with glucose for 80 mins. After 50 mins of perfusion, either 0.1 mmol/L or 0.7 mmol/L BAL, DMSA, DMPS or BAPSA were added to the perfusate. In these experiments BAPSA was significantly more effective in the overall elimination of arsenic than DMSA, DMPS, and BAL. The treatment with chelating agents may cause a substantial shift to fecal excretion by the increase in biliary excretion (BAL < DMSA < DMPS < BAPSA).

Heavy metals cause irreversible neurobehavioral damage in developing animals, but the mechanisms of toxicity are unknown. The influence of heavy metals on lethality, development, behavior and learning was studied using the fruit fly, Drosophila melanogaster. This animal was used because a) it has been extensively characterized at the molecular level, b) several of them may be used very easily in individual experiments and c) it is a system in which toxicological questions may be answered by using molecular genetic techniques. The larval LC50 ± standard error for triethyllead chloride, lead acetate, or cadmium chloride was found to be 0.006 ± 0.004 mM, 5.60 ± 0.54 mM, or 0.42 ± 0.04 mM, respectively. The tested compounds produced a dose related delay in development as shown by an increased time for the development of pupa from larva. For tests to measure phototaxis, locomotion, and learning, new larva were reared on medium containing 0.06 mM triethyllead chloride, 3.07 mM lead acetate, or 0.11 mM cadmium chloride. No neurobehavioral effects were observed under the experimental conditions, which may indicate Drosophila will not be an appropriate animal for the genetic dissection of the neurobehavioral effects of heavy metals.

Arsenic (As) bioavailability from mining waste soil was determined in male and female rabbits (5/sex/dose level) following a single oral administration. The test soil which contained 3900 ppm arsenic was formulated into capsules and administered at three dose levels (0.78, 1.95, or 3.9 mg As/kg BW). Standard groups (5/sex/group) included untreated controls, intravenous sodium arsenite solution (1.95 mg As/kg BW) and oral gavage sodium arsenite solution (1.95 mg As/kg BW). For all groups, samples of urine, cage rinse and feces were collected at 24-hour intervals for 5 days. All samples were analyzed for total arsenic concentration using graphite furnace atomic absorption or inductively coupled plasma - atomic emission spectroscopy. Bioavailability of arsenic in the soil was determined as the percent of arsenic excreted in the excreta of the capsule (soil) dosed animals. Results obtained following administration of the soil were compared to data from rabbits that were given a single intravenous or oral gavage administration of a soluble arsenic solution.

Transfer RNA and Cadmium Toxicity in Drosophila.

Transfer RNA (tRNA) is a derivative of guanine, found in the first position of the anticodon of the transfer RNAs for Asp, Ala, His and Tyr. The transcripts of these tRNAs contain a guanine in this position. This guanine is enzymatically oxidized and replaced by quinone. The ratio of quinone-containing or (q+) tRNA to its precursor or (q-) tRNA changes throughout the drosophila life cycle. In the egg 10% of the tRNA is (q+). During the three larval stages this ratio drops to zero. In the one day old adult it is about 10%. It has previously been shown that when flies are selected for the ability to grow in the presence of cadmium, the tolerant flies had 100% (q+) tRNA at the first day after pupation instead of 10%. However, it was not known whether the elevated level of (q+) tRNA was a coincidence or if the elevated levels of (q+) tRNA were protective. We explored this problem using germfree drosophila. The first thing was to determine if drosophila can synthesize quinone. Sterilized eggs were seeded onto sterile chemically defined medium. The flies were grown to the adult stage. This study showed that drosophila like mammals cannot synthesize quinone. A second result of this research was the demonstration that we could alter the ratio of (q+) to (q-) tRNA by adding exogenous quinone to the medium e.g. at 0.008 mM quinone the (q+) tRNA was 95% instead of < 5% in the last instar stage. Finally, we investigated whether or not quinone gave protection against cadmium. The results were that when the flies were grown in the presence of 0.2 mM cadmium quinone at 0.008 mM gave a statistically significant increase in the number of survivors.
NIKEL-INDUCED TRANSFORMATION OF CHINESE HAMSTER (CH) embryo cells frequently yields tumorigenic male cell lines, many of which exhibit deletions involving the heterochromatin region on the long arm of the X chromosome. By microcell-mediated chromosome transfer, an intact CH X has been introduced into several of these cell lines and the predominant outcome is senescence of the previously immortal recipient cells. Transfer of the CH X from either of two independent mouse A9 donor cell lines to the Ni-2/TG recipient cell line with a complete Xg deletion resulted in senescence of 100% of the resulting HA/TG hybrid clones. Similarly, transfer of the intact X from later passage A9 donor cells also induced senescence in 75% of the hybrids resulting from fusions to another nickel-transformed cell line, Ni-6/TG. These recipient cells do not have a visibly deleted X, but nevertheless may have sustained submicroscopic X chromosome damage as a result of the nickel treatment. A reduced incidence of senescence (47%) was also observed with chromosome transfer to the Ni-2/TG cells using later passage (P-6-10) CH X bearing A9 donor cells. To determine whether the CH X may have been inactivated by methylation during its extended residence in the mouse cells, late passage A9 donor cells were pretreated with 3′M 5-azacytidine for 48 or 72 hrs prior to microcell fusion to Ni-2/TG cells. Following this treatment, senescence was again observed in 96% of the resulting hybrids. The data suggests that a putative senescence gene located on the CH X chromosome may be under methylation regulation. (This work was supported by NIH #ES05312)

63 §8Ni²⁺ UPTAKE AND ITS DISTRIBUTION IN 3T3 CELLS AS EXAMINED BY A COMBINED AUTORADIOGRAPHY/IMMUNOFLUORESCENCE STAINING TECHNIQUE. K. Lin and J. N. Chou. Depts. Microbiology and Pathology, Boston Univ. School of Medicine, Boston, MA.

Ni²⁺ induced microtubule (MT) perturbation has been extensively investigated in our laboratory using mouse 3T3 cells as a model. Dramatic perinuclear bundling of MT was seen in cells treated with 1.5 or 2.0 mM Ni²⁺ for 20 h. To study the kinetics of Ni²⁺ uptake and its distribution, growth arrested 3T3 cells were incubated with §8Ni²⁺ (5 μCi/ml) in the presence of 2.0 mM NiCl₂, and processed for scintillation counting at a 5-6 h interval. The uptake was linear from time 0 to about 10 h, and then slowly tapered off and reached a plateau level. Studies on the effect of various Ni²⁺ doses on §8Ni²⁺ uptake showed that §8Ni²⁺ uptake peaked and plateaued at 1.0 mM Ni²⁺. To localize the cellular destination of the §8Ni²⁺ taken up by 3T3 cells, we have devised a fluorescence microscopic procedure by combining autoradiography with conventional immunofluorescence staining to visualize the §8Ni²⁺ radioactivity grains and the MT distribution within the cell. Using this procedure, we observed a discrete nuclear and possibly perinuclear concentration of §8Ni²⁺ grains. These results suggest a possible preferential target site(s) for Ni²⁺ insults in 3T3 cells.


We are developing an antibody-based method for detecting poorly-repaired DNA-protein crosslinks (DPC) as a way to measure previous exposure to chromium (Cr) compounds. Cr-induced DPCs were generated in a T-cell line (MOLT-4) in order to prepare rabbit anti-human lymphocyte-DPC antibodies to screen human for Cr exposure. Treatment of MOLT-4 cells with K₂CrO₇, in concentration resulting in time- and dose-dependent increases in DPC formation. However, there were major differences observed with these cells as compared with rodent (Chinese hamster ovary, CHO) or human (HOS osteosarcoma) fibroblast lines we have previously studied: 1) MOLT suspensions were 100-fold more radio-sensitive when DNA and protein were labelled with [³¹]H-TdR and [³⁵]S-Met, 2) MOLT DPC formation was considerably slower and required a change from a standard 2 hr metal treatment to 12-24 hr of exposure to assure significant DPC formation, and 3) the major rabbit anti-DPC antibody generated recognizes a 75-80 Kd antigen rather than a 59 Kd species obtained with CHO DPC material. Besides representing an easily-obtained long-lived human cell line, the major advantage in using the MOLT-4 is that compared with our other cell lines, these cells yield significantly more protein and DNA as DPCs after treatment with an equal dose of K₂CrO₇. This work was supported by ES04895 and ES04715 from the NIH and US EPA grant #RR14762.

65 CHRONIC EXPOSURE OF RATS TO CHROMATE INDUCES PERSISTENT ELEVATED TISSUE CONCENTRATIONS OF CHROMIUM. K. S. Squibb, S. Costantino, M. Costa and C. A. Snyder, Inst. of Environ. Med., NYU Medical Center, New York, NY.

Hexavalent chromium (Cr⁶⁺) is a well established lung carcinogen, however, knowledge of Cr metabolism and other health effects is limited. Studies were conducted to determine tissue accumulation of Cr in rats exposed to 100 ppm Cr (as potassium chromate) in their drinking water for up to 6 weeks. Clearance rates were measured over a period of 84 days after cessation of exposure. Cr concentrations were measured by AA spectrophotometry. Blood levels of Cr did not increase significantly between 3 and 6 weeks of exposure, but Cr accumulated continuously with time in the liver, kidney and spleen. After cessation of exposure, Cr elimination from the liver and kidneys was slower than that reported in single dose Cr clearance studies, while concentrations of Cr in the blood and spleen suggested a remobilization of Cr from other compartments during the clearance period. Concentrations in blood increased and decreased with a periodicity of approximately 25 days; concentrations in the spleen decreased initially (tₜ=2 days) but subsequently increased to peak concentrations 42 days after cessation of treatment. After an initial decrease, hepatic Cr concentrations remained constant, while kidney concentrations decreased in an apparent triexponential manner. Results demonstrate that chronic exposure to Cr induces elevated tissue concentrations of this metal for extended periods of time. (Supported by NIH grant ES 04895).

Chromium-51 was used to determine chromium uptake and distribution within cells of the blood. Isolated red blood cells (RBC) and enriched white blood cells (WBC) populations were exposed in vitro to potassium chromate (CrO₄⁻). Exposure of either rat or human blood cells to 50 μM CrO₄⁻ for 2h resulted in greater accumulation of chromium within WBC than RBC. Uptake by rat WBC was significantly greater than that of human whereas, uptake by human RBC was greater than that of the rat. Exposure of human whole blood to 50 μM CrO₄⁻ prior to isolation of WBC, also resulted in increased uptake of chromium by WBC. F-344 rats were exposed either i.p. or i.v. to a single dose of CrO₄⁻, and the distribution of chromium within blood cells was determined 1h, 24h or 7d following exposure. Regardless of the route or time of exposure, WBC chromium levels were consistently greater than those of RBC. However, the absolute levels of chromium did change with time. A comparison of chromium distribution 24h following a single oral exposure (1 ppm CrO₄⁻) to the distribution 7d following exposure demonstrated a reduction in chromium levels for RBC (10 fold) and for WBC (2.5 fold). In contrast, RBC Chromium levels were not significantly different when compared 1h, 24h and 7d following i.v. exposure. Although no difference in WBC chromium content was observed at 1h and 24h after i.v. exposure, an approximate 1.7 fold decrease in chromium content was detected at day 7. The accumulation of chromium by WBC support their use in the development of biomarkers to assess chromium exposure. (Supported by grant #ES04895 and #ES05454)

LEAD AND CADMIUM INHIBITION OF HUMAN OSTEOTOBLAST-LIKE OSTEOSARCOMA CELLS (HOS/TE85). C.R. Angell, D.J. Thomas, and S.A. Swanson. Dept of Pediatrics, Univ Nebraska Med Ctr, Omaha, NE.

Epidemiological and experimental data indicate that lead (Pb) and cadmium (Cd) are osteotoxins in humans. The relative sensitivity of human osteoblast-like cells from a human osteosarcoma cell line (HOS/TE85) was tested by incubations in serum-free media without added growth factors. Cytotoxicity ~ IC₅₀ (MTT assay) was induced by Cd < 0.5 μM, zinc 50 μM but not by Pb ≤ 100 μM or gallium ≤ 500 μM. The extreme sensitivity of HOS/TE05 to Cd is similar to that of rat osteosarcoma cells ROS 17/28 (Cd IC₅₀ ~ 0.3 μM). Exposure to 10 μM Pb for 24 hrs or 1 μM Pb for 48 hrs significantly inhibited thymidine incorporation into DNA. Removal of Pb from media after a 24 hr exposure to 10 μM Pb did not reverse the inhibition of DNA synthesis within 4 to 12 hr of washout. HOS/TE85 cells provide a sensitive human derived cell line for the study of the mechanisms of osteotoxicity of Pb and Cd. (Supported by the Itiner Fund for Pediatric Research, UNMC.)

CHROMIUM CONTENT OF BONE AFTER ORAL AND INTRAPERITONEAL (IP) ADMINISTRATION OF CHROMIUM (VI) TO RATS. C.M. Witmer and R. Harris, Rutgers University, Piscataway, N.J. and S.I. Shupeck, Villanova University, Villanova, PA.

Previous studies in our laboratory of the bioavailability of chromium VI compounds (Cr₆⁺) in rats, indicated that only about 55% of the administered Cr was recovered when the following tissues were analyzed for Cr content: lung, kidney, liver, blood, adrenal, spleen, brain, muscle, testes or as well as feces and urine. To determine whether bone is a Cr repository, analyses for Cr using Inductively Coupled Plasma were carried out after both oral and intraperitoneal administration of Cr. After 3 days of oral administration of Na₂CrO₄ at 100 μmol/kg, the Cr content of bone was 0.00 ng/g while liver contained 49% ng/g and lung content was 201 ng/g. After CaCrO₄ treatment (at the same level), bone content was 720 ng/g while Cr was at control levels in liver and lung (165-230 ng/g). If Na₂CrO₄ administration (100 μmol Cr/kg) on alternate days for a total of 2 treatments, resulted in bone content of 17.4 μg/g or 1.87% of the total administered dose (based on bone as 4.3% of body weight). These findings suggest that Cr content of bone is at least as high as that of tissues such as liver. It also suggests that Cr may be found in bone when it is not found in other tissues, particularly after treatment with insoluble Cr compounds.

SENSITIVE ASSAY FOR CADMIUM (Cd) IN BIOLOGICAL SAMPLES. D.P. Peterson, E.A. Huff, and M.H. Bhattacharyya. Argonne National Laboratory, Argonne, IL.

A sensitive method was developed for analyzing Cd in whole blood, urine, and plasma by electrothermal atomic absorption spectrophotometry (ETAAS). Prior to ETAAS, Cd was concentrated on an anion exchange column, resulting in a detection limit of 0.02 ppb for an 8-ml blood sample. Recovery of 10⁹Cd from the column was 92.2 ± 0.9% (mean ± SE, n = 35). The column separated Cd from 99.8% of the cations in deproteinized whole blood, allowing comparison of unknown samples to aqueous acidic standards using commercially available acids. The mean intra-assay coefficient of variation (CV) was 12 ± 3% (n = 6) for blood, plasma, and urine samples having 0.1-0.5 ppb Cd. The interassay CV was 13% (n = 7) for a blood sample containing 0.6 ppb Cd. The recovery of known amounts of Cd (0.2-0.5 ppb) added to blood, plasma, and urine was 97 ± 6% (n = 4). Current methods are not sensitive enough to accurately determine Cd concentrations in the blood of persons not occupationally exposed (ca. 0.2 ppb). With this assay, we can for the first time determine the extent to which low levels of Cd released from industrial or municipal waste sites might contribute to the Cd exposure of persons living nearby. Work supported by the U.S. Department of Energy, Office of Health and Environmental Research, under Contract No. W-31-109-EN-38 and by NIH grant ES-04816.
The effects of nutritional factors on the intestinal absorption and tissue distribution of Cd were studied in rats and the effects of Fe, Zn, and Cd deficiency in rats using an in situ model where an intestinal loop of 5 cm was incubated with CdCl₂ or Cd-Mt for 30 and 60 min. The intestinal uptake of Cd from CdCl₂ or Cd-Mt was not affected by nutritional deficiencies, although the Cd uptake from CdCl₂ was always higher than that from Cd-Mt. However, Fe deficiency markedly increased the tissue distribution of Cd. After 30 min incubation with CdCl₂, Cd was deposited only in liver in control and SH deficient rats while Cd was detected also in kidney and pancreas of both Fe and Zn deficient rats. At 60 min incubation with CdCl₂, the deposition of Cd in the liver, kidney and pancreas of Fe deficient rat was significantly higher than that in the control. Cd after Cd-Mt incubation was mainly deposited in kidney and nutritional deficiencies had little effect on tissue deposition of Cd. The results suggest that the intestinal uptake mechanism of Cd from CdCl₂ and Cd-Mt is different and the renal deposition of Cd was specifically increased in Fe deficient rats when incubated with CdCl₂. (Supported by HRC, Canada).

Cadmium, mercury and calcium uptake in hepatocytes of the small skate (Raja erinacea). Z.A. Sheikh, M.E. Blaska and H Yoshida. Desert Island Biological Laboratory, Salisbury Cove, ME.

Cadmium and mercury are present in marine ecosystems and accumulate in the liver of many aquatic organisms. Cd and Hg interact with the essential metals for transport across the plasma membrane. Therefore, we studied the uptake and accumulation of radiolabeled 3 μM Cd, Hg and also Ca in isolated liver hepatocytes in the presence or absence of other metals (i.e. Ba, Cd, Cu, Fe, Hg, La or Zn) at 4 or 14°C for up to 30 min. The accumulation of Cd, Hg and Ca was biphasic. During the initial rapid phase the uptake of Hg was 3 times greater than that of Cd. Incubation of hepatocytes at 4°C decreased both Cd and Ca accumulation by 30 and 70%, respectively, but had no effect on Hg accumulation. Furthermore, Hg uptake was completed within 3 min, while Cd and Ca continued to be taken up over 30 min. 100 μM Zn decreased Cd uptake by 27%. In contrast, 10 μM Hg and 100 μM Cd increased Cd uptake by 3- and 1.6-fold, respectively. Hg accumulation was depressed 31% by 100 μM Cu, but 100 μM Cd or Zn had little effect. Cd (3-100 μM) reduced Ca accumulation in a concentration-dependent manner to a maximum of 64%. The accumulation of Ca was also reduced by 100 μM Ba and Cu (20%), 100 μM Zn (57%) and 10 μM Hg (72%). In contrast, 100 μM La increased Ca accumulation by 22% after 30 min. These results demonstrate striking differences in hepatic uptakes of Cd, Ca and Hg and show that other metal ions do not always affect their uptake and accumulation in a similar manner (Supported by a NIB Center Grant ES-03826).

Cadmium toxicokinetics in testicular interstitial cells from mouse resistant and susceptible to the testotoxic effects of cadmium. R.A. Barter and M.P. Wallack. National Cancer Institute-FRCCD, Frederick, MD.

Testicular toxicity and carcinogenicity of cadmium (Cd) in mice is highly strain dependent. To determine the differences in Cd uptake and the toxic effects of Cd toxicokinetics were examined in testicular interstitial cells (ICs) prepared using strains of mice both resistant (BALB/c) and sensitive (NFS) to Cd-induced testicular lesions. Testes were removed and decapsulated and ICs were isolated by collagenase dispersion. Cellular uptake of Cd was determined in IC preparations incubated with 10 μM Cd for up to 12 min with the layered oil technique. The uptake of Cd was found to be multiphasic in both strains of mice. Cd uptake tended to be greater in ICs from NFS mice, which accumulated 25% more than ICs from BALB/c mice at 12 min. Efflux of Cd was measured for 1 hour in ICs incubated with 100 μM Cd for 30 min and then placed in fresh buffer. Cd efflux from BALB/c ICs was 10% greater than NFS. Cd-binding protein levels in BALB/c and NFS ICs were also determined. ICs from BALB/c mice demonstrated a higher protein binding capacity for Cd than ICs from NFS mice (1275 ± 21 vs 882 ± 38.5 μg Cd/gm tests, respectively). Thus, it appears that differences in toxicokinetics may contribute to the strain difference in susceptibility to Cd toxicity in the tests, although other aspects also need to be considered.

Stress protein synthesis induced in rat liver by cadmium proceeds hepatotoxicity. P. Goering, BR Fisher, PP Chaudhary, and CA Dick. Center for Devices & Radiological Health, Food & Drug Administration, Rockville, MD.

Adverse environmental stimuli increase the synthesis of a class of proteins referred to as stress proteins. We are exploring alterations in protein synthesis as biomarkers of chemical exposure/toxicity. CdCl₂ was used as a model hepatotoxin and the effects of acute exposure to this compound on hepatotoxicity in male rat liver were evaluated. Tissue slices were incubated with [35S]-methionine for 1.5 hr, subjected to SDS-PAGE and [35S]-labeled proteins were detected by autoradiography. De novo synthesis of 70, 90-, and 110-kilodalton (kd) proteins was enhanced at 2-4 hr and decreased at 8-16 hr after iv injection of 2 mg Cd/kg. Synthesis of a 68-kD protein was inhibited 2 hr after exposure and then increased at 16-24 hr. Dose-related increases in the synthesis of 70-, 90-, and 110-kD proteins and inhibition of the 68-kD protein were observed 4 hr after injection of 1 and 2 mg/kg, but not after 0.5 mg/kg. Western blot analysis using monoclonal antibodies identified the Cd-induced 70- and 90-kD proteins as members of the eukaryotic heat shock, or stress, protein family. Liver toxicity as assessed by increased plasma sorbitol dehydrogenase and desalted microsomal N-demethylase activities was observed, but only 8-16 hr after injection of 2 mg/kg. No changes in kidney protein synthesis or toxicity were observed. The data demonstrate that alterations in protein synthesis occur prior to cell injury in target organs and may be useful as biomarkers of exposure/toxicity.
Western blotting technique is very useful for detection of trace amounts of proteins in biological samples. The technique was applied for the detection of metallothionein (MT). Cadmium-containing rat MT-I and -II were subjected to SDS/polyacrylamide gel electrophoresis (SDS-PAGE) without prior reduction with 2-mercaptoethanol (2-ME), because reduction of MTs resulted in diffused bands. After electrophoresis, gels were reduced by incubating in buffer containing 2-ME, and then electrophoretically transferred to a polyvinylidene fluoride membrane. Non-reduced MTs were not found to be immobilized on the membrane. Prior to electrophoresis the zinc, in zinc-containing MT samples, must be replaced with cadmium by addition of excess cadmium chloride. MTs immobilized on the membrane were detected by two methods: 1) binding of radioactive cadmium (109Cd) and 2) immunochromatography staining using rabbit anti-rat MT-I serum and protein A-gold colloidal gold conjugate. The detection limit was found to be 0.4 and 0.06-0.16 μg MT, respectively.

The relationship between hepatic MT and plasma corticosterone (CORT) concentrations, and induction of hepatic MT by Zn, was assessed in pregnant and lactating C57B1/6J mice. MT and CORT were measured on gestation day 16 (GD) and lactation day 1, 4, 7, 10, 14, 16, 18 and 20 (LD) for both pregnant and lactating mice. MT concentration prior to GD 8 was low (3.4-5.1 μg/g liver), comparable to MT levels in non-pregnant mice. Hepatic MT concentration increased to a mean of 43.5 μg/g on GD 10 and remained at this level through GD 16. MT levels fell subsequently to approximately 30 μg/g, and remained at this level through lactation. Induction of hepatic MT by Zn (300 nmol/kg ip, 18 hr before sacrifice) was assessed on GD 6, 10, 14, 18; LD 4, 10, 17. Despite changes in hepatic MT level, MT induction by Zn was comparable at all stages. CORT concentration on GD 10 (mean of 540 μg/ml) doubled from earlier values. Unlike MT level, CORT concentration continued to rise, reaching values 8-10 fold higher than early gestational values before decreasing to a mean of 2000 μg/ml on GD 18. Thus, although endogenous glucocorticoids may regulate hepatic MT in pregnant and lactating mice, CORT and MT levels are not closely correlated, and MT may not be coordinately regulated by CORT during this period.

Chronic exposure to arsenic has biological consequences that may include lung and/or skin cancer. Short term results include modulation of synthesis of heat shock and stress-related proteins. We investigated the effect of arsenic treatment on metallothionein (MT) synthesis and half-life in liver, kidney, and pancreas of rats. Male adult Sprague-Dawley rats were injected with As3+ (25 to 90 μmol/kg) or As5+ (50 to 200 μmol/kg). As3+ resulted in a dose-dependent increase in hepatic MT (20-fold), zinc (1.6-fold) and arsenic (25-fold) levels. As5+ had little effect. Arsenic injection caused no change in renal MT, Zn, or Cu content. Zinc pretreatment resulted in a 40-fold enhancement of As3+-mediated MT induction in liver. The half-life of hepatic MT (as measured by the decrease of the appearance of (55Fe)-MT) was longer in rats treated with As3+ plus Zn (βt=43.2 h) than in rats treated with Zn alone (βt=18 h). The half-life of the bulk of hepatic proteins was also slightly increased by As5+ treatment. Treatment with hepatic MT-I and MT-2 mRNA were induced by As3+, but total accumulation was less than that resulting from zinc induction. UV spectrophotometry showed that As3+ bound apo-MT and Zn-MT at acidic pH. Binding was abolished at neutral or alkaline pH. At acid pH, As-MT was more resistant to enzymatic degradation than Zn-MT. At alkaline pH, Zn-MT was more stable than As-MT. These results suggest that As3+ induces MT in vivo in a tissue-specific manner, and that MT binding to As may stabilize the polypeptide and protect it against proteolytic degradation.

Supports by grants from the MRC of Canada

Antisense oligonucleotides (ODN) with sequence complementary to the messenger RNA coding for human metallothionein II were prepared and tested for their ability to inhibit both constitutive and cadmium induced metallothionein protein synthesis in neuroblastoma-IRMA and Chang liver cells in culture. The ODN was also examined as a measure of sequence specificity. Metallothionein protein synthesis was determined by 35S-cysteine metabolic labelling and analysis in SDS-PAGE gels with authentic metallothionein as a molecular weight marker. The antisense inhibition of metallothionein protein synthesis rendered both cell lines more sensitive to cadmium toxicity. However, the sense ODN had no effects on either metallothionein protein synthesis or sensitivity to cadmium. Phosphorothiate oligonucleotides (ODN) are more potent than the phosphodiester backbone structures. These ODN (ODN) targeted to the splice donor region between exon 1 and intron 1 was significantly more potent as an inhibitor than those targeted to either the 5' or the 3' end of the mRNA or within exon 3. Thus, not all ODN antisense sequences are equally potent as an inhibitor and prediction of the most potent sequence is not possible. These studies may provide a useful tool for the study of metallothionein function. Additionally, these studies indicate that metallothionein is an essential gene in at least the neuroblastoma and Chang liver cells.


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78 DEGRADATION OF METALLOTHIONEIN BY CATHEPSINS. J M McKm Jr, S Choudhuri and C D Klaassen. Univ Kansas Med Ctr, Kansas City, KS

Metallothionein (MT) is a small (M, = 8800) cysteine-rich protein, which has been shown to reduce the toxic effects of metals and some chemotherapeutic drugs. Although several studies have suggested that lysosomal proteins may play an important role in MT degradation, there is currently no information concerning the susceptibility of MT to degradation by cathepsins, the primary lysosomal proteases. To examine this question, purified cathepsins (C, D) were incubated with radioiodinated MT. Incubations were conducted at pH 5.5 with or without 2 mM diithiothreitol at 37°C. Degradation over time was monitored using SDS-PAGE. MT bands were excised from gels, solubilized and quantitated by measuring their 35S content. Using these in vitro conditions, cathepsins B, C, and D degraded thionein at rates of 7.5 ± 0.9, 1.1 ± 0.1 and 0.15 ± 0.01 mol MT/mmol cathepsin/min, respectively. In comparison, both ZnMT and CdMT were extremely resistant to degradation by all cathepsins, with rates 1/400 to 1/5,000 of thionein. Because it is known that low pH, such as that found in lysosomes, dissociates metals from MT, it seems plausible that lysosomal degradation of MT occurs mainly after metals are displaced. These data suggest the following: (1) thionein is sensitive to degradation by cathepsins, (2) cathepsin B is the most potent of the cathepsins tested in degrading MT, followed by cathepsin C and then D, and (3) MT is much more resistant to degradation when metals are bound to it. (Supported by NIH Grants ES-01142 and ES-07079).

79 LYSOSOMAL DEGRADATION OF METALLOTHIONEIN (MT). S Choudhuri, J M McKm Jr and C D Klaassen. Univ Kansas Med Ctr, Kansas City, KS

Protein synthesis and degradation regulate cellular protein levels. Several studies have been performed on the synthesis of MT, but little is known about its degradation. Therefore, the purpose of this study was to examine the ability of lysosomes to degrade both metal saturated (CdMT, ZnMT) as well as metal-free thionein. Hepatic lysosomes, prepared by differential centrifugation, were enriched 10-15 fold over the homogenate as determined by marker enzyme assays (acid phosphatase, N-acetyl-D-glucosaminidase, aryl sulfatase). S-thiolated thionein, ZnMT or CdMT was added to incubation mixtures containing lysosomal protein, acetate buffer (pH 5.5) and 2 mM diithiothreitol. Degradation of the MTs was monitored using SDS-PAGE. S-MT bands were excised from gels, solubilized and quantitated. As little as 0.6 µmol of lysosomal protein degraded approximately 60% of the metal-free thionein in 1 hr. The degradation rate was approximately 70 ± 5 n mole of thionein/mg lysosomal protein/min. In contrast, the degradation of metal-saturated thioneins (CdMT and ZnMT) was much slower. Degradation of 50% of CdMT or ZnMT required more than 100 times as much protein and extended incubation times. Specific inhibitors of cathepsins reduced the lysosomal degradation of metal-free thionein. In conclusion, the lysosomal cathepsins are very effective in the degradation of metal-free thionein, but metals bound to MT protect it from this degradation. (Supported by NIH Grants ES-01142 and ES-07079).

80 EFFECT OF SODIUM BUTYRATE HOMOLOGUES ON METALLOTHIONEIN INDUCTION IN RAT HEPATOCYTE PRIMARY CULTURES. J Liu, J M McKm Jr, J Y Liu and C D Klaassen. Univ Kansas Med Ctr, Kansas City, KS

Sodium butyrate (NaB), a 4-carbon fatty acyl, has been reported to activate metallothionein (MT) genes in certain carcinoma cell lines. Because the effects of NaB are dependent on the cell type investigated, this study was conducted to determine if NaB and its homologues induce MT in normal cultured rat hepatocytes. Hepatocytes were grown on monolayers for 12 h and subsequently treated with sodium formate, acetate, propionate (NaP), NaB and valeric acid for 10-56 h. To examine their interactions with known MT inducers, cadmium (Cd), zinc (Zn) or dexamethasone (Dex) was added to some cultures. MT protein in the cells was quantitated by the Cd/hemoglobin assay; MT-1 and MT-II mRNAs were analyzed by Northern blot hybridizations with oligonucleotide probes, and quantitated by slot-blot analysis. Among the 1-5 carbon carboxylic acids, only NaP (3 carbon) and NaB (4 carbon) induced MT. NaP and NaB alone produced a moderate increase in MT (2-4 fold over control), and when combined with Cd or Dex, an additive increase was observed. However, when combined with Zn, a synergistic increase was detected. While NaB and Zn synergistically increased MT protein, they produced only an additive increase in MT mRNAs suggesting the involvement of some post-transcriptional event(s) in the NaB-Zn induction of MT. In conclusion, NaP and NaB induced MT in normal cultured rat hepatocytes, producing an additive increase in MT with Cd and Dex and a synergistic increase in MT with Zn. (Supported by NIH Grant ES-01142 and ES-07079).

81 METALLOTHIONEIN INDUCTION BY DIETHYL MALEATE. J W Bauman, J M McKm Jr, J Liu, Y P Liu and C D Klaassen. U Kansas Med Ctr, Kansas City, KS

Diethyl maleate (DEM) is a glutathione depleting agent that increases hepatic levels of the sulfhydryl-rich protein metallothionein (MT). The goal of the current study was to characterize this increase in hepatic MT levels by DEM in mice and to examine the mechanism(s) by which DEM might increase MT levels. A time-course study demonstrated that MT concentration in livers of mice increased 22 fold and reached a peak between 12 and 24 hours after DEM (5 mmol/kg, sc) treatment. There was an increase in both MT-I and MT-II mRNA as shown by Northern blot hybridizations using MT-I and MT-II specific oligonucleotide probes. This increase was quantitated by slot blot analysis, which revealed a 9-fold increase in MT-I and a 5-fold increase in MT-II. DEM does not appear to be a direct MT inducer as shown by the lack of a significant increase in MT levels when added to cultured mouse hepatocytes. The increase in MT does not appear to be directly related to the decrease in liver GSH (60%) after DEM, because phorone, which decreases liver GSH (80%), produces only a 4-fold increase in liver MT. The adrenal glands do not appear to be necessary for MT induction, as adrenalecotomy did not abolish the increase in MT caused by DEM. Zn redistribution to the liver was not the cause of MT induction, because liver Zn levels did not increase prior to the increase in MT. In summary, DEM is a highly effective inducer of MT which increases MT at the transcriptional level. However, the mediator for this induction has not yet been determined. (Supported by NIH Grants ES-01142 and ES-07079).

The processes involved in the uptake of cobalt across epithelial cells remains to be elucidated. Most chemical forms of cobalt are incompletely absorbed from the gastrointestinal tract (GIT). The in vivo absorption of cobalt naphthenate (CoNap), a slightly soluble organic compound, was investigated. In addition, the ability of the absorbed cobalt from CoNap and cobalt chloride (CoCl₂), a water soluble compound, to stimulate heme oxygenase was also investigated. Absorption studies were conducted after the single oral dose to male Fischer 344 rats of 2.8 or 280 mg/kg CoNap dissolved in Emulphor/ethanol (1:2), ca. 0.001 and 0.1 x L.D₅₀. Cobalt levels were determined over after 36 hr in the urine, feces, and tissues by atomic absorption spectrophotometry. Both the low and high dose groups excreted approximately 30% of the dose in the urine by 36 hr, with the majority of the dose excreted in the feces. Tissue levels peaked at approximately 8 hr. Those tissues found to have the highest cobalt levels were the liver, heart, and kidneys. The heme oxygenase results indicated that the oral administration of either CoNap or CoCl₂ at the 280 mg/kg dose level was not sufficient to induce activity. The liver cobalt levels from these animals were approximately equal, indicating that the ability of the GIT to absorb cobalt was dependent on the rate of uptake and not the rate of dissolution of the compounds.

INFLUENCE OF VARIOUS DIETARY CONSTITUENTS ON GASTROINTESTINAL ABSORPTION AND URINARY EXCRETION OF ALUMINUM IN RATS.

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Eight groups of female Sprague-Dawley rats were treated with 281 mg Al(OH)₃/kg/day by gastric intubation five times a week for five weeks. Concurrently, animals in seven groups received ascorbic acid (56.3 mg/kg/day), citric acid (62 mg/kg/day), glucuronic acid (60.7 mg/kg/day), lactate acid (2.8 mg/kg/day), malic acid (42.9 mg/kg/day), and citric acid (25.8 mg/kg/day), and taurine (45 mg/kg/day) in the drinking water. The eighth group did not receive any dietary constituent in the water and was designated as control group. Animals were placed in plastic metabolic cages and urine was collected during the treatment period. The liver, spleen, kidney, brain, and bone aluminum levels of each rat were measured, as well as the total amount of aluminum excreted into urine. All the dietary constituents significantly increased the aluminum concentrations in most of the tissues, with ascorbic and citric acids showing the highest rate of aluminum accumulation. In contrast, no significant differences between control and treated rats were observed in the concentrations of aluminum excreted into urine. In view of these results, we suggest that a careful surveillance of the intake of aluminum patients is performed.


Heavy metal poisoning is often associated with reproductive and metabolic disorders. Since endocrine function is directed in part by pituitary hormones, the effect of mercury, zinc, cadmium, nickel, and lead on pituitary release of growth hormone (GH) and prolactin was evaluated using rat anterior pituitary cells in vitro. Cultured cells were exposed to either control or metal-containing media for one hour and accumulation of hormone was measured by RIA. Incubation of cells with the chloride salt of zinc, nickel or cadmium did not alter basal release of GH but reduced basal prolactin levels. Incubation of cells with mercuric chloride resulted in higher basal levels of both GH and prolactin but this effect did not consistently reach statistical significance. All of the metals were effective in reducing GH release stimulated by growth hormone releasing hormone (GRF). The threshold concentration for inhibition of GRF action was 10⁻⁷M for mercury, 5 x 10⁻⁶M for zinc, 10⁻⁴ for cadmium and 10⁻⁴M for nickel. In contrast, incubation of cells with lead nitrate did not affect basal or GRF-stimulated hormone release. These data suggest that pituitary somatotrophs, lactotrophs are potential targets for the toxic effects of mercury, zinc, cadmium and nickel.

THE EFFECTS OF ZINC ON DIETHYLDITHIOCARBAMATE (DDC) CYTOTOXICITY ON GLIAL CELLS. G. Barry, H. L. Trombetta. Toxicology Program, College of Pharmacy. St. John's University, New York, NY.

A primary culture of rat cerebral astrocytes were grown in Dulbecco's Modified Eagle's medium with 10% FBS. Cells were treated as follows: 1 hour with 35μg/ml DDC in medium, simultaneously treated with 50μM ZnCl₂ and 35μg/ml DDC in medium, pretreated with 50μM ZnCl₂ and 35μg/ml DDC, then washed in buffer and treated with DDC for 1 hour, or pretreated with ZnCl₂ washed in buffer for 1 hour, then treated with DDC for 1 hour. After treatment, the cells were fed complete medium. Cell adherence was determined after a 24 hour time period as a percent of control. 12% of the DDC treated cells remained adherent after 24 hours. Cells treated simultaneously with ZnCl₂ and DDC had a slight protective effect with a mean adherence of 50%. All cells pretreated with ZnCl₂ had a marked protective effect with a mean adherence of 92%. A significant difference in adherence was seen among groups (p< .001).
Vanadium (V) accumulates in human placenta and can generate superoxide due to free-radical redox cycling. V⁴⁺ oxidizes to V⁵⁺ under aerobic conditions. V⁴⁺ immediately reacts with superoxide, forming peroxo-vanadyl complex which absorbs at 328 nm. To determine peroxidative effects of (hydro)peroxo-vanadyl, human term placental microsomes were incubated with V⁴⁺ or V⁵⁺ in the presence of benz(a)pyrene (BP) or BP-7,8-dihydrodiol. The products of co-oxygenation were measured by HPLC and radiometry. V⁴⁺, but not V⁵⁺, caused a dose-dependent increase in macromolecular binding of BP and BP-7,8-diol, trans-anti-BP-tetrahydroteratol and total products of BP-7,8-diol. It is postulated that the (hydro)peroxo-vanadyl can initiate lipid peroxidation in human term placental microsomes, leading to production of lipid peroxyl radicals which co-oxygenate BP-7,8-diol to its ultimate carcinogen, BP-dihydriodiol epoxide.

Supported in part by Grant from The Council for Tobacco Research USA, Inc.

Recently, we have shown that NADPH-dependent metabolism of 1,3-butanediene by mouse liver microsomes or H₂O₂-dependent metabolism by chloroperoxidase yields both BM and CA. In the present study, incubations of 1,3-butanediene with myeloperoxidase from human leukocytes and H₂O₂ resulted in the formation of BM. Metabolism of 1,3-butanediene by myeloperoxidase was dependent on pH (optimal at pH 7.4), H₂O₂ concentrations, and incubation time. The myeloperoxidase-dependent metabolism of 1,3-butanediene to BM occurred at slower rates than with chloroperoxidase. To determine whether myeloperoxidase was inactivated during catalysis, reactions with the inclusion of ascorbic acid, which is known to convert inactive myeloperoxidase to an active form, were studied. Addition of ascorbic acid at 15 min after the beginning of the enzymatic reaction significantly enhanced the formation of BM compared to control incubations. Moreover, the use of ascorbic acid allowed the detection of both BM and CA. These results indicate that myeloperoxidase may play a significant role in 1,3-butanediene-induced toxicity. (Supported by NIH grant GM-40375)

Chronic inhalation exposure of rats to VA induces benign tumors of respiratory regions (RES) and malignant tumors of olfactory regions (OLF) of rat but not mouse nasal cavity. We propose the mechanism of action involves, but may not be limited to, nasal esterase-mediated hydrolysis of VA to the genotoxic agent acetaldehyde and the cytotoxic agent acetic acid. Kinetic studies were performed to measure the rate of hydrolysis of VA to acetaldehyde in RES and OLF of rats and mice. The Vₐ₅₀/Kₐ value for male and female rat OLF was approximately 5 times greater than that of RES. Vₐ₅₀/Kₐ for RES and OLF from female rats was approximately 1.5 times greater than that of male rats. For mice, the Vₐ₅₀/Kₐ value for male and female OLF was approximately 4 times greater than that of RES. Vₐ₅₀/Kₐ was similar when comparing male and female RES or OLF. No differences were detected when comparing Vₐ₅₀/Kₐ across species. These results correlate with the bioassay results showing that OLF is more sensitive to the chronic toxicity and carcinogenic action of VA than RES. However, species differences in the carcinogenic effects of VA were not accounted for by differences in Vₐ₅₀/Kₐ. In total, the results support the hypothesis that metabolism of VA to acetaldehyde and acetic acid is important in the mechanism of VA-induced nasal carcinogenesis.

Recent investigations from our laboratory have established that lipoxigenase is capable of xenobiotic metabolism. In this study we examined the soybean lipoxigenase dependent co-oxidation of 2-AF, a putative carcinogen and teratogen. 2-AF metabolism was followed spectrally as a decrease in absorbance at 286 nm. In the presence of 2.8 mM linoleic acid, 34 μg soybean lipoxigenase and 250 μM 2-AF, the rate of metabolism was found to be 1.2 umoles/min/mg protein. The generation of electrophilic 2-AF intermediate(s) capable of covalent binding to macromolecules was investigated radiometrically. Preliminary results indicated that the magnitude (umoles/min/mg) of binding to protein and calf thymus DNA was 2.65 and 0.40 respectively, suggesting clearly bioactivation of 2-AF. Pre-incubation of enzyme with 33μM Eicosatriynoic acid or 20 μM Nordihydroguaiaretic acid resulted in >50% inhibition of 2-AF oxidation and covalent binding to protein and DNA. These results strongly suggest that hydroperoxidase activity of lipoxigenase is capable of 2-AF metabolism and this may represent yet another pathway for bioactivation of this aryamine. To our knowledge, there is no previous report on the lipoxigenase catalyzed 2-AF metabolism. Supported in part by funds from The Council for Tobacco Research, Inc. USA.
1,3-Butadiene is more carcinogenic to mice than rats; BM, a mutagenic metabolite of 1,3-butadiene, has been implicated in 1,3-butadiene-induced toxicity, but the role of GSH S-transferases in BM metabolism/toxicity was not previously investigated. In this study, the rate of the NADPH-dependent 1,3-butadiene metabolism to BM by mouse liver microsomes was found to be nearly 2-fold higher than that of rat liver microsomes. The GSH-dependent metabolism of BM to S-(2-hydroxy-3-buten-1-yl)glutathione (I) and S-(1-hydroxy-3-buten-2-yl)glutathione (II) was localized (>90%) in the cytosolic fraction of rat liver with conjugate I constituting nearly 80% of the total conjugates formed. The combined rates of conjugate I and II formation by mouse and rat liver cytosol were similar (Vmax = 102 ± 22 and 72 ± 10 nmol/mg protein/min; mean ± SD) and were nearly 10 and 20-fold higher than the rates of BM formation, respectively. These results indicate that species differences in 1,3-butadiene oxidation to BM, rather than BM conjugation with GSH, may be useful in predicting species susceptibility to 1,3-butadiene-induced toxicity. (Supported by NIH grants GM-40375 and ES-07015)

**METABOLISM OF WR 6026, AN 8-AMINOQUINOLINE WITH ANTIILEISHMANIAL ACTIVITY.** B. Chang, P. Brennan, D. Johnson, M. Goldman, P. Adams, and A. Buczkow. Walter Reed Army Institute of Research, Washington, DC and School of Veterinary Medicine, UC Davis, Davis, CA.

WR 6026, 8-(6-Diethylaminohexylamino)-6-methoxy-4-methylquinoline, a highly efficacious antileishmanial drug, is currently in Phase I clinical trials. Earlier work has demonstrated a number of biotransformation products arising from WR 6026 (Xenobiotica 20: 31, 1990). The present studies compare the profile of metabolites in precision cut liver slices to earlier results in perfused liver and microsomal preparations. Haemster liver slices, prepared with a Kruedioc liver slice, were incubated with ^14C-WR 6026 in either Ham's F12 (containing 25 mM glucose and 10 mM HEPES) or Waymouth's medium (10 mM HEPES) for 4 hr. Viability of the slices, as assessed by LDH leakage and ATP content, was appreciably better in slices incubated in Waymouth's medium. The primary metabolite isolated from the incubation medium was the O-demethylated derivative; levels of this metabolite produced by slices incubated with Waymouth's medium were 3 fold higher than in Ham's. Trace quantities of the N,N-dideethyl metabolite and the carboxylic acid derived by deamination were observed. None of the unconjugated 4-hydroxyethyl metabolite, a major biotransformation product in the rat and hamster, was observed in liver slice medium. These studies indicate that metabolite profiles differ qualitatively from those obtained in other systems. Supported by USAMRDC contract No. DAMD 17-86-C-6177.


NDS was originally developed as an agricultural fungicide, but was subsequently found to be nephrotoxic in rats. Although the mechanism of this toxicity remains to be elucidated, a role for metabolism has been proposed. In the process of examining the metabolism of NDS, we found evidence for two previously unreported minor metabolites. Reversed phase HPLC was used for resolution of metabolites and LSC of collected fractions for their quantification. Metabolites were tentatively identified by co-chromatography with authentic standards. Hepatic homogenates were prepared from animals pretreated with phenobarbital and were then incubated in vitro (N=4) with ^14C-NDS (2.0 mM). Under these conditions, a small amount (0.9%) of N-(3,5-dichlorophenyl)-hydroxysuccinimide (NDS-P-OH) was detected, in addition to several other previously identified metabolites. Production of NDS-P-OH was NADPH-dependent. This compound was not detected in renal homogenate incubations. NDS-P-OH was previously proposed as the intermediate in the production of two of the known metabolites of NDS. This metabolite was also detected in hepatic homogenates (2.3%) prepared three hours after animals (N=4) were dosed with 0.6 mmol/kg ^14C-NDS (i.p.). In both hepatic and renal homogenates prepared from these animals, small amounts of N-(3,5-dichloro-4-hydroxyphenyl)succinimide (NDS-P-OH) were also detected. Some of the possible roles of NDS-P-OH in the toxicity of NDS have been previously examined, but the possible relationship of NDS-P-OH to NDS-induced toxicity remains to be investigated. Supported by P.H.S. grant ES05189.

**SPECIES AND ORGAN DIFFERENCES IN THE METABOLIC ACTIVATION OF 1,3-BUTADIENE.** G A Csanady and J A Bond. CIIT, Research Triangle Park, NC.

Butadiene (BD) is carcinogenic at multiple organ sites in Sprague-Dawley rats and B6C3F1 mice. Mouse display a considerably higher sensitivity to inhaled BD than rats. Knowledge of the metabolic capacity of target organs toward BD in both species will be important in extrapolation of BD metabolism and toxicity to humans. The goal of this study was to characterize the in vitro metabolism of BD to butadiene monoeoxide (BMO), a potential DNA reactive BD metabolite, in liver and lung microsomes of rats and mice. Reactions were carried out in sealed reaction flasks and the headspace was analyzed for BD and BMO using gas chromatography. The maximal rate of BD disappearance (Vmax) in liver microsomes of mice 1.80 (1.04 · 1.56 at 95% CI) nmol/min/mg protein was 4 times greater than that observed in rats 0.35 (0.28 - 0.42 at 95% CI) nmol/min/mg protein. Lung microsomes metabolized BD at rates similar to those measured in liver microsomes. In both species metabolism of BD was inhibited at concentrations above 10,000 ppm. In rat lung microsomes, further metabolism of BMO occurred to a lesser extent than in liver microsomes and accumulated in reaction flasks. These data suggest that the rate of metabolism of both BD and BMO may be important considerations in species sensitivity to BD. The data from this study will be used to develop a physiologically-based pharmacokinetic model for BD.
Nicotine (NIC) is extensively metabolized in humans via phase I xenobiotic metabolizing enzymes to two major metabolites, cotinine (COT) and trans-3-hydroxycotinine (3HC). A recent study reported indirect evidence that nicotine and these major metabolites were excreted as glucuronide conjugates in the urine of smokers. We have confirmed this observation using a newly developed thermospray LC/MS technique which permits the direct determination of NIC, COT, and 3HC in human urine. In this study, smokers' urine was analyzed without extraction or derivatization for NIC, COT and 3HC. The urine was then extracted with methylene chloride to minimize the concentration of free NIC and its metabolites. Treatment of the extracted urine with β-glucuronidase at 37°C for 24 hours produced a dramatic increase in the amount of free NIC, COT, and 3-HC in the urine. These aglycons were identified and quantified using thermospray LC/MS method. The specificity of β-glucuronidase for these conjugates was confirmed by inhibiting conjugate hydrolysis with p-sulfanilic acid 1,4-diacetate. Optimum conditions for the hydrolysis of these glucuronide conjugates were established: 4380 units of β-glucuronidase/ml of 0.1M sodium acetate buffer, pH 4.5 and a 24-hour incubation. These results demonstrate that smokers metabolize nicotine via both phase I and phase II metabolic pathways and that glucuronide conjugates of NIC, COT and 3HC are major human metabolites of nicotine.

Over 30% of benzo(a)pyrene (BP), an environmental carcinogen and teratogen, is eliminated by glucuronidation, which compiles cytochromes P-450 catalyzed bioactivation to a carcinogenic reactive intermediate. A genetic deficiency in bilirubin UDP-glucuronyl transferase (UGT) occurs in 5 to 7% of the population, and this deficiency could predispose such people to benzo(a)pyrene bioactivation and toxicity. In previous studies, this hypothesis was confirmed in Gunn and RHA rats, which are similarly deficient in GT, and which demonstrate reduced BP glucuronidation, enhanced BP bioactivation (FASEB J. 4: 4699, 1990) and enhanced susceptibility to BP embryopathy (FASEB J. 3: A1025, 1989). In the current study, a noninvasive method was developed to measure the GT activity for BP in humans. [7,10-14C]Benzo(a)pyrene was incubated with homogenates of human blood lymphocytes (HL)/20 - 50 x 10^6 cells per incubation), NADPH and UDPGA for 120 min at 37°C. BP and its metabolites, including glucuronides, were meared by high-performance liquid chromatography with a radioactivity detector. The identity of BP glucuronides was confirmed by incubation without UDPGA, and by reincubation with beta-glucuronidase. In HL obtained from 8 healthy volunteers, the glucuronides of BP constituted 0.04% to 0.25% (0.17±0.03%, mean±SE) of the total radioactivity, and the formation rate of BP glucuronides was 0.02 to 0.47 (0.23±0.07) pmol/sec/10^6 cells/hr. BP glucuronidation was measurable in all subjects, and the observed counting efficiency of [7,10-14C]BP was 90±5%. There appear to be remarkable interindividual differences in BP glucuronidation, and the use of HL may provide a useful approach for toxicological studies of polycyclic aromatic hydrocarbons in people with GT deficiencies. (Support: Medical Research Council of Canada)

Induction of the glutathione S-transferase (GST) has been examined in rat hepatic tissue following the treatment with thiazole, pyrazine, pyridazine, pyrimidine, thiophene and triazole. GST activity towards 1-chloro-2,4-dinitrobenzene (CDNB) was increased -2-fold in the cytosol isolated from thiazole- and pyrazine-treated rats relative to control, whereas pyridazine, pyrimidine and thiophene increased GST activity only by 50%, respectively. Triazole failed to induce GST activity. SDS-PAGE and immunoblot analyses revealed a time-dependent increase in the expression of GST subunits 1, 2 and 3-4 relative to control with a 3-fold increase in the intensity of subunit 1 monitored at day 3 posttreatment with thiazole, pyrazine and pyridazine. The results of GST activity measurements using the inhibitors Cibacon Blue and triethyltin bromide are consistent with the observation that form 1-1 is induced by -3-fold. Hybridization analyses revealed that the levels of a-class mRNAs were increased 8- to 11-fold and 2- to 8-fold posttreatment with thiazole and pyridazine, respectively, relative to control. Pyrazine also increased a-class mRNAs by 12- to 14-fold. These results reveal that these compounds are efficient inducers of GSTs and that the induction involves large increases in a-class mRNAs. Supported by NIH grant GM42620.

Induction of the glutathione S-transferase (GST) was examined in rabbit hepatic tissue following treatment with 4-picoline. Male New Zealand white rabbits, 2.0-2.5 kg, were injected with 4-picoline, 100 mg/kg, ip, for 4 d. Cytosol was isolated and subjected to SDS-PAGE analysis which revealed the presence of a 28 kDa polypeptide band which was not observed in the cytosol of untreated animals. Western blot analysis using goat antirat class a specific IgG confirmed that this 28 kDa band was detectable only in cytosol from 4-picoline-treated rabbits and was immunochemically related to rat class a isoenzymes. The 28 kDa protein selectively bound to an S-hexylglutathione agarose affinity column and was subsequently purified using NaCl gradient elution from a CM-Sepharose column followed by chromatofocusing on a PBE118 column. The purified protein had a molecular weight of 24 kDa and a pi of 8.0 determined by gel filtration and isoelectric focusing, respectively. This purified GST displayed a Vmax of 73.5 μmoles conjugate formed/min/mg protein and a Km of 1.60 mM towards 1-chloro-2,4-dinitrobenzene, and a Vmax of 6.92 U/mg and a Km of 1.02 mM towards cumene hydroperoxide. Although the N-terminus was blocked, sequencing of a cyanogen bromide cleavage fragment yielded a consensus class a sequence MKLVQTRAFNYAA. Supported by NIH grant GM42620.
BILIRUBIN-GLUCURONIDE EXCRETION REMAINS UNCHANGED DESPITE CIRCADIAN VARIATION IN UDP-GLUCURONIC ACID AND BILE FLOW IN RATS. D Y Mitchell, C Machu, J W Bauman and C D Klaassen, Univ Kansas Med Ctr, Kansas City, KS.

Bilirubin is a potentially neurotoxic, degradation product of heme catabolism. Bilirubin biosynthesis is a two-step process catalyzed by heme oxygenase (HO) and biliverdin reductase. Elimination of bilirubin requires glucurono-nidation utilizing glucuronyl transferase (UDP-GT) and the co-substrate UDP-glucuronic acid (UDP-GA). As circadian variation has been attributed to the toxicity of a number of xenobiotics, the purpose of this study was to determine if bilirubin formation, metabolism or excretion is affected by diurnal changes in enzyme activities, UDP-GA levels or bile flow. Every 4 hrs during a 24-hr cycle, bilirubin-glucuronide (BIL-GLUC) concentration, bile flow, enzyme activities and UDP-GA levels were determined in Sprague-Dawley rats. BIL-GLUC excretion remained unchanged during the 24-hr cycle, although BIL-GLUC concentrations were elevated by 20% from 2 to 10 pm. The increased BIL-GLUC concentrations correlated with a 15% decrease in bile flow. UDP-GA concentration was at its nadir at 2 pm, with a 250% increase by 10 pm. The marked change in UDP-GA concentration was not reflected by an alteration of BIL-GLUC concentration or excretion, but did correlate inversely with UDP-glucose and glycogen levels. HO, UDP-GT and 7-glucuronidase activities were unchanged during the 24-hr cycle. In summary, BIL-GLUC excretion remained unchanged despite circadian variation in UDP-GA levels and bile flow. (Supported by NIH Grants ES-03192 and ES-07079)

HOMEOSTASIS OF SULFATE AND 3'-PHOSPHO-ADENOSINE 5'-PHOSPHOSULFATE (PAPS) IN RATS FOLLOWING ACETAMINOPHEN (AA) ADMINISTRATION. H J Kim, P Rozman and C D Klaassen, Univ Kansas Med Ctr, Kansas City, KS

AA is a drug whose biotransformation by sulfation is easily saturated. We have previously demonstrated that these dose-dependent kinetics appear to be due to a depletion of PAPS. In order to determine if the depletion of PAPS might be due to a lack of sulfate, we characterized the effect of AA not only on PAPS homeostasis but also of its precursor, sulfate. The maximum excretion of AA-SO₄ was observed after 75 mg/kg AA, ip, and higher dosages did not increase its excretion. AA dosages between 150-600 mg/kg, ip, 2 hr after dosing depleted 60-80% of hepatic PAPS concentrations. Hepatic PAPS levels returned to control values 16-20 hrs after dosing with 600 mg/kg AA. AA decreased serum sulfate to a similar degree (80%) and duration (16 hr) as did hepatic PAPS. AA also lowered hepatic sulfate concentrations, but to a somewhat lesser extent (65%) than in serum, and returned to control values at 16-24 hrs after dosing with AA. Even though AA did not alter renal PAPS concentrations, it did produce a 65% decrease in the renal sulfate levels. In summary, these studies demonstrate that AA markedly depletes PAPS concentrations in liver, but not in kidney, and drastically decreases serum and tissue sulfate concentrations. The depletions of hepatic PAPS and sulfate levels are restored concomitantly. Our findings suggest that the hepatic sulfation of AA is limited by the availability of sulfate in liver. (Supported by NIH Grants ES-03192 and ES-07079)

HOMEOSTASIS OF SULFATE AND 3'-PHOSPHO-ADENOSINE 5'-PHOSPHOSULFATE (PAPS) BY DIETARY ALTERATION OF SULFUR. P Rozman, H J Kim, C Machu and C D Klaassen, Univ Kansas Med Ctr, Kansas City, KS

This study was designed to determine the role of dietary organic and inorganic sulfur on PAPS homeostasis. Organic sulfur was altered by adding various amounts of methionine (0.15, 0.3, 0.6 or 1.2%) to a sulfhydril-deficient diet. Inorganic sulfur was altered by providing rats with no sulfate or sulfate in their diets (0.12%) and distilled or tap water (1.8 mM). Rats received these diets for 5 days. The two lowest methionine-containing diets produced a 60% reduction in liver glutathione (GSH) concentrations, and the addition of sulfate to the diets did not restore hepatic GSH levels. Urinary sulfate excretion was reduced by 95% in rats fed the three low-methionine diets. Addition of sulfate to these diets increased the urinary excretion of sulfate, but did not return sulfate levels to control values. The three low methionine-containing diets decreased serum and liver sulfate concentrations about 50% and addition of sulfate to these diets largely restored them to control levels. Hepatic PAPS concentration was decreased (10%) only in the group receiving the lowest methionine content in their diet, and addition of sulfate had no effect on hepatic PAPS. In summary, dietary alterations of sulfur lowered the GSH concentration in the liver as well as decreased sulfate levels in serum, liver and urine, but had minimal effects on hepatic PAPS concentrations. Therefore, it appears that hepatic steady-state PAPS levels are not highly dependent on the sulfur content of the diet. (Supported by NIH Grants ES-03192 and ES-07079)

SPECIES COMPARISON OF URINARY 3-METHYLINDOLE METABOLITES. M L Appleton, G L Skiles, D J Smith, J R Carlson, and G S Yost, 1 Dept. Pharmacol. & Toxicol., Univ. of Utah, Salt Lake City, UT and 2 Dept. of Animal Sciences, Washington State Univ., Pullman, WA

3-Methylindole (3MI) is a pulmonary toxin formed by bacterial fermentation of dietary tryptophan. Species susceptibility to 3MI toxicity is quite variable. Urinary 3MI metabolites from species have been identified; goats, a highly susceptible species and rats and mice, both relatively nonsusceptible. Nine significant metabolites have been identified from goats; 4 glucuronides, 3 sulfates, 1 mercapturic acid, and an unconjugated derivative. Four metabolites have been identified from mice; 2 glucuronides and 2 unconjugated derivatives. A mercapturic acid was isolated from rats. Both goats and rats, but not mice produce the mercapturate, 3-(N-acetylcysteine-S-yl)-methylindole. This mercapturate indicates that an electrophilic 3-methylindole imine is formed in vivo. This reactive intermediate has been suggested to be responsible for the toxicity. All of the metabolites found in mouse urine are also found in goat urine although the conjugation patterns differ. Seven of the 9 metabolites from goat and 3 of the 4 in mice are oxidodizes, thus this oxidation appears to be a general pathway. Although these 3 species differ significantly in their susceptibility to 3MI toxicity, their urinary metabolic profiles do not reflect this difference in toxicity. Supported by USPHS Grant HL13643. G SY is a USPHS Research Career Development Awardee (HL02119).
102 ENHANCED BILIARY EXCRETION OF THYROID HORMONES BY MICROSOMAL ENZYME INDUCERS. C D Klaassen and R A Barter. Univ Kansas Med Ctr, Kansas City, KS

Microsomal enzyme inducers have been shown to reduce thyroid hormone levels by an extrathyroidal mechanism. Experiments were performed to determine if these inducing agents increase the catabolism and excretion of thyroid hormones to produce this reduction. Thyroidectomized male rats had osmotic minipumps implanted to deliver 125I-Thyroxine (T4) and 3,5,3'-triiodothyronine and three days later began diets containing phenobarbital (PB; 1200 ppm), 3-methylcholanthrene (3MC; 250 ppm), pregnenalone-16a-carbonitrile (PCN; 1000 ppm), or polychlorinated biphenyls (PCB; 250 ppm) for ten days. On the tenth day of treatment, bile, urine, livers and kidneys were collected. No change in renal concentrations of radioactivity or urinary excretion was observed. Hepatic accumulation of radioactivity was increased by treatment with PB, PCN and PCB. Total biliary excretion of 125I-T4 and its metabolites was increased after treatment with 3MC, PCN and PCB. Biliary metabolites of 125I-T4 were separated by HPLC and quantitated by gamma- scintillation spectroscopy. The biliary excretion of T3-glucuronide was dramatically increased by 3MC, PCN and PCB, whereas PB produced a moderate increase. An increase in 125I-holode excretion was observed with 3MC, PCN and PCB. Thus it appears that the various microsomal enzyme inducers differentially affect hepatic uptake, biotransformation and biliary excretion of thyroxine. (Supported by NIH Grants ES-03192 and ES-07079, and a Procter & Gamble Fellowship).

104 COMPARISON OF METABOLISM OF 3,4-(METHYLENEDIOXY) METHAMPHETAMINE (MDMA) IN RATS AND MICE. H K Lim, S Zeng, C O Sakashita, D M Chel and R L Foltz. Center for Human Toxicology, Dept. Pharmacology and Toxicology, Univ. of Utah, SLC, UT. (Sponsor: D E Moody)

There are marked species differences in MDMA-induced neurotoxicity: rats and mice are most and least susceptible to the development of neurotoxicity, respectively. Consequently, the comparative metabolism of MDMA was investigated in these two species. All but one of the previously reported metabolites in rats (Lim and Foltz, Chem. Res. Toxic. 1, 370, 1988) are also formed in mice and they include 4-hydroxy-3-methoxyamphetamine (HMA), 4-hydroxy-3-methoxyamphetamine (HMA), 3,4-(methylenedioxy)-amphetamine (MDA), 3,4-dihydroxymethamphetamine, (3,4-dihydroxyphenyl)acetic acid, (4-hydroxy-3-methoxyphenyl)acetic acid and 3,4-(methylenedioxyphenyl)acetic acid. A PPT2A enhanced NIH-3-PCI-11131 assay linear from 2 to 1000 ng/ml, has been developed for simultaneous quantitation of MDMA, MDA, HMA and HMA in urine. The 24 h urinary excretion of MDMA, MDA, HMA and HMA account for 16.34, 6.34, 14.30 and 2.05%, respectively of the administered dose (10 mg/kg) to rats. For mice, the 24 h urinary excretion of MDMA, MDA, HMA and HMA account for 43.02, 7.52, 11.16 and 2.47%, respectively of the administered dose. HMA is the major metabolite in both rats and mice urines. The similar 24 h urinary excretion of MDA, HMA and HMA in mice and rats suggests that these metabolites and/or their precursors are unlikely to account for the observed species differences in MDMA-induced neurotoxicity. The 24 h urinary excretion of MDMA in rats is approximately 3 times less than in mice which suggests greater MDMA metabolism in rats. (Supported by NIDA grant 1RO1 DA 05860-01).

103 URINARY METABOLITES OF 2-METHOXYETHANOL DETERMINED BY NMR SPECTROSCOPY. SCJ Summar, DO Clarke, F Welsh, and TR Fennell. CIIT, Research Triangle Park, NC

2-Methoxyethanol (2-ME) is teratogenic. Studies in several species have revealed 2-methoxyacetic acid (2-MAA) and 2-methoxy-N-acetylglycine (2-MNAG) as major urinary metabolites of 2-ME. Other metabolites have been proposed, and the purpose of this study was to elucidate their structures. Pregnant CD-1 mice (gestation day 11) were dosed p.o. with 250 or 25 mg/kg uniformly 13C labelled 2-ME. Urine was collected for 24 hours and examined by one- and two-dimensional NMR spectroscopy without separation or purification of metabolites. The 13C spectra of urines contained signals from enriched carbons which showed that at least 6 metabolites were present at the high dose. Signals for 4 metabolites were detected at the low dose. At both doses, 2-MAA and 2-MNAG were the predominant metabolites. 2-ME was excreted as the parent compound, appeared to form a glucuronide conjugate and to be metabolized via ethylene glycol to glycolic acid and glycine. Signals consistent with metabolites formed by incorporation of 2-MAA into the citric acid cycle and fatty acid synthesis were detected. Some of the metabolites of 2-MAA may contribute to the teratogenic effects of 2-ME.

105 SPECIES DIFFERENCES IN METABOLISM OF INHALED BUTADIENE. P L Sabatini, L T Burka*, A R Dahl, W E Bechtold and K P Henderson. Inhalation Toxicology Research Institute, Albuquerque, NM. *NIEHS, RTP, NC

Chronic exposure of B6CF1 mice and Sprague-Dawley rats to butadiene (BD) produced a very high incidence of cancer in mice while the incidence in rats was much lower with different tissues affected. Studies at this Institute indicate that for equivalent exposures, the blood BD epoxide concentrations in mice is 10-fold higher than in rats and > 10-fold higher than in Cynomolgus monkeys. In this study, the profiles of urinary metabolites of butadiene were determined in Cynomolgus monkeys, F344/N rats, Sprague Dawley rats, B6CF1 mice and Syrian hamsters. Species containing widely divergent hepatic epoxide hydrolase (EH) activities. Animals were exposed for 2 hr to 8,000 ppm [14C]BD and 24-hr urine samples were analyzed for metabolites. Two major urinary metabolites were identified, N-acetyl-S-(1 or 2)-3-buten derivative and 3-buten-2-one (3'-cy) (1) and N-acetyl-S-(4-butenyl-2,3-diol)cysteine (11). Monkeys exposed by inhalation produced primarily metabolite 11, while rodent species produced 1-4 times as much of I compared to 11. The ratio of 1/11 formation was related to the hepatic epoxide hydrolase activity in different species. The high 11/1 ratio in monkeys was consistent with the lower blood epoxide levels in this species. BD metabolism by humans is similar to that in the monkey, exposure of humans to BD may result in lower tissue concentrations of reactive metabolites than an equivalent exposure of rodents. This has important implications for assessing the risk to humans of BD exposure based on rodent studies. (Supported by NIEHS through Interagency Agreement ES-9800 with the U.S. DOE/HER Contract DE-AC04-76EV01013.)
106 IDENTIFICATION OF THE URINARY METABOLITES OF [1,2,3-\textsuperscript{13}C]-TRICHLOROPROPANE IN THE RAT BY \textsuperscript{13}C NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY. \textsuperscript{a} I. G. Sipes, S. M. Winter, M. E. Mackenzie and J. G. Weber. Dept. Pharm/Tox and Pharm Sci., University of Arizona, Tucson, AZ.

1,2,3-Trichloropropene (TCP) is used as an intermediate in the manufacturing of pesticides and polysulfide rubbers and has been identified as a ground water contaminant. TCP has recently been shown to be tumorigenic in a chronic bioassay conducted by the National Toxicology Program. Since the metabolites of TCP may be unstable, polar and thus difficult to isolate, \textsuperscript{13}C NMR was used to identify directly the urinary metabolites of 99\% \textsuperscript{13}C-enriched TCP. Male Fischer-344 rats received [1,2,3-\textsuperscript{13}C]TCP (30 mg/kg in soybean oil) together with a radioactive tracer, [2,\textsuperscript{13}C]TCP. By 48 hr approximately 50\% of the dose was recovered in the urine. Urine collected at 6, 12, 24 and 48 hr post dosing was investigated directly using proton-decoupled \textsuperscript{13}C and 2D homonuclear correlated NMR spectroscopy. The urine from TCP treated rats revealed major, time dependent, signals from at least 5 \textsuperscript{13}C-enriched metabolites of TCP containing 2 and 3 \textsuperscript{13}C units. Signals have been assigned to 2,3-dichloropropionic acid and N-acetyl-5-(3-chloro-2-hydroxypropyl)cysteine by comparison to spectra of authentic standards. No unchanged TCP was detected. Assignment of other signals is in progress and will be used to elucidate the metabolic fate of TCP. Supported by NTP ES-85230.

107 PREMERCAPTURIC ACID METABOLITES OF BROMOBENZENE-2,3- AND -3,4-OXIDE. J. Zheng and R. P. Handrik. Department of Medicinal Chemistry, University of Kansas, Lawrence, KS.

Bromobenzene 1 has been studied as a model of halogenated aromatic compound metabolism and toxicity. It is suggested to be metabolized to epoxides 2 and 3, which are believed to cause hepatotoxicity. The only evidence for formation of 3 is a reported mass spectrum of the corresponding dihydriodiol. To verify that 3 is a real metabolite of 1, we searched for evidence of its conjugation with glutathione. Rats pretreated with either phenobarbital or \(\beta\)-naphthoflavone were treated with 1, and their urine fractionated which afforded premercapturic acids 4, 5 and 6. These structures were elucidated by FAB-MS and COSY-NMR; parallel studies with BB-3,5-d2 and BB-2,4,6-d2 facilitated interpretation of the spectra. Acid dehydrogenation of 4 and 5 (mixture) gave only \(p\)-bromophenylmercapturic acid, indicating a shift of sulfur from \(meta\) to \(para\) in the reaction of 5. Acid dehydrogenation of 6 gave both \(o\)- and \(m\)-bromophenylmercapturic acids. Base dehydrogenation of 4 and 5 (mixture) gave a mixture of \(p\)- and \(m\)-bromophenylmercapturic acids (i.e. no rearrangement) and base dehydrogenation of 6 gave only \(m\)-bromophenylmercapturic acid.


2,5-Dimethylbenzene (2,5-DMB), a chemical commonly found in fuels and also a component of hydrocarbon based smog, was administered by gavage to male Fischer 344 rats. The urinary metabolites of 2,5-DMB as determined by gas chromatography and gas chromatography/mass spectrometry techniques included 2,5-dimethyl-1-hexanoic acid, 2,5-dimethyl-1,2-hexanediol, 2,5-dimethyl-1,5-hexanediol, 2,5-dimethyl-2,5-hexanediol and 2,5-dimethyl-5-hydroxy-1-hexanoic acid. The kidneys from the dosed rats showed a moderate hydrocarbon-induced nephropathy characterized by hyaline droplet formation. The extent of renal tubular damage was less than that found for 2,2,4- and 2,3,4-trimethylpentane.


A genetic disorder in the jaundiced Gunn rat and in the human Crigler-Najjar Type I syndrome results in unconjugated hyperbilirubinemia and is due to the lack of bilirubin UDP-glucuronosyltransferase activity. Liver microsomes of Gunn rats pretreated with 2, 3, 7, 8 tetrachlorodibenzo-p-dioxin were incubated with DMB, NADPH, glutathione (GSH), molecular oxygen, and bis-(nitrophenyl) phosphate to inhibit demethylation. This resulted in the formation of four pigment products. Formation of two of these pigments, recently reported as hydroxylated GSH conjugates, was found to be directly proportional to GSH concentration. Of the remaining two pigments, the formation of one (pigment A) was found to be inversely proportional to GSH concentration, while the other (pigment B) was independent. The formation of these four pigments was not attenuated by the antioxidant enzymes superoxide dismutase and catalase. Inhibition and induction studies implicate involvement of the cytochrome P-450 IA family. The kinetics of formation suggest that pigment A is a DMB dihydrodiol related to the two GSH conjugates through a common epoxide intermediate and pigment B may reflect the presence of another pathway. This hypothesis is consistent with the lack of attenuation by superoxide dissipate and catalase. The formation of these conjugates demonstrates that, through induction, an alternative pathway for the detoxication of bilirubin exists, and may be of benefit to patients with Type I Crigler-Najjar syndrome.
IDENTIFICATION OF THE BILIARY METABOLITES OF
2,3,7,8-TETRACHLORODIBENZOFURAN IN THE RAT. L T
Burka, S R McGown and K B Toner. NIENH, RTP, NC.
Sponsor: D B Matthews

2,3,7,8-Tetrachlorodibenzofuran (TCDF) is a
highly toxic member of a class of
polychlorinated aromatic compounds, the
polychlorodibenzo-furans, which are widespread
contaminants in the environment. Previous
studies have demonstrated that TCDF is
metabolized and that the metabolites are
primarily eliminated in bile. In this study
TCDF metabolites were obtained by cannulation of
the common bile duct of male F344 rats. iv
administration of 270 mg/kg TCDF/kg body weight,
and collection of bile for 6 hr. Bile was
washed with glucuronidase/sulfatase. The freed
phenolic metabolites were methylated and
subjected to GC/MS analysis. Two metabolites
were present in a ratio of about 8 to 1.
Chemical structures of the metabolites 4-
hydroxy-2,3,7,8-tetrachlorodibenzofuran (the
major metabolite) and 3-hydroxy-2,7,8-
trichlorodibenzo-furan, were confirmed by
comparison of retention times and mass spectra
with synthetic standards. These metabolites
suggest that metabolism of TCDF is primarily
directed to the vicinity of the furan oxygen.
This is consistent with previous observations
that chlorine substitution at the 4 position in the
corresponding pentachloro compound decreases the rate of chlorodibenzofuran metabolism.

A SENSITIVE ASSAY FOR THE METABOLITES OF THE
EXPERIMENTAL DRUG DICHLOORACETATE (DCA). G N
Henderson, P O Whalen, S H Curry and P W Staapoe. Deps. of
Medicine and Pharmaceutics, Univ. of Florida, Gainesville, FL.
Sponsor: M O James

DCA is effective against a number of metabolic disorders, such as
lactic acidosis, diabetes, hyperlipidemia, ischemic heart disease and
hepatitis. It is metabolized to glyoxylate, oxalate, glycine and
glycolate by isolated rat hepatocytes. The quantitative importance
of these metabolites is unknown in humans. Clinically, oxalate is the
most important metabolite because of its potential toxicity. There
are wide variations of plasma oxalate levels in normals as measured
by various reported methods (1 to 200 μmoles/L). Because of this,
we have developed a sensitive and specific HPLC method to
measure oxalate and its precursor glyoxylate in plasma. Plasma
ultrafiltrate (100 μl) containing oxalate and glyoxylate is reacted
with o-phenylenediamine (1 mL, 0.1 M) at 100°C to give highly
fluorescent quinoxaline derivatives. We found ZnCl2 catalyzes the
reaction and use of the catalyst substantially reduced the reaction
time (90 min). A small sample (20 μL) is analyzed on HPLC using
C8 reverse phase column, fluorescent detector (ex. 340 nm; em. 420
nm) and an eluent mixture of MeCN, i-PrOH and H2O (oxalate 5.8
min, glyoxylate 11.3 min and 7-hydroxycoumarin (internal std.) 20.6
min). Calibration gives a straight line for the assay with R values
of about 0.99. For normals (n=10), plasma oxalate and glyoxylate
levels were found to be 2-6 μmoles/L (intra day CV 3.1%, inter day
CV 5.3%) and 1-3 μmoles/L (intra day CV 3.4%, inter day CV 5.8%)
respectively, with the detection limit of 50 nmol/L. To our
knowledge, this is the first method that allows the simultaneous
measurement in plasma of both glyoxylate and oxalate.

KINETICS OF LEAD DISPOSITION IN HUMANS. E J
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Cincinnati, Cincinnati, OH.

A model of lead disposition in humans has been
developed. The model consists of kidney, liver,
bone, and other well-perfused and poorly-
perfused tissues. Fractional absorption of lead
domestic alcohol intake is dependent on
age but not on magnitude of exposure.
Clearance, in urine and bile, is also dependent
on age. The rates of all transfers of lead out
of the blood are related to the concentration of
lead in plasma. Interchanges of lead between
plasma and bone are modeled as partially
interactive events linked in series. A rapid,
flow-limited exchange takes place at all bone
surfaces in contact with blood. In metabolically
active regions of bone, lead is
deposited along with calcium as newly-formed
bone is mineralized, and returned to blood as
bone is resorbed during modeling or remodeling.
Exchange of lead between canalicular fluid and
bone is modeled as a radial diffusion outward
from canalicules into a set of concentric
cylindrical shells surrounding each canalicule.
The predictions of the model are compared with
data relating oral lead intake to blood lead
levels in adults and in infants up to three
months old. (Supported by US. EPA Assistance
Agreement CR-815820-01-0).

METABOLISM OF PHENANTHRENE IN THE RAT AND
GUINEA PIG. I Chu, K M E Ng, P M Benoit and
D C Villeneuve. Environmental Health
Directorate, Health Protection Branch, Ottawa,
Ontario, Canada

Phenanthrene and its alkyl derivatives are the
major polynuclear aromatic hydrocarbon
components in certain liquid fuels. This
compound has also been detected in automotive
exhaust, sidestream cigarette smoking and coal
tar. The object of the present study was to
investigate the biotransformation of
phenanthrene in the rat and guinea pig. C-14
labelled phenanthrene was administered by
gavage in corn oil to Sprague Dawley rats (100
mg/kg b.w./day) and guinea pigs (50 mg/kg
b.w./day) and urine and the animals were
collected for analysis of metabolites.
Phenanthrene was metabolized by the rat and
guinea pig to conjugates of glucuronides,
sulfates and glutathiones. The percentage of
conjugates, expressed as the total urinary
radioactivity, were 39% glucuronides, 24%
sulfates and 18% glutathiones for rats; and
39% glucuronides, 23% sulfates and 28%
 glutathiones for guinea pigs. Enzymatic
hydrolysis of glucuronides and sulfates
resulted in the formation of free 1,2-3,4-
and 9,10-dihydroidoles of phenanthrene and 1-2-
3-4-hydroxyphenanthrene in both
species.
ROLE OF THIOYL STATUS IN CYTOTOXICITY INDUCED BY THE ALKYLATING AGENT ETHYL METHANESULFONATE (EMS). M. W. Fariss, C. S. Brown and V. R. Humaw. Environmental and Molecular Toxicology, Department of Pathology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA

Using freshly isolated hepatocytes in suspension, the addition of 50 mM EMS resulted in the loss of intracellular ATP and K+ as well as promoted lipid peroxidation, bieb formation and cell death. However, the addition of alpha tocopherol succinate (TS, 25 μM) to hepatocyte suspensions prevented each of these toxic manifestations of EMS administration. This system provides an excellent model to test the importance of thiol status to cell survival during a toxic insult. The administration of EMS alone resulted in the rapid depletion of cellular GSH (to < 5% of initial concentration), no GSSG formation, the inhibition of cellular GSH efflux and the loss of protein SH (to < 70% of initial). Under protective conditions (EMS + TS) only the depletion of protein thiol status was reversed. All other EMS-induced alterations in GSH status were identical for protected and non-protected cells. These results suggest that the maintenance of cell viability and protein SH are not dependent on cellular GSH content and support the premise that maintenance of the protein redox status is important for cell survival during a toxic insult. (Supported by a grant from the Jeffress Memorial Trust).

THE EFFECTS OF DIETHYL DITHIOCARBAMATE (DDC) ON ASTROCYTE MICRO TUBULES. M. F. McManus and L. D. Trombetta. Toxicology Program, College of Pharmacy. St. John's University, New York, NY.

A primary culture of rat hippocampal astrocytes were grown in Dulbecco's modified Eagle's medium with 10% FBS. Cells were treated for 1 hour with 35 μg/ml DDC (1.5 x 10^-4 M) in medium. After treatment the cells were given complete medium or medium containing 10 mM glutathione. Control cells were given complete medium alone or medium with glutathione. At 1, 4, and 12 hours cells were fixed and immunohistochemically stained for β-tubulin using colloidal gold with silver enhancement. Control cells showed a complex network of microtubules radiating from the nuclear region into the cell processes. Cells treated with glutathione appeared similar to control cells. Post DDC treated cells appeared similar to controls after 1 hour. By 4 hours many cells showed retracted processes containing condensed reaction product. At 12 hours post DDC treatment cells contained abnormal patterns of microtubules, fragmented microtubules and condensed reaction product. Cells post treated with glutathione were protected from the effects of DDC and appeared normal.

HEPATIC GLUTATHIONE SUPPRESSION BY THE SELECTIVE α-ADRENERGIC RECEPTOR AGONISTS CLONIDINE AND PHENYLEPHRINE. B. C. James, R. D. Harbison, and S. M. Roberts. Center for Environmental & Human Toxicology, University of Florida, Gainesville, FL.

Intraperitoneal administration of either phenylephrine, a selective α1-adrenoceptor agonist, or clonidine, a selective α2-agonist, to male ICR mice resulted in a dose-dependent suppression of hepatic glutathione (GSH) content. Clonidine was approximately 10-fold more potent than phenylephrine in producing this effect, and the GSH depression from clonidine had a somewhat different time course. The selectivity of phenylephrine and clonidine for α-adrenoreceptor subtypes in their effects on GSH was confirmed in experiments using the selective adrenergic antagonists prazosin and yohimbine. Clonidine was also effective by the intracerebroventricular (i.c.v.) route, but i.c.v. phenylephrine had no effect on hepatic GSH levels. These observations suggest that systemic doses of phenylephrine probably diminish hepatic GSH concentrations through peripheral actions while central actions of clonidine may be responsible, at least in part, for its effects on GSH in the liver. Central actions of clonidine on hepatic GSH levels do not appear to be mediated through opioid peptide release, since clonidine effects were not inhibited by the opioid antagonist naltrexone.

GLUTATHIONE DEPLETION INHIBITS TRANSMEMBRANE SIGNAL TRANSDUCTION IN HUMAN CD4+ AND CD8+ LYMPHOCYTES. T. J. Kavanagh, A. Grossman, J. C. Jinneman and P. S. Rabinovich. Departments of Medicine, Environmental Health, and Pathology, Univ. of Washington, Seattle, WA. Sponsor: E. Faustman.

Glutathione status has been shown to be an important determinant of T lymphocyte proliferation. We have shown that proliferation of human CD4+ lymphocytes sorted by flow cytometry on the basis of GSH content is highly correlated with GSH level. These sorted cells also show differences in transmembrane signal transduction as measured by anti-CD3 monospecific antibody (α-CD3 mAb) stimulated intracellular calcium ([Ca2+]i) mobilization. We hypothesized that chemical depletion of GSH would similarly inhibit α-CD3 mAb induced [Ca2+]i mobilization. Peripheral blood lymphocytes from healthy human donors were isolated by density gradient centrifugation and exposed to chloroform/ dibenzene (CDNB) or diethylmaleate (DEM) and subsequently labelled with FITC conjugated α-CD4 or α-CD8 mAb. They were then stained with either monochlorobimine (to measure the extent of GSH) or with indo-1 (to measure intracellular calcium). There was a dose dependent decrease in [Ca2+]i mobilization and GSH when cells were treated with either CDNB or DEM. High doses of CDNB caused an increased baseline (unstimulated) [Ca2+]i. CD4+ and CD8+ cells were equally sensitive to the effects of CDNB and DEM. These results indicate that agents which deplete lymphocyte GSH may depress the immune response via suppression of an early event in lymphocyte activation.
GLUTATHIONE AND MICROTUBULE SENSITIVITY TO METHYLMERCURY. R.D Graf3, M A Philbert and K R Reuhl. Neurotoxicology Laboratories and JGPT, Rutgers College of Pharmacy, Piscataway, NJ.

Methylmercury (MM) toxicity may be mediated in part by perturbation of microtubules (MTs). It has been proposed that glutathione (GSH) may protect the cell against MM, perhaps by sequestering MM from intracellular target sites. In order to examine the relationship between cellular GSH and MT integrity, embryonic carcinoma cells in monolayer culture were exposed to 0-7.5uM MM for 2h, 0-10uM diethylnalate (DEM, a sulhydryl binder) for 0.5h, or 0-10uM buthionine sulfoximine (BSO, a glutathione synthetase inhibitor) for 12h. Total GSH in cultures was determined using an enzymatic cycling assay. MTs were labelled with antibodies to tyrosinated and acetylated α-tubulins and examined by indirect immunofluorescence microscopy. MM exposure resulted in a concentration-dependent disassembly of MTs without significant depletion of GSH. Conversely, exposure to either DEM or BSO resulted in substantial depletion of GSH without observable changes in MT pattern. In order to determine whether cells with reduced GSH were more susceptible to MT disassembly by MM, cultures were pretreated with either 10uM DEM or 10uM BSO (resulting in 40% and 60% GSH depletion respectively) and subsequently exposed to 0-7.5uM MM for 2h. Exposure to MM following treatment with DEM or BSO resulted in complete depletion of GSH without altering MT susceptibility to MM. These data indicate that, while MM in combination with DEM or BSO may lower GSH, MT sensitivity to MM-induced disassembly is not dependent on intracellular GSH content. (Supported by ES04976.)

DOXORUBICIN INDUCED ALDEHYDE FORMATION AND GLUTATHIONE DEPLETION IN CARDIAC MICROSONES. L C Trost and K R Wallace, Department of Pharmacology, School of Medicine, University of Minnesota, Duluth, MN.

Doxorubicin is a broad spectrum antineoplastic agent effective in the treatment of a variety of neoplasms. However, clinical usefulness is limited by the cumulative cardiomyopathy associated with doxorubicin therapy. It has been proposed that doxorubicin cardiotoxicity is mediated by the free radical induced generation of lipid peroxides. Lipid peroxides may be converted to biologically reactive aldehydes which can react with glutathione (GSH) to form thioethers and thus decrease intracellular glutathione. The purpose of this investigation was to determine whether lipid aldehydes are formed during the incubation of cardiac microsomes with doxorubicin, and to investigate the role of GSH in inhibiting aldehyde accumulation. Cardiac microsomes were incubated with 100uM doxorubicin in the presence of an NADPH-regenerating system. Resulting aldehydes and remaining GSH were quantified by spectrophotometric techniques. Doxorubicin was found to stimulate the NADPH-dependent accumulation of aldehydes during the course of the reaction. In the presence of GSH, aldehydes did not accumulate and GSH was depleted. However, including EDTA in the reaction mixture prevented both aldehyde accumulation and GSH consumption. The data suggest that doxorubicin stimulates the iron-dependent generation of aldehyde products of lipid peroxidation. The mechanism of inhibition of this reaction by GSH appears to be via the formation of thioethers, which may account for the depletion of GSH associated with doxorubicin treatment.

CELLULAR LOCALIZATION OF GLUTATHIONE IN THE DEVELOPING MOUSE NERVOUS SYSTEM. C M Beiswanger, T Roscoe, K R Reuhl, and H E Lowndes. Neurotoxicology Laboratories, Rutgers University College of Pharmacy, Piscataway, NJ.

Biochemical studies have shown postnatal decreases in glutathione (GSH) in brain, but provided no information about its cellular distribution. A histofluorescent study of GSH distribution in the developing and adult mouse nervous system was undertaken to determine developmental changes and for comparison between mice and rats. The brain and spinal cord/DRG were removed at gestational day E13; the olfactory bulbs, cerebral cortex, cerebellum, brainstem and spinal cord/DRG at day E17 and post-natal (PN) days 3, 13, 32 and from adult mice. Frozen sections (8μ) stained with mercury orange were examined by fluorescence microscopy. The nervous system of the mouse exhibited a developmental change in the cellular distribution of GSH. At E13 uniformly positive staining of the nervous system indicated no obvious differences in the distribution of GSH between neurons and glia. By E17, however, large GSH-negative neuronal cell bodies in the anterior horn could be distinguished from surrounding positive glia, whereas large neurons in the cerebellum, brainstem and olfactory bulbs still exhibited positive GSH staining. Postnatally, GSH-negative neurons became increasingly prominent in all regions of the brain. The DRG neurons exhibited high levels of GSH throughout development. The adult pattern of GSH distribution, apparent by PN 15, was identical to that seen in adult rats (Philbert et al, in press). With the exception of the DRG neurons, glutathione was not apparent in neuronal cell bodies. The physiological basis and neurotoxicological consequences of a shift towards predominantly macroglial localization of GSH during development are unknown. (Supported by NS23325 and ES04976.)
122 POLARITY OF GLUTATHIONE EFFUX IN HEPATOCYTES OF A PRIMITIVE VERTEBRATE. T W Simmons and N Ballatori. Environ. Health Sciences Center, Univ. of Rochester School of Medicine, Rochester, NY.

Glutathione (GSH) turnover in mammalian hepatocytes is regulated by its efflux across basolateral and canalicular plasma membranes. In the rat, GSH is released at about equal rates into plasma and bile (Am. J. Physiol. 1989, 256:G482-G490), whereas GSSG and GS-conjugates are secreted almost exclusively into bile. To investigate the evolutionary development of these efflux pathways, the present study examined release of GSH and a GS-conjugate in isolated perfused livers of the little skate, Raja erinacea. Skate livers were perfused for 6 h at 15°C with 100 ml of recirculating Ringer solution. Biliary excretion of GSH decreased during the first 2 h, then stabilized at a rate of 0.83±0.13 nmol GSH equiv/h·g liver⁻¹, about 0.5% of that observed in rats. Levels of perfusate GSH increased during the first 2 h, after which no change could be detected, indicating an excretion rate of <0.5 nmol GSH equiv/h·g liver⁻¹. The presence of 200 μM acivicin (an inhibitor of γ-glutamyltransfere) in the perfusate had no effect on GSH concentrations, indicating that the lack of GSH accumulation in perfusate was not due to its catabolism. Therefore, biliary secretion of GSH accounted for at least 60% of the total hepatic release. When 0.5 μmol 1-chloro-2,4-dinitrobenzene was infused into the liver, 60% (299±38 nmol) was recovered in bile as dinitrophenyl-conjugates, over 5 h. Conjugates were not detected in perfusate (<50 nmol), indicating that dinitrophenyl-SG is predominantly excreted into bile, as seen in mammals. Thus, biliary transport mechanisms for GSH and GS-conjugates have evolved in this marine fish and may play an important role in regulating GSH turnover.

123 STATUS OF GLUTATHIONE IN NASAL EPITHELIUM. L Finch, J R Udinsky, and D W Potter. Rohn and Haas Co., Spring House, PA. Sponsor: C B Frederick

During inhalation exposure to air-born toxicants, the nasal epithelium may be subjected to local toxicity. Since glutathione (GSH) is often involved in xenobiotic metabolism, GSH status in these tissues has been examined. GSH content and apparent first-order rate constants for GSH turnover and synthesis were determined for respiratory epithelium covering the anterior ventral septum, olfactory epithelium covering the dorsal posterior septum, and olfactory epithelium of the dorsal meatus from male Fischer rats. Three tissues had similar concentrations of GSH (approximately 3.3-3.4 μmol/g tissue) as determined by the Ellman's assay or by HPLC equipped with an electrochemical detector. Animals were administered [³⁵S]Cyosine (CyS) by tail vein injection and rate constants were estimated, after incorporation of Cy into tissue GSH pools, by the decrease in GSH specific activity 1-102 hr after administration. Total [³⁵S]GSH was analyzed by HPLC with a flow-through radioactivity detector. The three nasal epithelial tissues had similar apparent biphasic rates of GSH turnover, with rapid-phase half-lives of <10 hr and slow-phase half-lives of approximately 30 hr. The high GSH concentrations and the apparent rapid GSH turnover may facilitate the GSH-mediated detoxification within nasal tissue.

124 REDUCTION OF GLUTATHIONE DISULFIDE TO GLUTATHIONE IN RAT SMALL INTESTINE. L J Dahm, H Zhong, and D F Jones. Dept. of Biochemistry and Winship Cancer Center, Emory University, Atlanta, GA.

Previous studies from our laboratory have shown that reduced glutathione (GSH) is transported intact from the jejunal lumen into the mesenteric circulation in rats. This process may provide a mechanism by which dietary GSH can be utilized in detoxication reactions in jejunal enterocytes and cells capable of taking up GSH from plasma. Glutathione disulfide (GSSG) was not transported from intestinal lumen to vasculature in these studies, although the concentration of GSH in the vasculature increased. To determine whether intestinal reduction of GSSG to GSH with subsequent absorption of GSH might account for this effect, we monitored the reduction of GSSG (200 μM) to GSH in intestinal sacs treated with buthionine sulfoximine and acivicin to inhibit GSH synthesis and degradation, respectively. After a 10 min incubation at 37°C, duodenal and jejunal sacs converted 15-35% of GSSG to GSH. Efflux of GSH from intestinal epithelial cells could not account for the increase in luminal GSH concentration, since intestinal sacs devoid of GSSG had measurable, but much lower, GSH concentrations. The other major product appeared to be cysteiny1-glutathione disulfide (25-40%), and unchanged GSSG comprised the remainder. These results indicate that rat small intestine reduces GSSG to GSH, possibly by thiol disulfide interchange between GSSG and cysteine released from intestinal epithelium. This mechanism might function to elevate luminal GSH concentration and subsequent plasma GSH concentration after dietary GSSG intake. (Supported by NIH grant HL 39968.)

125 EFFECTS OF ARSENITE ON THE CYTOESKELETON AND CELLULAR GSH IN CULTURED CELLS. W Li, S Yang, Z Cao, C D Andry, and L N Chou. Dept. Microbiology and Pathology, Boston Univ. School of Medicine, Boston, MA.

The effects of As³⁺ (NaAsO₂) on DNA synthesis, cytoskeletal organization, cytoskeletal and cytosolic (soluble) protein sulhydryls (SH groups), and cellular GSH levels were examined in quiescent mouse 3T3 cells. As³⁺ treatment for 15 h produced a dose-dependent inhibition of mitogen-stimulated DNA synthesis (50% inhibition at 1.25 μM As³⁺). Exposure of cells to 2.5 μM As³⁺ resulted in microtubules (MT) bundling with increased actin microfilament (MF) cables. At higher doses (>20 μM), As³⁺ treatment resulted in loss and redistribution of MT and MF. Enzymatic (Tietze) assay and HPLC analysis demonstrated that As³⁺ exposure increased the cellular GSH synthesis since the elevated cellular GSH content in As³⁺ treated cells could be abolished by treatment with buthionine sulfoximine (BSO), an inhibitor of GSH biosynthesis. As determined by the (³⁵S)-N-ethylmaleimide (NEM) incorporation assays, As³⁺ exposure also increased the amount of protein SH groups in both the cytoskeletal and the cytosolic (soluble) protein fractions. Furthermore, a greater increase in protein sulphydryls was observed in the cytoskeletal fraction than in the soluble fraction. As a result, As³⁺ exposure altered the ratio of cytoskeletal protein SH groups/total cell protein SH groups. These results suggest that the cytoskeleton could be a cellular target for injury by As³⁺ exposure. The elevated cellular GSH content induced by As³⁺ could provide a protective mechanism against further injury from this metal insult.
ALTERNATION OF PULMONARY GLUTATHIONE STATUS AND ANTIOXIDANT ENZYME ACTIVITIES BY AMIODARONE (AM). R G Leeder and T E Massey, Dept. Pharmacology and Toxicology, and Medicine, Queen's University, Kingston, ON, Canada. Sponsor: J Brodeur.

AM is a potent anti-arrhythmic agent restricted in use by adverse effects, particularly the development of pulmonary fibrosis. Although the etiology of amiodarone-induced pulmonary toxicity (AIFT) has not been established, a direct toxic mechanism involving oxidative stress has been proposed. The purpose of this study was to determine the role of oxidative stress in AIFT by evaluating pulmonary glutathione status and antioxidant enzyme activities after administration. Intratracheal administration of AM (1.8 mg) to male golden Syrian hamsters significantly increased lung GSSG/(GSH+GSSG) (control; 0.45 ± 0.11%, AM; 1.79 ± 0.67%) 2 hours post dosing. AM also increased activities of glutathione reductase (by 89%) 3 days post dosing, and glutathione peroxidase (by 110% and 45%) and total superoxide dismutase (by 58% and 35%) both 3 and 7 days post dosing. However, treatment of hamsters with the antioxidant agent butylated hydroxyanisole (BHA, 150 mg/kg/day s.c.) for 3 days prior to AM had no effect on elevations in pulmonary antioxidant enzyme activities. BHA treatment also had no effect on development of AM-induced pulmonary fibrosis as determined by increases in hydroxyproline content 21 days post dosing. Although treatment with BHA was ineffective, alterations in glutathione status and antioxidant enzyme activities are consistent with direct toxicity by an oxidant mechanism. (Supported by the Ontario Thoracic Society)

STUDIES OF GLUTATHIONE SYNTHESIS AND TURNOVER IN CHANNEL CATFISH. E P Gallagher and R T Di Giulio, School of Forestry and Environmental Studies, Integrated Toxicology Program, Duke University, Durham, NC. Sponsor: M B Abo-Donia.

Glutathione (GSH) synthesis and turnover were investigated in channel catfish by employing chemical and physical techniques that cause GSH depletion in rodents. Buthionine sulfoximine (BSO, 1000 mg/kg ip), an inhibitor of glutathione synthetase (GCS), did not deplete hepatic GSH. Hepatic GSH concentrations also remained stable in catfish starved over a 3 day period. Administration of diethyl maleate (DEM, 0.6 mg/ml ip) resulted in 84% hepatic GSH depletion at 6 h, however, hepatic GSH levels were restored by 24 h. Co-administration of BSO and DEM (1000 mg/kg, 0.6 ml/kg) depleted hepatic cysteolic and mitochondrial GSH pools by 98% and 92%, respectively. Gill cystolic and mitochondrial GSH were depleted 94% and 42%, respectively, at 24 h. Different rates of replenishment in liver and gill cystolic and mitochondrial GSH indicated metabolically distinct pools of GSH in those compartments. Gill GSH was restored at a rate exceeding liver in BSO/DEM treated catfish. Substantial GCS activities were measured in liver and gills of control catfish, and gills exhibited greater GCS activity than liver. Gill GSH was elevated 150-200% in control fish and to 400-800% in fish without 72 h exposure to the electrophilic fungicide chlorothalonil. The results of these experiments indicate that channel catfish can rapidly synthesize and maintain normal or elevated levels of GSH in liver and gills after chemical challenge. Unlike rodents, channel catfish do not possess a labile pool of hepatic cystolic GSH.

REGULATION OF INTRACELLULAR GLUTATHIONE IN A RAT, DIPLOID HEPATIC EPITHELIAL CELL LINE S R Channel, D Eaton and T J Kavanagh, Dept. Environmental Health, University of Washington, Seattle, WA.

A rat-derived hepatic cell line is used to demonstrate the intracellular control of glutathione synthesis emphasizing the rate controlling enzyme y-glutamylcysteine synthetase (y-GCS). Experiments tested the pattern of induction of y-GCS during cellular challenge by chemical inducers of phase I and II xenobiotic metabolizing systems. Both the phase I and the phase II agent induced y-GCS activity. Transcriptional and translational control schemes were tested by specific inhibition with actinomycin D (ACD) and cycloheximide (CXC). GSH recovery was followed by flow cytometry. When transcriptional activity was inhibited > 75% with ACD cells retained the ability to replenish GSH lost to depleting agents. Likewise, with protein translation reduced > 90% by CXC, GSH repletion ability not only remained but was present at a higher rate than without CXC. The studies support cellular redox state, perhaps acting through the GSH/GSSG ratio, and cysteine/cystine transport to be central regulating mechanisms by which cells maintain GSH pools.


Previous studies using cytotoxic rabbit lung Glutathione S-transferases (GSH-T) have demonstrated stereoselectivity (R/S ratio 0.35 — 0.75) in the conjugation of glutathione (GSH) with the prochiral substrate pyrene 4-oxide. This study was carried out to determine if similar stereospecificity of these enzymes occurred in intact rabbit lung. Lungs were surgically prepared from male New Zealand White rabbits and perfused in a recirculating system using cell free medium consisting of Krebs-ringer bicarbonate buffer fortified with BSA and glucose. The lungs were perfused for 1 hr with an initial 9H-pyrene oxide (51 µCl/µmol) concentration of 36 µM. Perfusion medium samples were periodically taken and analyzed by HPLC to determine the diastereomeric composition of the GSH-conjugate produced. Tissue samples were similarly analyzed at the end of the perfusion period to verify that stereoselective tissue retention of products had not occurred. The results from the perfused lungs were in sharp contrast to the results obtained from the experiments using cytotoxic: In perfused lungs, the diastereomeric mixture produced indicated that GSH attack had occurred predominantly at the prochiral R carbon (R/S > 7.0). Studies carried out using rabbit lung slices produced very similar results (R/S ratio >7.0). The changes in observed stereospecificity that occur during cytotoxic preparation may be due to selective loss of specific GSH-T isozymes, or may be due to differences in the GSH concentration in the assay of cytotoxic preparations compared to that in the intact tissue. Ongoing experiments may resolve these questions.
A ROLE FOR THE GLUTATHIONE CONJUGATE AND
RENAL CYTOCHROME P450 IN ACETAMINOPHEN
(APAP) INDUCED NEPHROTOXICITY IN THE CD-1
MOUSE. S G Emeligh Hart, D S Wyand, Ed Khairallah
c. and Cell Biol. and Pathobiol., Univ. of Connecticut,
Storrs, CT.

In the male CD-1 mouse, hepatotoxic doses of APAP
cause renal proximal tubular necrosis which is cytochrome
P450 dependent. The present study was done to deter-
mine the importance of renal uptake and metabolism of
the APAP-glutathione conjugate to nephrotoxicity. Fasted
(18 hr), 3 month old, male mice were injected ip with
either AT-125 (50 mg/kg) or probenecid (150 mg/kg) 30
min, or aminoacyclic acid (AODA, 109 mg/kg) 1 hr before
APAP (600 mg/kg, ip). They were killed 12 hr after APAP
for determination of SDH, BUN and histopathology. Ad-
tional mice were castrated at 2.5 months of age (to selec-
tively decrease renal cytochrome P450) and were allowed to
recover for 3 weeks before APAP treatment. APAP-
induced renal necrosis was prevented completely by ca-
stration and probenecid (blocks transport), and diminished
by AT-125 (blocks y - glutamyl transpeptidase) pretreat-
ments. By contrast, AODA (blocks - lysse) had no detectable
effect. None of the treatments altered hepa-
toxicity. Results suggest that active uptake of APAP-
conjugated derivatives and activation by renal cytochrome
P450 mediates APAP nephrotoxicity in the male CD-1
mouse. (Supported by NIH GM31460, ES07163 and the Center for Biochemical Toxicology)

1-NAPHTHYLISOTHIOCYANATE INDUCED DEPLETION
OF GLUTATHIONE (GSH) IN FRESHLY ISOLATED
HEPATOCYTES. P A Jean, L C Deje, D H Marchand, R A
Roth* and D J Reed. Dept. Biochemistry & Biophysics, Oregon State
University, Corvallis, OR; *Dpt. Pharmacology &
Toxicology, Michigan State University, East Lansing, MI.

1-Naphthylisothiocyanate (ANIT) has long been utilized to
cause cholestasis in laboratory animals. Various biochemical
and morphological changes occur in the liver including biliary
epithelial and parenchyma cell necrosis. Although the mechanism(s) for these effects is not understood, large
quantities of mercapturic acids are formed in vivo. With
freshly isolated hepatocytes, ANIT induced a dose- and time-
dependent depletion of cellular GSH which preceded cell death.
Analysis of the incubation medium indicated that the majority
of the cellular GSH which was lost was present extracellularly
as GSH or as a GSH-releasing compound. This is in agreement
with earlier work by others (Bruggeman, J.; Temmink, J.; van
showing that the glutathione and cysteine conjugates of allyl
and benzyl isothiocyanates are reversible in nature. Mixing
ANIT with GSH at pH 7.5 yielded a compound which was
characterized by HPLC and FAB-MS as an ANIT-GSH conju-
gate. This ANIT-GSH conjugate was not stable under the
conditions of cell incubations, 37° and neutral pH. These data
indicate that ANIT may deplete hepatocytes of glutathione
through a reversible conjugation, which may contribute to
ANIT's toxicity.

132 N-ACETYLCYL/D-DEACETYLATION AS DETERMIN-
ANTS OF QUINOLINE THIOETHER NEPHROTOXICITY.
Templeton: J Monks, Patricia H. Kirner and
Serrine S. Lau. Division of Pharmacol. & Toxicol., College
of Pharmacy, University of Texas at Austin, Austin, TX, 78712.

Cysteine (CYS) and N-acetyl-CYS (NAC) conjugates of 2-
bromohydroquinone (2-BHQ) are nephrotoxic when adminis-
tered to male Spraque-Dawley rats. The CYS conjugates are
more potent than the corresponding NAC analogs; the dicYS
and diNAC isomers are more toxic than the mono-substituted
conjugates; and mono-NAC conjugates exhibit differential
toxicity. We have now examined the relative rates at which
each of the 2-BHQ-thioethers undergo either N-acetylation or
N-deacetylation and whether differences in the cycling of the
compounds contribute to their relative toxicity. Renal cortical
cytoxic N-deacetylation and renal cortical microsomal cysteine
S-conjugate N-acetyltransferase (N-AcT) activity toward 2-BR-
(diNAC)HQ and 2-BR(diCYS)HQ respectively, was lower
than toward the mono-thioethers. The relative rates of both N-
acetylation and N-deacetylation of the mono-NAC conjugates
in the order 2-B-6=2-B-5>>2-B-3. Interestingly, liver cysteol did not exhibit N-deacetylase activity toward these
substrates. Whereas the specific activity of renal N-AcTr was
approximately 20-fold higher than the N-deacetylase, total renal
N-deacetylase activity was less than 4-fold higher. Thus, although
the relative activity of the two renal enzymes probably favors
NAC formation, the cycling of 2-BHQ-thioethers may be
limited by the availability of the acetyl-CoA cofactor. In
addition, omission of 1 mM ascorbate from assays of N-AcTr
activity resulted in a decrease in the rate of N-acetylation
probably due to oxidative cyclization of the 2-BR(HQ)HQ
substrates. Thus, the concentration of renal reductants (GSH,
NADPH, etc.) will also influence the amount of 2-BR(CYS)-
HQ available for N-acetylation. (ES04662, GM39338).

133 CYSTEINE CONJUGATE ß-LYASE AND THE NEPH-
ROTOXICITY OF THE ß-THIETHERS OF 2-BROMOHY-
DRQUINONE. Serrine S. Lau, Heng-Huang Lao, Barbara A.
Hill and Terrence J. Monks, Div. of Pharmacol. & Toxicol.,
College of Pharmacy, Univ. of Texas at Austin, TX 78712.

2-Br-(mono-cystein-3-yl)hydroquinone (2-Br[CYSH]HQ; 50-
150 µmol/kg) and 2-Br-(mono-N-acety-cystein-3-yl)hydroquinone
(2-Br[NAC]HQ; 150-200 µmol/kg) conjugates are renal proximal
nephrotoxic tubular toxicants in the rat. We have now investigated the role of cysteine conjugate ß-lyase (ß-lyase) and renal organic
transport in the toxicity of 2-Br-6-(NAC)HQ and a 2:1 mixture of
2-Br-6- and 2-Br-5-(CYS)HQ (2-Br-5&6-CYS)HQHQ).
The nephrotoxicity of both thioethers (150 µmol/kg) was inhibited
by pretreatment of animals with aminooxyacetic acid, a ß-lyase
inhibitor, as evidenced by decreases in blood urea nitrogen
(BUN), and in the urinary excretion of glucose, lactate
dehydrogenase (LDH) and y-glutamyl transpeptidase (y-GT).
Premtreatment of animals with probenecid, an inhibitor of the
organic anion transporter, had no effect on the toxicity. The data
suggest that, in contrast to ß-lyase (di-glutathion-S-yl)HQ, metabolism by ß-lyase plays an important role in the nephro-
toxicity of 2-Br-5&6(CYS)HQ and of 2-BR-6-(NAC)HQ. The
differential toxicity of mono- and di-substituted thioethers was
investigated further. 2-BR(diCYS)HQ caused elevations in
BUN and increases in the urinary excretion of glucose, LDH and
y-GT at a dose of 25 µmol/kg. 2-BR(di-NAC)HQ caused
glucosuria and enzymuria at 50 µmol/kg and increases in BUN
at 100 µmol/kg. Thus, 2-BR(diCYS)HQ was more toxic, on a
per dose basis, than 2-BR(diNAC)HQ, and both compounds
were more potent nephrotoxics than their corresponding
mono-substituted analogs. Whether ß-lyase plays a role in the
nephrotoxicity of 2-BR(diCYS)HQ and 2-BR(di-NAC)HQ, and
whether the mechanism of toxicity of mono- and di-
substituted thioethers is similar, remains to be determined. (ES04662).
ELECTROCHEMISTRY OF 2-BROMOHYDROQUINONE-Ethioethers. Heng-Hsiang Lo, Serina S. Lau and Terrence J. Monks, Division of Pharmacology & Toxicology, College of Pharmacy, University of Texas at Austin, TX.

We have previously reported that glutathione (GSH), γ-glutamyl transpeptidase and the mercapturic acid pathway are effective modulators of 2-bromohydroquinone oxidation (2-BrHQ). Thus, 2-Br-3-(GSy)HQ and 2-Br-3-(N-acetylcysteinyl-S-yl[NAC])HQ were more stable to oxidation than either 2-BrHQ or 2-Br-3(cysteinyl-S-yl[CYS])HQ. We now report that this modulation appears to be even more pronounced with a series of disubstituted 2-BrHQ-thioethers, which are more potent nephrotoxins than the corresponding mono-thioethers. The half-wave oxidation potentials, determined by hydrodynamic voltammetry (citrate buffer, pH 4.0) of 2-Br(diCYS)HQ, 2-BrHQ, 2-Br(diNAC)HQ and 2-Br(diGSY)HQ were ~85, ~66, ~49 and +286 mV respectively. Thus, 2-Br(diCYS)HQ is far easier to oxidize than 2-Br(diGSY)HQ. Differences in the half-wave oxidation potentials between pH 4.3 and 7.4 for 2-BrHQ and 2-Br-3-(GSy)HQ were indicative of a typical, thermodynamically reversible, two proton, two electron transfer reaction. In contrast, the change of ~95mV/pH for 2-Br-3-(CYS)HQ, which corresponds to a three proton, two electron redox process. The third proton is probably due to the β-amino group of the CYS substituent in the quinone. The effect of pH on the half-wave oxidation potential of 2-Br-3-(NAC)HQ (~25mV/pH) suggest that this conjugate is a very weak acid. 2-Br-(CYS)HQ conjugates are more potent nephrotoxins than 2-Br-(NAC)HQ thioethers. Differences in their electrochemical behaviour may therefore contribute to this differential toxicity. (ES 04662, GM 39358).

REACTION OF 4-NITROSOSIPHENYL WITH HEMOGLOBIN AND GLUTATHIONE IN INTACT RAT ERYTHROCYTES. John S. Wheeler, Fred F. Radlubar, and Jack A. Hinson, National Center for Toxicological Research, Jefferson, AR.

4-Aminobiphenyl (4-ABP) is known to be metabolized to the reactive metabolite 4-nitrosobiphenyl (4-NBP) which reacts with hemoglobin (Hb) to form 4-ABP adducts at the 938 cysteine. The formation of these adducts has been utilized as a biomonitor of 4-ABP exposure. In a previous work we demonstrated that 4-NBP also reacts with glutathione (GSH) to form the adduct N-(glutathion-S-yl)-4-ABP S-oxide and that in vitro formation of Hb-4-ABP adducts from 4-NBP can be inhibited by the addition of GSH. In this work we have demonstrated that addition of 4-NBP to intact erythrocytes results in formation of 4-ABP-hemoglobin adducts within the erythrocytes under conditions which does not result in hemolysis. Adduct formation was concentration-dependent. Concurrent with the formation of Hb adducts, intracellular GSH decreased with the concomitant formation of N-(glutathion-S-yl)-4-ABP S-oxide. These data indicate that GSH is an important host factor in 4-ABP metabolism.


The effect of some inducers, i.e. phenobarbital and methylcholanthrene, on liver glutathione S-transferases (GST) is well studied in vivo. However, little is known about the influence of trans-stilbene oxide (TSO) in this study, male Sprague-Dawley rats (180-200 g) were injected (IP) with 600 mg TSO/kg during 5 days. Corn oil (used to dissolve TSO) injected rats were used as controls. GST activity increased 2.9 and 2.0 fold towards the substrates 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene respectively. Separation by BPLC of the different GST subunits revealed the appearance of subunit 7. Furthermore a specific effect of the epoxide on subunits 1, 2 and 3 was observed. Subunit 4 remained unchanged.

Immunocytocchemical analysis demonstrated a homogeneous staining of the treated liver for subunit 7. Northern blot analysis confirmed the appearance of subunit 7 in vivo. The results indicate that TSO treatment in vivo induces rat liver GST subunits 1 and 7 especially. Increased GST steady-state mRNA levels confirm the changes observed on the GST proteins.

GLUTATHIONE (GSH) CONJUGATION OF HEXACHLOROBENZENE (HCB) IN LIVER OF MALE AND FEMALE RATS. M D'Auzan and M Chartonnoeau, Dép. de Médicine du Travail et d'Hygiène du Milieu, Université de Montréal, Québec, Canada.

HCB induces hepatic porphyria and liver cancer in female rats, whereas liver toxicity is minimal in male rats. HCB is biotransformed to sulfur-containing metabolites originating from conjugation to GSH. Conflicting results have been reported on HCB-induced depletion in rats. To verify if HCB causes depletion of liver GSH and if there exist a sex-specific response, groups of male and female Sprague-Dawley rats (N-5) were given (po, 100 mg/kg, 10 ml/kg in corn oil) 5 consecutive doses of HCB (2 bid (7:30), 15:30) + 1 sid (7:30)). This cumulative dose produced porphyria in female rats after a delay period of 5 weeks. The animal were killed 0, 6, 12, 18 or 24 hr after treatment. Hepatic GSH levels showed a diurnal cycle in both sexes, but it was more pronounced in male rat livers; minimum level was observed at 12 hr (19:30). GSH levels in HCB-treated male rats were significantly lower than controls at 6, 12 and 24 hr, but no significant differences were observed for female rats. Bilirubin excretion of pentachlorothiophenol, a metabolite originating from GSH conjugation, was higher in male rats. Results suggest that GSH conjugation of HCB is more important in male than in female rat liver. This may be related to the reduced liver toxicity observed in HCB-treated male rats compared to female rats. (Supported by MCRI and FRSQ).

NA results in dose-dependent necrosis of pulmonary Clara cells in mouse lung; injury to Clara cells in hamsters or rats is not observed even at doses 8 to 16 fold higher than cytotoxic doses in the mouse. We have compared NA metabolism in target cell populations of sensitive and nonsensitive species using airways isolated by blunt dissection. NA was metabolized at easily measurable rates in mouse trachea, lobar bronchi, major and minor daughter and distal bronchi. The rate of NA turnover in distal bronchi was twice that in trachea; no activity was detected in blood vessels or parenchyma. NA metabolism in mouse airways was stereospecific. 1R,2S-NA oxide, trapped as a glutathione (GSH) adduct, was easily measured in airway levels; corresponding GSH adducts from 1S,2R-NA epoxide were not detected. In contrast, airways of the rat and hamster metabolized NA to both enantiomeric epoxides with the formation of 1S,2R-epoxide predominating in both cases. The total rate of NA metabolism in hamster and rat airways was < 30 and 20%, respectively, of that in the mouse. Antibodies to a P450 isozyme isolated from mouse liver that steroselectively metabolizes NA to the 1R,2S-oxide (Nagata et al., Drug Metab. Dispo, in press) have been used to study the localization of this isozyme in the lung. Intense labelling was observed at all airway levels of the mouse but was not detected in rat or hamster. These data suggest that the stereochirality of NA metabolism, catalyzed by a unique P450 isozyme localized in target cells of the mouse, is intimately involved in the species and cell selective toxicity of NA. NIEHS 04311.

METABOLITES OF NEPHROTOXIC CYSTEINE CONJUGATES FORM COVALENT ADDUCTS WITH RAT KIDNEY MITOCHONDRIA PHOSPHOLIPIDS. C.J. Welsh, P.J. Hayden, Y. Yang*, A.J. Ward* and J.L. Stevens. W. Alton Jones Cell Science Center, Lake Placid, NY and *Clarkson University, Potsdam, NY.

Nephrotoxic cysteine conjugates derived from a variety of halogenated ethylenes are enzymatically activated via the β-lactase pathway to yield reactive sulfur-containing metabolites which bind covalently to cellular macromolecules. Mitochondria contain β-lactase enzymes and are primary targets of binding and toxicity. Previously, protein and/or DNA have been considered as molecular targets for metabolite binding. We now report that metabolites from nephrotoxic cysteine conjugates form covalent adducts with mitochondrial phospholipids. Rat kidney mitochondria were incubated with the 2S-labeled conjugates S-(1,2,3,4-tetrafluoroethyl)-L-cysteine (TFEC), S-(1,2,3,4-tetrachloroethyl)-L-cysteine (TCC), S-(1,2-dichlorovinyl)-L-cysteine (DCVV) and S-(1,2,3,4,5-pentachlorobutadienyl)-L-cysteine (PCBC). Total lipids were extracted and separated by thin layer chromatography. New 3S-containing phospholipids were identified from TFEC and TCC treated samples. 3S-products from DCVV and PCBC could not be resolved from normal phospholipids by TLC. Fatty acid composition, 1H and 31P NMR spectroscopy indicate that the TFEC adduct is the difluorothioamide of phosphatidyl ethanolamine. Work on isolation and structural elucidation of the other adducts continues.

METHYLYATION OF CYTOSOLIC GLUTATHIONE S-TRANSFERASES (GSTRS) FROM RAT BRAIN, KIDNEY, LIVER, LUNG AND TESTES. J.A. Johnson and F.L. Siegel. Dept. of Physiological Chemistry and Pediatrics, the Environmental Toxicology Center and the Waisman Center, Univ. of Wisconsin, Madison, WI. Sponsor: C.R Jefcoat.

Previous reports from this laboratory described the calmodulin-stimulated in vitro methylation of a Mr 29,000 GST in rat liver cytosol (Siegel & Wright, 1985: Neal et al., 1988). To determine the GST isoform specificity of protein methylation, methylated GST dimers and subunits were isolated by chromatofocusing and reverse-phase HPLC, respectively. The Mr-class isozyme (3-3, 3-4 and 4-4) were the primary liver GSTs methylated and their methylation was stimulated by calmodulin (Johnson et al., 1990). GST methylation was observed in kidney, liver and lung cytosols and GST subunits 3 and 4 isolated from all tissues were methylated by partially purified liver GST methytransferase. The a-class subunits were minimally methylated and methylation of the a-class subunit 7 was greater than that of a-class but less than that of a-class GSTs. The Mr-class GST subunit 11, found only in testis and brain, was substantially methylated compared to subunits 3 and 4 and on a mole-to-mole basis, methylation reached 80%. The high level of stoichiometry of subunit 11 methylation will allow us to determine the effect of methylation of GST conjugating activity. (Supported by NIH grants GM36497, NS24669, HD03352).

DIFLUOROTHIOACYL-PROTEIN ADDUCTS FROM S-(1,2,3,4-TETRAFLUOROETHYL)-L-CYSTEINE METABOLITES: NUCLEOPHILIC CATALYSIS OF ADDUCT FORMATION BY HISTIDINE AND TYROSINE. P.J. Hayden, Y. Yang*, A.J. Ward* and D. Duflick* and J.L. Stevens. W. Alton Jones Cell Science Center, Lake Placid, NY, *Clarkson University, Potsdam, NY and **SmithKline Beecham Pharmaceuticals, King of Prussia, PA.

1H NMR spectroscopy was used to demonstrate that difluorothioaldehyde-protein adducts are formed from metabolites of the nephrotoxic cysteine conjugate S-(1,2,3,4-tetrafluoroethyl)-L-cysteine (TFEC). The reactivity of TFEC metabolites with a variety of N-acetylaminos acids was also investigated. An N-difluorothioacetyl-N-acetylylsine (DFTAL) adduct was isolated and characterized by NMR and mass spectrometry. Histidine and tyrosine were found to operate as nucleophilic catalysts to facilitate the formation of both protein and DFTAL adducts. 1H NMR data and other evidence indicate that the protein adduct is a difluorothioamide formed from lysyl residues. N-Acetylylsine, at concentrations >100-fold in excess of protein lysine, was not effective in preventing binding to protein. We conclude that nucleophilic catalysis is an important mechanism for the difluorothioacetylation of specific lysyl residues in protein. TFEC metabolites were very reactive with GSH and N-acetylcysteine. The predicted difluorothioesters from these reactions could not be isolated. Both stable difluorothioamide and less stable difluorothioester adducts may play a role in TFEC mediated nephrotoxicity.
MOUSE STRAIN DIFFERENCES IN GLUTATHIONE S-TRANSFERASE ACTIVITY AND AFLATOXIN B1 BIOTRANSFORMATION. K I Borroz, H S Ramsdell and D L Eaton. Environmental Health, U. of WA, Seattle, WA

The resistance of the mouse to the hepatocarcinogenic effects of aflatoxin B1 (AFB) is thought to result from expression of a glutathione S-transferase (GST) isoenzyme with high activity toward AFB, a-8,9-oxygenated aflatoxin B1 (AFB1) in interstrain differences in GST conjugation toward AFB are as large as variations previously reported for other substrates (up to 6.2x), then there may be mouse strain differences in susceptibility to hepatocarcinogenesis by AFB. The hepatic microsomal and cytosolic biotransformation of AFB was studied in 8 different strains of mice fed a purified diet using livers pooled from 8-10 mice each. GST activities toward AFB, 1-chloro-2,4-dinitrobenzene (CDNB), 3,4-dichloronitrobenzene (DCNB), ethacrynic acid (ECA) and cumene hydroperoxide (CHP) were determined, and ranged from 1.5-2.1, 2830-5370, 81-144, 38-69 and 32-73 nmol/mg protein/min, respectively. The cytochrome P450-mediated microsomal oxidation of AFB to AFBO, aflatoxins Q1, M1, and P1 was measured by an HPLC assay, with specific activities ranging from 209-465, 36-70, 161-326, and 252-426 pmol/mg protein/min, respectively. No striking differences among mouse strains were observed for any of the cytochrome P450- or GST-mediated activities, although some small, but statistically significant, differences were found. Mice fed a standard rodent chow diet showed evidence of microsomal and cytosolic enzyme induction compared to mice fed a purified diet. The lack of striking differences in AFB biotransformation activities between mouse strains suggests limited interstrain variations in the hepatocarcinogenic effects of AFB in mice. (Supported by NIH grants ES-03933, ES-04696, and CA-47561.)

CONJUGATION OF AFLATOXIN B1-8,9-EPOXIDE BY GLUTATHIONE S-TRANSFERASES FROM HUMAN AND RODENT LIVER. H S Ramsdell, D H Slone, and D L Eaton. Department of Environmental Health, University of Washington, Seattle, WA.

The reactive metabolite of the carcinogen aflatoxin B1 (AFB), AFB-8,9-epoxide (AFBO), is conjugated with glutathione (GSH) by cytosolic GSH S-transferase (GST). Pooled hepatic cytosol samples were prepared from rats, mice, hamsters and human healthy donor samples (male). GST isoenzymes were isolated from the rodent cytosols by affinity chromatography and chromatofocusing. GST conjugation of AFBO (generated in situ by microsomal oxidation of AFB or added exogenously) was determined by HPLC. Using microsomal generation of AFBO, GSH conjugation rates of 0.031, 10.3, and 1.1 nmol/mg protein/min were measured for cytosol from the rat, mouse, and hamster, respectively, whereas GST activity in human liver cytosol was not observed at a detection limit of 0.002 nmol/mg protein/min. In each of the rodents studied, α-class GSTs had AFBO activity 5-75-fold greater than ψ-class isoenzymes from the same species. The relative activity of GST isoenzymes from a species varied little with different amounts of AFBO generated from 13-128 μM AFB added to the incubation mixtures. Incubation of synthetic AFBO (80 μM) with human cytosol resulted in GSH conjugation at a level 5% that observed in the presence of rat cytosol. Thus, human liver appears to have relatively low GST activity toward AFBO. Our results are also consistent with previous suggestions that α-class GSTs are the principal isoenzymes responsible for the detoxification of AFBO. (Supported by NIH grants ES-03933, ES-04696 and CA-47561.)

A NOVEL APPROACH TO ASSESS CHEMICAL FETOTOXICITY IN HUMANS: AN APPLICATION TO ETHYLENE DIBROMIDE (EDB) TOXICITY USING HIGHLY PURIFIED HUMAN PLACENTAL GLUTATHIONE-S-TRANSFERASE (GST). AK Mitra, S Govindwar, DR Hilbelink, J Hartsfield, JJ Dvornik and AP Kulkarni. Toxicology Program, University of South Florida, Tampa, FL.

EDB, a putative mammalian carcinogen, is bioactivated by GST. The fetotoxic potential of this high volume chemical in humans is currently unknown. Due to the lack of necessary bioactivating enzyme in developing embryos, in vivo tests with lab. animals are likely to result in false negative data. Therefore, we have developed a novel protocol in which rat embryos, serving as passive targets, are grown in a bioactivating system consisting of 1 mM GSH, 0.75 mM EDB and desirable amounts of highly purified human placental GST. 17 different parameters were examined to determine fetotoxicity. The results indicate that yolk sac diameter and malformative composite score were significantly reduced by the dose-dependent increments of GST. Specific activities of GST with EDB ranged from 800-8000 molecules/min/mg protein. This is the first report on the use of human fetal tissue enzyme in elucidation of fetotoxicity of environmental chemicals.

Supported in part by funds from U.S. EPA.


2,4,5,6-Tetrachlorothiphenol (Chlorothalonil) forms conjugates with glutathione and these conjugates are metabolized to thiol analogs. These thiol analogs may be responsible for the nephrotoxicity produced in rats following long term administration of chlorothalonil. In vitro studies have shown that the parent compound is completely metabolized to polar compounds before translocation between the mucosal and serosal surfaces of the duodenum. It is believed that gastrointestinal bacteria in the rat contribute to the metabolism of chlorothalonil to precursors of the thiol analogs and that excretion of the thiol in urine may reflect the susceptibility of different species to nephrotoxic effects. In the rat, 1.6% of a 50 mg/kg oral dose of chlorothalonil is excreted in urine as di- and tri-thiol analogs. Similar experiments have been conducted in the germ-free rat, dog and monkey, and the amounts of thiol analogs of chlorothalonil that were excreted by these animals ranged from no thiol in the dog to 0.06% in the germ-free rat. These data suggest that conjugation of chlorothalonil with glutathione and subsequent conversion to thiols occurs to a lesser degree in the germ-free rat, dog and monkey than in the intact rat, and that these other animals may be less susceptible to the nephrotoxic effects of long term administration of chlorothalonil.
146 INHIBITION OF HEPATIC GLUTATHIONE-S-TRANSFERASES BY FATTY ACIDS AND FATTY ACID ESTERS. S Govindwar, AK Mitra and AP Kulkarni. Florida Toxicol. Research Center, College of Public Health, University of South Florida, Tampa, FL.

The effect of fatty acids and fatty acid esters on female rat and mouse hepatic glutathione-S-transferase (GST) was investigated. Cytosolic GST was assayed using CDNB as a substrate. I-50 values (ranged from 423 uM - 15 uM) indicate that arachidonic acid is approximately 25 times more potent in inhibiting rat GST than palmitic acid which was the least effective. Linoleic and arachidonic acids and the esters of saturated fatty acids, ascorbyl stearate and ascorbyl palmitate were equipotent in inhibiting mouse liver cytosolic GST. Both linoleic and arachidonic acids significantly inhibited dichloronitrobenzene and 4-nitropyridine N-oxide conjugation with GSH but were ineffective as inhibitors when ethacrynic acid was used as substrate for measuring GST activity in rat cytosol. The results suggest potential for use of this class of GST inhibitors for discriminating different isoforms of mammalian GST. Supported in part by a grant from the Environmental Protection Agency, Washington D.C.

148 THE COMPARATIVE REACTIVITY OF SELECTED ACRYLATE ESTERS TOWARDS GLUTATHIONE AND DEOXYRIBONUCLEOSIDES IN VITRO. TJ McCarthy and G Witz. Joint Graduate Program in Toxicology, Rutgers University/UMDNJ R W Johnson Medical School, Piscataway, NJ.

The toxicity of acrylate esters, important plastic monomers, is thought to involve alkylation of cellular nucleophiles by Michael addition. As part of ongoing research of structure-activity relationships, previous studies on the electrophilic reactivity of methyl acrylate and methacrylate, butyl acrylate and tetrahydropropylene glycol diacrylate towards glutathione (GSH) in a cell-free and red blood cell system were expanded to include ethyl acrylate and -methacrylate, butyl methacrylate, tetrahydropropylene glycol and ethylene glycol dimethacrylate. α-Methyl substitution greatly decreased the electrophilic reactivity of the esters in both systems; increased alcohol chain length moderately decreased the second order rate constants for their spontaneous reaction with GSH, and bifunctional esters were more than twice as reactive as monofunctional esters. Ethyl acrylate (0.1 mM), a carcinogenic ester, and the non-carcinogenic ethyl methacrylate (0.5 mM) formed no detectable adducts with deoxyguanosine (DG, 0.5 mM) or other deoxyribonucleosides (dC, dA, dT) in vitro when incubated at pH 6.7 or 7.4 and 37 °C for up to 24 hrs, as well as at 50 °C for up to 8 hrs based upon HPLC analysis using UV-Vis detection at 265 nm. These limited studies in vitro suggest that mechanisms other than direct alkylation may play a role in the mechanism of action of carcinogenic acrylates. (Sponsored in part by a fellowship from the New Jersey Department of Environmental Protection and the Environmental and Occupational Health Sciences Institute.)

147 CYSTEINE CONJUGATE B-LYASE AND THIOL-S-METHYLTANSFERASE ACTIVITY IN INSECTS. S C Gardner and W C Daverman. N.C. State University, Department of Toxicology, Raleigh, N.C.

The enzymes, cysteine conjugate B-lyase and thiol-S-methyltransferase operate in concert in the detoxication process of a broad range of substrates. Several insect species were examined to establish if these enzymes are present in insects. Cysteine conjugate B-lyase activity was determined spectrophotometrically by measuring the formation of 2-mercaptobenzothiazole at 316 nm from the substrate S-2-benzothiazolyl-L-cystine. Strong activity was demonstrated in the supernatant of the tobacco hornworm, which facilitated further developmental and tissue distribution studies. This enzyme was shown to be localized primarily in the cytosol of the cuticle with some activity present in the fat body. Thiol-S-methyltransferase activity was found in the microsomal fraction of the tobacco hornworm by spectrophotometrically measuring the disappearance of 2-mercaptobenzothiazole at 316 nm in the presence of S-adenosylmethionine. Activity of both of these enzymes were measured in the tobacco budworm and CSMA (insecticide susceptible) and 594vb (insecticide resistant) strains of house fly.

149 INTRAHEPATIC CONVERSION OF A XENOBIOTIC TO ITS MERCAPTURIC ACID. CA Hinchman, H Matsumoto, TW Simmons and N Hallacy. Environ. Health Sciences Ctr. Univ. of Rochester School of Medicine, Rochester NY.

Because of the low hepatic activity of γ-glutamyltranspeptidase (γGT) in the rat, the liver is generally considered to play only a minor role in the degradation of GSH conjugates, a limiting step in mercapturic acid (MA) formation. Recent findings from this laboratory indicate, however, that the liver may have a more prominent role in this process in species other than the rat. To examine the contributions of liver to MA biosynthesis, MA formation was measured in isolated perfused livers from rats and guinea pigs dosed with either 0.3 or 3.0 μmol 1-chloro-2,4-dinitrobenzene (CDNB). Chemically synthesized MA and intermediate metabolites of CDNB were used as standards in the HPLC analysis of bile and perfusate samples. Biliary excretion accounted for almost all of the recovered metabolites. A marked species difference was observed in the pattern of CDNB metabolism. Rat livers dosed with 0.3 μmol CDNB excreted 55 and 8.2 % of the total biliary metabolites as the GS-conjugate and the MA, respectively, whereas guinea pig livers excreted only 4.8 % as the GS-conjugate and 47% as the MA. MA formation was also dose dependent, with a larger fraction of MA formed at the lower dose (6.2 vs 3.7 % in the rat; 47 vs 19 % in the guinea pig). Conversion of the GSH conjugate to the MA was markedly inhibited in both species by pretreating the animals with acivicin, an inhibitor of γ-GT activity. These findings provide direct evidence for extensive intrahepatic biosynthesis of MA. Thus, GSH conjugates synthesized within hepatocytes are secreted into bile and broken down to cysteine-conjugates; the latter are then presumably reabsorbed by the liver, N-acetylated to form the MA and re-excreted into bile.
The bioactivation of the nephrotoxin hexachlorobutadiene involves hepatic formation and biliary excretion of S-(pentachlorobutadienyl)-glutathione (PCBG). The intestinal absorption of PCBG, which is required for nephrotoxicity, was studied in vivo by introducing PCBG or its metabolite S-(pentachlorobutadienyl)-L-cysteine (PCCG) into rat intestines with a biliary cannula and monitoring PCBG and PCCG concentrations in the blood. When PCBG was infused, 62.5% was found as PCCG in the blood; the highest metabolite concentrations (0.9 nmol/ml for PCBG and 1 nmol/ml for PCCG) were observed after 30 min. Higher PCBG concentrations (7.3 nmol/ml) were observed after PCGG infusion. Transport studies in Caco-2 cells—a human intestinal cell line, showed that application of PCBG (0.1 mM) to the apical side of the cells resulted in a 2.4-fold accumulation of PCBG in the basolateral chamber after 24 hr; in this time, the cells transformed 69% of the applied PCBG to PCCG. Addition of glutathione (1 mM), γ-glutamyl nitroanilide (1 mM), or probenecid (1 mM) to the apical chamber diminished the active transport of PCBG. With PCBG no active apico-basolateral transport was observed. The experiments demonstrate for the first time intestinal absorption of the γ-conjugates of HCBD, an important step in their translocation from the liver to the kidney. (Supported by NIEHS grant ES03127 and AFOSR grant 86-0302.)

Maintenance of Adult Primate Liver in Organ Culture: Potential Use in Toxicity Testing.

Adult Rhesus monkey liver slices were incubated using a dynamic organ culture method to determine hepatocyte viability, drug biotransformation potential and the in vitro response to the hepatotoxicant, allyl alcohol (AA). After 1, 2, 4, or 8 hr, slices were removed from culture and analyzed for incorporation of [3H]-leucine into acid-precipitable material, and medium alanine aminotransferase (ALT) activity was determined. Separate slices were taken for histological evaluation and for evaluation of microsomal 7-ethoxy-4-trifluoromethyl coumarin-O-deethylase (EFCOD) activity. Incorporation of [3H]-leucine into slices was linear over the period of incubation and was specifically inhibited by cycloheximide (10 μM) at all time points. In the absence of AA, enzyme leakage was minimal over 8 hr. Marked ALT leakage occurred with 1 mM AA. Control slices had an initial fall to 55% of in vivo EFCOD activity that stabilised at 40-50% activity by 8 hr. Histological evaluation of control slices indicated that there was minimal cellular degeneration and that, in PAS-stained sections, glycogen accumulation occurred over the incubation period. This system allows for maintenance and viability of adult primate liver slices in culture for at least 8 hr and may be useful for in vitro toxicity and biotransformation studies.

The interactive toxicity of two non-toxic concentrations of CHCl₃ and BrCCl₃ in precision-cut rat liver slices. A.J. Gandolfi, S. Azri, H.P. Mata, K. Brendel. Department of Anesthesiology, University of Arizona, Tucson, AZ.

The interactive toxicity of two non-toxic concentrations of CHCl₃ and BrCCl₃ were examined in precision-cut rat liver slices. Liver slices were prepared from male Sprague-Dawley rats (220-250 g) pretreated with phenobarbital for 4 days. The order of toxicant addition was found to be crucial in these studies. The addition of 0.2 mM CHCl₃ prior to 0.125 mM BrCCl₃ resulted in a time dependent loss of intracellular K⁺ which was significant relative to controls at 9 hr (1.56%). Cytochrome P-450 loss was significant as early as 6 hr in slices given CHCl₃ prior to BrCCl₃. BrCCl₃ alone caused a similar loss of cytochrome P-450 in slices given both BrCCl₃ and CHCl₃ in that order. Suicide inactivation of cytochrome P-450 by BrCCl₃ appears to prevent CHCl₃ bioactivation thereby preventing the interactive toxicity from being manifest. Slices GSH levels and LDH levels were identical to all controls over the duration of the experiment. The centrilobular enzyme glucose-6-phosphate dehydrogenase fell only slightly in slices exposed to CHCl₃ and BrCCl₃. Based upon these studies, the mechanisms responsible for the interactive toxicity of these two toxicants in non-toxic concentrations can be further elucidated in precision-cut slices (NIH 1-P42-ES-04940, GM 38290, F 32 ESO 5474).
EVALUATION OF TWO NEW ANESTHETICS BY PRECISION-CUT GUINEA PIG LIVER SLICES. H. Ghantous, J. Fernando, A. J. Gandolfi, K. Brendel. Department of Anesthesiology, University of Arizona, Tucson, AZ 85724.

Since precision-cut guinea pig liver slice culture has been shown to be a sensitive system for evaluating the hepatotoxicity and biotransformation of volatile anesthetics, this system was used to evaluate two new anesthetics (sevoflurane and desflurane) and compare their effects to the widely used anesthetic, isoflurane. Liver slices (250-300 μm) were prepared and incubated (6-24 hr) in sealed vials with supplemented Krebs-Henseleit buffer at 37° C under 95% O₂. Desflurane, sevoflurane and isoflurane were vaporized to produce medium concentrations of 2.2, 2.1, and 2.3 mM, respectively. Intracellular K⁺ content, protein synthesis and secretion were measured as viability markers. The common metabolite, F⁻, was measured with specific ion electrodes. Sevoflurane did not affect intracellular K⁺ content of the liver slice while desflurane and isoflurane caused a 20% drop. However, sevoflurane and isoflurane had a significant effect (40-50% of control) on protein synthesis whereas desflurane did not. None of the anesthetics affected protein secretion. The biotransformation of desflurane was minimal while F⁻ production from sevoflurane was 3 times more than that of isoflurane. Since protein synthesis is often a more sensitive indicator of toxicity for subtle toxicants than intracellular K⁺ content, sevoflurane and isoflurane appear to be more toxic than desflurane to the guinea pig liver slices. In addition, desflurane is resistant to biotransformation while the ramifications of the extent of sevoflurane remain to be investigated. (NIH GM 45678)

SUBANESTHETIC HALOTHANE IS HEPATOTOXIC IN GUINEA PIGS: PLASMA ICDH VS ALT AS AN INDEX OF INJURY. RC Lind, AJ Gandolfi. Department of Anesthesiology, University of Arizona, Tucson, AZ.

Inhalation of an anesthetic concentration (1% v/v) of halothane (H; CF₃CBrCIH) for 4 hr produces centrilobular necrosis in guinea pigs. Although the mechanism appears to be covalent binding of H biotransformation intermediates to hepatic proteins, reduced hepatic blood flow and oxygenation during anesthesia have been proposed as contributing factors. Thus, subanesthetic H was evaluated. Plasma IC DH and ALT were compared as indices of injury. OUTbred male Hartley guinea pigs (600-700 g, N=8) were exposed to 0.1% (v/v) H for 4 hr in a 180 l exposure chamber. One-half were killed immediately after exposure. The others were bled at 0, 24, 48 and 72 hr post and killed at 96 hr. 0 hr blood [H] = 45 ± 5 μM, 1/10 of that previously seen with 1% H; liver [H] = 61 ± 14 nmoles/gm wet wt, 1/23 of 1% H levels. 0 hr plasma trifluoroacetic acid (oxidative metabolite) concentrations (929 ± 143 μM) were 2x previous 1% H values (p < 0.001) while fluoride ion (reductive metabolite) was 4.4 ± 0.7 μM (66% of 1% H values; p < 0.001). Hepatic necrosis developed in 2/4 animals whose plasma ALT activities increased ~10x 24-48 hr post whereas ICDH increased ~30-70x. As previously seen with 1% H, glutathione depletion prior to exposure potentiated necrosis. Physiologic changes during H anesthesia do not appear to be a factor in H-induced hepatic toxicity. Plasma ICDH seems to be a more sensitive index of injury. Covalent binding of H biotransformation intermediates to hepatic macromolecules will be compared with 1% H.


To maximize availability of human liver tissue for research, storage of the organ at low temperature must be optimized. For organs which are harvested for transplant but are not used, a cold storage time of 48 hr has to be anticipated. This study investigates rat tissue viability of livers after cold perfusion or cold immersion for 12, 24, 36 and 48 hr in solutions of Belzer's, Euro-Collins, Sacks, and saline. After predetermined cold storage times the rat livers were precision cut and incubated for 4 hr in Waymouth's tissue culture medium and intracellular K⁺ content, LDH release and protein synthesis measured as viability parameters. Belzer's cold preservation solution yielded the most viable livers followed by Sacks, Euro-Collins and saline. This rank order was maintained for both perfused and non-perfused livers while perfused livers had overall better viabilities. These data suggest that livers should be perfused and stored in Belzer's for optimal cold preservation a condition which is routine for most human liver tissue procured for transplantation. (NIH GM 38290)
The metabolic capability of 12 Sprague Dawley rat livers was examined after 60 hr cold preservation in physiological saline, (NaCl), Krebs Bicarbonate (KB), Sacks (S), Euro-Collins (EC), Belzer's (UW) solutions. Rat livers were initially perfused with NaCl to remove blood and then reperfused with one of 4 above mentioned cold preservation solutions and kept at 4°C for the duration of the experiment. Every 12 hr cylindrical cores were cut from each liver and sliced using a mechanical tissue slicer. These slices were exposed to 7-ethoxycoumarin (100μM) in organ culture (4 hr at 37°C) to assess phase I and II metabolism. Metabolite production of cold preserved liver slices was then compared to their fresh counterparts sliced after the initial reperfusion. After 60 hr cold preservation total metabolic production and the coupling of phase I and II reactions were maintained best in UW and followed a rank order: UW > S > KB > EC > NaCl. (NIH GM 38290)

Rat liver slices were kept in organ culture to assess maintenance/induction of xenobiotic metabolism. Phenobarbital (PB) and 3-methylcholanthrene (3MC) were used at concentrations of 1 mM and 50 μM, respectively. Slices were exposed to these agents for 72 h at 37°C in organ culture. Throughout the course of the experiment slice ability to metabolize a model substrate, 7-ethoxycoumarin (7EC), was assessed at intervals. The substrate 7EC which was used at a nontoxic concentration of 100μM is first O-deethylated to 7-hydroxycoumarin (7HC) (Phase 1) and the latter conjugated to the sulfate and glucuronide. The coupling of Phase I and II reactions was used as a critical indicator of functional viability. Phase I and II reactions remained coupled throughout the experiment in slices that were exposed to PB and 3MC, however slices exposed only to medium exhibited a decline in both phases of metabolism at 72 h. (NIH GM 38290)

Perturbation of intracellular Ca²⁺ homeostasis has been proposed as a final common pathway for liver cell death. This presumably is a result of calcification of mitochondria, uncoupling of oxidative-phosphorylation and inability to synthesize ATP. Calmodulin has been implicated in mitochondrial Ca²⁺ uptake and TFP, a calmodulin antagonist, protects against liver necrosis induced by ccl₁ or galactosamine. The objective of the present study was to investigate the effects of 100 μM TFP on 0.3 mM AA or acrolein (AC) mediated hepatotoxicity. Markers of toxicity were Ca²⁺ uptake and K⁺ retention as well as ATP content. Results showed partial protection from AA intoxication by TFP on all three parameters but a lack of protection from AC toxicity. After a 2 h preincubation with TFP, slices were exposed to AA or AC for 2 h. The direct effect of TFP on AA activation by alcohol dehydrogenase as an alternative mechanism are currently under investigation. (NIH GM 38290)

Rat liver slices were kept in organ culture to assess maintenance/induction of xenobiotic metabolism. Phenobarbital (PB) and 3-methylcholanthrene (3MC) were used at concentrations of 1 mM and 50 μM, respectively. Slices were exposed to these agents for 72 h at 37°C in organ culture. Throughout the course of the experiment slice ability to metabolize a model substrate, 7-ethoxycoumarin (7EC), was assessed at intervals. The substrate 7EC which was used at a nontoxic concentration of 100μM is first O-deethylated to 7-hydroxycoumarin (7HC) (Phase 1) and the latter conjugated to the sulfate and glucuronide. The coupling of Phase I and II reactions was used as a critical indicator of functional viability. Phase I and II reactions remained coupled throughout the experiment in slices that were exposed to PB and 3MC, however slices exposed only to medium exhibited a decline in both phases of metabolism at 72 h. (NIH GM 38290)

Primary hepatocyte cultures represent a valuable in vitro system for investigating the metabolism and toxicity of xenobiotics. In this study we have compared the use of various substrates with culture medium supplemented with dimethyl sulfoxide (DMSO) which has been shown to maintain rat hepatocytes for extended periods. Hepatocytes were obtained from male Sprague-Dawley rats by collagenase perfusion and the cells maintained in either RPMI 1640 medium containing 2% DMSO (RPMI/DMSO medium) or on various substrates in medium without DMSO (RPMI medium). Hepatocytes cultured in RPMI/DMSO medium for 21 days maintained their differentiated function and had measurable levels of cytochrome P-450 and 7-ethoxycoumarin O-deethylase (7EC) activity. Cells cultured in RPMI medium on a 3T3 cell feeder layer were also still differentiated after 21 days but had very low 7EC activity. The use of either matrigel or fibronecta as substratum appeared to offer no advantage over RPMI/DMSO medium. In contrast, the use of a collagen sandwich technique with RPMI medium was successful in maintaining viable and differentiated hepatocytes. The addition of 2mM phenobarbital (PB), 10μM β-naphthoflavone (BNF) or 50μM nafenopin (NAP) in both collagen sandwich and RPMI/DMSO systems maintained cytochrome P-450 levels above those in untreated cultures. PB and BNF, but not NAP, induced 7EC activity, whereas only NAP induced peroxisomal fatty acid β-oxidation activity. Generally, a better response was obtained with the collagen sandwich technique than with RPMI/DMSO medium. These results demonstrate that the collagen sandwich technique may be a useful system for maintaining viable and differentiated rat hepatocytes for prolonged periods without supplementing the medium with DMSO. (Supported by U.K. Ministry of Agriculture, Fisheries and Food).

Partial hepatectomy (70%, PH) model was used to test our hypothesis that the slow hepaticcellular regenerative response after CCl4-induced liver injury is responsible for the high sensitivity of gerbils to CCl4 toxicity. PH-stimulated cell division in male gerbils 5 days after PH (PH5) was 4 to 5-fold higher than in those 15 days after PH (PH15) or sham operated gerbils (SH), as indicated by [3H]thymidine ([3H]-T) incorporation into hepatocellular nuclear DNA. Each group was injected ip with either a low (15 μg/kg) or a LD50 dose of CCl4 (80 μl/kg). The elevation of serum enzymes (ALT, AST) was less in PH5 gerbils, indicating less injury, than in PH15 or SH groups after administration of either dose. The LD50 dose of CCl4 caused a mortality of 21% in PH5 gerbils, significantly less than 50%. Histopathological examination was consistent with these findings. In vivo metabolism of 14C-CCl4 was not significantly different among the groups, suggesting that the mitigation of CCl4 toxicity in PH5 gerbils is unlikely to be due to reduced CCl4 bioactivation. [3H]-T was further augmented 24 hr after the challenge of a low dose of CCl4 in PH5 gerbils, whereas it remained low until 48 hr in PH15 or SH group. The findings support the concept that gerbil’s high sensitivity to CCl4 hepatotoxicity is due to a sluggish stimulation of tissue repair and suggest that PH-induced hepatocellular regeneration provides protection against CCl4 toxicity. (Supported by AFSOR-88-0009).


The metabolism of the reproductive toxicant bis(2-methoxyethyl)ether (CAS Reg. No. 111-96-1; diglyme) was studied in isolated rat hepatocytes and in the intact rat. Male Sprague-Dawley rats (190-220 g) were used in both studies. Hepatocytes, isolated by a two-step in situ collagenase perfusion of the liver, were cultured as monolayers and incubated with [14C]diglyme at 1 to 50 μM for up to 48 hr. The in vivo study, rats were given single ip doses of [14C]diglyme at 5.1 mmol/kg body wt, and the urine was collected for 48 hr. Radioactive compounds in the culture medium or in the urine were separated by reverse-phase HPLC and quantified with an in-line radioactivity flow monitor. Metabolites were identified by GC-MS. The principal metabolite from hepatocytes and in the urine was (2-methoxyethoxy)acetic acid (MEA). In 48-hr culture medium, MEA increased with diglyme concentration up to 40 μM and accounted for approximately 36% of the dose. In 48-hr urine, MEA accounted for 67% of the dose. Other prominent metabolites common to both systems included methoxyacetic acid and 2-methoxyethanol. Preliminary results indicate that the diglyme metabolite profiles from urine and from hepatocytes are qualitatively similar.

EFFECT OF DICHLOROACETATE AND TRICHLOROACETATE ON THE INCORPORATION OF [3H]-THYMIDINE IN PRIMARY RAT HEPATOCYTE CULTURES. A. J. VanderPol, S. A. Bruschi, R. E. Bull. College of Pharmacy, Washington State University, Pullman WA.

Trichloroacetate (TCA) and dichloroacetate (DCA) have been shown to be hepatocarcinogenic in B6C3F1 mice. Subsequent experiments indicated that both DCA and TCA induced increased [3H]-thymidine incorporation into hepatic DNA in vivo. However, these effects were not associated with increases in cell division. To further characterize this response we have attempted to replicate these observations in cell culture using primary rat hepatocyte monolayer cultures. The hepatocytes were allowed to attach to a collagen substrate for 8 hrs. followed by 18 hrs. of treatment with the test compound in the presence of [3H]-thymidine and hydroxyurea. [3H]-thymidine was quantitated using liquid scintillation counting and expressed as DPM/μg DNA. Both DCA and TCA increased [3H]-thymidine incorporation up to 1 mM extracellular concentration. Higher concentrations significantly decreased [3H]-thymidine incorporation with respect to controls in a dose-related manner. The mechanism of these responses is currently unknown, questions of whether these effects are due to alterations in thymidine pool size or unscheduled DNA synthesis are being investigated. [Supported by NIH grant # 3RO1ES04648].


Research on mechanisms of cell death is hindered by the lack of efficient methods to monitor continuously irreversible injury. Previously, we developed an assay to monitor cell viability non-destructively in cell suspensions (Am J Physiol 255, C315). Here, we describe a cytotoxicity screening assay suitable for cultured cells. Overnight cultured rat hepatocytes plated on 96-well microtiter plates (1-5x10^5/well) were incubated in Krebs-Ringer-Hepes buffer containing 10-50 μM propidium iodide (PI). As cells lost viability, their nuclei were labeled by PI with fluorescence enhancement measured with a multi-well scanner. Diginon (375 μM) was used to permeabilize all cells and generate a fluorescence signal corresponding to 100% cell death. The increase of fluorescence correlated linearly with LDH release. Employing this technique, two reputed protective agents, U74006F and calciphor, were evaluated during a chemical hypoxia with KCN plus iodoacetate and oxidative stress with n-butyl hydroperoxide (n-BuOOH). U74006F (10-50 μM), an inhibitor of lipid peroxidation, protected against both chemical hypoxia and n-BuOOH. Calciphor, a mixture of prostaglandin B1 oligomers, did not protect and was toxic at higher concentrations (≥50 μM). In conclusion, a simple high capacity cytotoxicity screening assay was developed for cultured cells. U74006F but not calciphor protected against lethal injury in models of chemical hypoxia and oxidative stress.
IN VITRO METABOLISM AND TOXICITY ASSESSMENT OF N-METHYLCARBAZOLE IN CULTURED RAT HEPATOCYTES. T. Jiang, W. Yang, P. J. Davis and D. Acosta. Dept. of Pharmacol. & Tox., Medicinal Chemistry, College of Pharmacy, Univ. of Texas at Austin, Austin, TX.

N-methylcarbazole (NMC), the active co-carcinogen and mutagen present in tobacco smoke, is converted to two major metabolites by cultured rat hepatocytes as measured by HPLC: N-hydroxymethylcarbazole (NHMC) and carbazole. The metabolites NHMC and carbazole have similar retention times and identical ultraviolet spectra as the synthetic NHMC and carbazole. The toxicity of NMC and its two metabolites was assessed by the neutral red test (NR) and lactate dehydrogenase (LDH). Treatment of the cultures with NMC and NHMC (5×10^{-4} M) resulted in LDH leakage that was 188% and 722% of control values, respectively. In contrast, 5×10^{-4} M carbazole did not cause LDH leakage. Treatment of the cultures with NMC, NHMC and carbazole (5×10^{-4} M) reduced NR uptake to 61, 14 and 75% of control values, respectively. This study showed that NHMC is a toxic metabolite of NMC and carbazole is most likely a detoxification metabolite.


Hepatic fat storing cells (FSC) are the primary vitamin A (VA) storing cells in the body. Following hepatic necrosis, FSC can lose their VA-storing ability and convert to collagen-secreting myofibroblasts. Therefore, it was hypothesized that supplemental VA may sustain the lipid storing function of FSC and suppress their transformation into collagen-secreting cells in response to liver necrosis. Rats were gavaged with VA vehicle or VA (retinol; 250, 000 IU/Kg/d) for 9 or 42 days. Rats received carbon tetrachloride (CCl4; 0.15 ml/Kg; i.p.) four times over a 44 day period. Hepatotoxicity was evaluated by serum alanine aminotransferase (ALT) activity, liver necrosis, and hepatic collagen deposition. Following CCl4 exposure, liver necrosis (p<0.05) and serum ALT activity (p<0.01) were significantly increased in VA-treated rats compared to controls that received CCl4 and the VA vehicle. The least necrosis but the most fibrosis was observed in rats receiving CCl4 after an initial 9 day treatment of VA. In contrast, rats maintained on VA for 42 days showed the most necrosis, but least fibrosis (p<0.05) following CCl4. These results indicate that excessive VA administration prior to CCl4 exposure decreases hepatic fibrosis by a mechanism that may involve impaired transformation of FSC to myofibroblasts.

Past results support using hepatocyte systems for ranking acute toxicity for specific chemical classes but not as a general screen. Hepatocyte monolayer cultures were examined under a variety of conditions to determine if correspondence might be improved. Hepatocytes isolated from young, male Sprague-Dawley rats with collagenase were attached to collagen-coated, 96-well dishes. Acetaminophen, aspirin, salicylic acid, diazepam, amitriptyline, digoxin, fosfomycin, tolazol, ethanol, isopropanol, or ethylene glycol were tested at various concentrations in media containing either 0.2% or 2.0% bovine serum albumin (BSA).

Cytotoxicity was assessed at 24 and 48 hr by measuring MTT (tetrazolium salt) reduction and total cellular protein using Coomassie blue dye. Cellular lactate dehydrogenase was evaluated as an alternate indicator. EC values were determined from log-probit analyses. Cytotoxic potency in general increased with incubation time and tended to be greater in 0.2% BSA than in 2.0% BSA-containing media. Linear regression analysis of the log transformed EC values with log oral LD50 data for the chemicals in the rat gave an optimal R2 of 0.86 for the EC10 at 24 hr with MTT as the indicator. Further refinements of the assay are possible and may improve this correspondence. (Supported by an International Fellowship from Kitasato Institute.)

ACETAMINOPHEN-INDUCED DNA FRAGMENTATION AND CELL DEATH STUDIED IN VITRO IN CULTURED MOUSE HEPATOCYTES. SD Ray, LM Kamendulis, W Shen, GB Corcoran. Toxicology Program, U. New Mexico College of Pharmacy, Albuquerque, NM.

Acetaminophen doses producing liver necrosis elevate nuclear Ca2+ and fragment DNA in vivo by 2 hr (Tox Appl Pharmacol, In Press). The present studies used a homogeneous population of cultured hepatocytes from male NIH Swiss mice to evaluate DNA changes during cell death. Cells isolated by 2-step collagenase perfusion were cultured in Williams' E containing nicotinamide, and exposed to 1-25 mM acetaminophen after nicotinamide removal. Cytotoxicity was assessed by leakage of lactate dehydrogenase and adenosine nucleotides (pre-labeled with 3H-adenine). Fragmentation of genomic DNA was determined by sedimentation analysis and agarose gel electrophoresis. 25 mM Acetaminophen produced cytotoxicity at 6 hr (180% control leakage), and all concentrations produced damage from 24 hr onward (>225% of control). 15-25 mM Acetaminophen caused DNA fragmentation (205% of control) from 1 hr onward, and 5 mM by 4 hr (200% of control). Agarose gels showed oligonucleosome length DNA fragments characteristic of Ca2+-endonuclease activation. Aurintricarboxylic acid, an endonuclease inhibitor, sharply reduced DNA cleavage and cytotoxicity. Collectively, the results suggest that early DNA cleavage is mediated by endonuclease activation and occurs at an early enough time point to contribute to acetaminophen-induced cytotoxicity in cultured mouse hepatocytes. (Supported in part by NIH GM-41564)

IN VITRO EVALUATION OF GALACTOSAMINE AND ETHIONINE-INDUCED CYTOTOXICITY IN CHANG LIVER AND H-4-II-E HEPATOMA CELL LINES. J P Bacon, D A Linnoila, and T L Hochstein. Investigative Toxicology, The Upjohn Company, Kalamazoo, MI.

The cytotoxic mechanisms of galactosamine (GALN) and ethionine (ETH), two metabolically activated hepatotoxins, were examined in vitro in the Chang human liver and H-4-II-E rat hepatoma cell lines. This study was conducted to determine if these hepatotoxins exert similar cytotoxic effects in these cell lines as observed in vivo. GALN and ETH are hepatotoxic in vivo by depleting UTP and ATP, respectively. Accordingly, toxicity can be inhibited by adding either UTP or ATP precursors. Methionine can also inhibit ETH toxicity. Subconfluent cultures were treated for 48 hrs with GALN (5 or 25 mM) + uridine or galactose (1, 2.5, 5, or 25 mM) or ETH (5 or 10 mM) + adenine (0.5, 0.75, or 1 mM), inosine (2.5 mM), or methionine (5 or 10 mM). Cytotoxicity was assessed by LDH leakage and cell proliferation. ATP levels were determined in Chang cells treated with ETH and cotreatments. Uridine had no effect on GALN-induced cytotoxicity, while galactose provided significant protection. ATP levels were not significantly depleted by ETH treatment. Adenine and inosine did not alleviate the cytotoxicity despite elevating ATP levels. Methionine, however, significantly inhibited the cytotoxicity without altering ATP content. The data suggests UTP and ATP depletion may not be the major mechanisms of GALN and ETH cytotoxicity in Chang and H-4-II-E cell lines. However, some other pathway related to galactose and methionine metabolism may be involved.

DIFFERENTIAL MYCOTOXIN CONTENT AND TOXICITY OF FUSARIUM MONOCLUMULOS (FM) ISOLATES. W P Normand and C W Bacon. Toxicology and Mycotoxin Research Unit, ARS/USDA, R B Russell Agricultural Research Center, Athens, GA

FM is a common fungal contaminant of corn. It causes diseases in corn, and mycotoxins produced by it threaten both animal and human health. FM produces the mutagen, fusarin C (FC), as well as the tumor promoter, fumonisin B1 (FB). Ten isolates of FM were examined for their ability to produce FC and FB when grown on seed corn. We also examined the cytotoxicity toward rat primary hepatocytes of organic or aqueous extracts of the cultures and of purified FC and FB. FB at concentrations up to 10-3 M had no effect on either release of lactate dehydrogenase (LDH) or uptake of [3H]valine (VI). FC was toxic at 10-6 M. Doses of 50, 100, and 200 mg equivalents of culture/106 cells/ml were used, and effects on LDH and VI determined. FC production by the isolates ranged from 7-346 ppm, and that of FB from 0-1000 ppm. Isolates that produced the most FB produced the least FC, and vice versa. Organic extracts had moderate effects on LDH, and inhibited VI at high doses. Water extracts had little effect on LDH, but did inhibit VI. There was no correlation between FB content of water extracts and cytotoxicity. FC content of organic extracts did correlate with cytotoxic effects, but some isolates that produced no FC were toxic. The results indicate that cytotoxicity to hepatocytes of extracts of FM cultures is due at least in part to mycotoxins other than FB or FC.

The aminocyclitol antibiotic, tropaneotomicin sulfate (6'-propylsaponinomycin), produces intracellular lamellar bodies in hepatocytes both in vivo and in vitro. Most, but not all, lamellar bodies appear to be lysosomal but their origin and fate are not clear. Analysis of tropaneotomicin-induced lamellar bodies from rat liver (isolated by sucrose density gradient ultracentrifugation) showed them to be phospholipid with an enrichment for anionic phospholipids. Based on this, we used a fluorescent phosphatidylcholine analog (NBD-PC) with cultured rat hepatocytes. Control cells incubated with NBD-PC liposomes 5-24 hr showed diffuse labeling when viewed by fluorescence microscopy. Co-treatment of hepatocytes with NBD-PC and tropaneotomicin or 6'-pentyloctapineomycin produced labelled intracellular inclusions, shown by electron microscopy to be lamellar bodies. For degradation studies, hepatocyte lysosomes were labelled with RITC-dextran. Isolated lamellar bodies were labelled with NBD-PC then microinjected into the hepatocytes; observations were made for up to 18 hrs. Fluorescence faded from the lamellar bodies, which remained in the cells but did not fuse with existing lysosomes. Our results indicate that tropaneotomicin-induced lamellar bodies form by a dynamic process that involves the plasma membrane, and are not degraded by pre-existing lysosomes.


Traditionally, all potential toxic compounds are first tested in rodents and rodent originated culture systems. However, species difference exists in xenobiotic biotransformation between rodents and human beings. We studied the genotoxic potential of a carcinogen in human hepatoma cell line, HepG2, as compared to that in primary rat hepatocyte culture. HepG2 is a well differentiated human hepatoma cell line with biotransforming capacity and no HBV integration. Rat hepatocytes were obtained by the standard two step in situ perfusion and cultured in William E with 10% FBS. By using benzo[a]pyrene, both HepG2 and primary rat hepatocyte culture showed unscheduled DNA synthesis with different sensitivity. In 32p-postlabeling analysis, the chromatogram showed quantitative and qualitative difference in HepG2 and primary rat hepatocyte culture when treated with 10 uM benzo[a]pyrene for 18 hr.

HepG2 formed 16.68 BPDEI-dGuo per 106 nucleotides, while rat hepatocyte culture only formed 2.22 adducts. In addition, rat hepatocyte showed minor adducts while such adducts were not significant in HepG2. Therefore, the human HepG2 cell line may be used as a model in risk assessment for environmental carcinogens.

EFFECTS OF ACETALDEHYDE AND GLUCOCORTICOIDS ON G6PDH ACTIVITY IN PRIMARY RAT HEPATOCYTES IN CULTURE. G Stevens, R Hetzel, R Ulrich, S Staplton, L Ginsberg. Dept. of Biological Sciences, Western Michigan University, and Upjohn Laboratories, Kalamazoo, MI. Sponsor: T Petry

Acetaldehyde, a metabolite of ethanol, has been shown to play a significant role in the development of alcohol induced fatty liver. Glucose-6-phosphate dehydrogenase (G6PDH; EC 1.1.1.49) is an important hepatic lipogenic enzyme. In primary rat hepatocytes incubated in a defined media containing 10 uM insulin and 15 uM hydrocortisone, the activity and mRNA levels of G6PDH are induced in the presence of 30 mM ethanol for 48 hours in culture. Under similar conditions, 100 uM acetaldehyde increases G6PDH activity. Hepatocytes incubated with 100 uM acetaldehyde alone increased G6PDH activity by 21% above control. In hepatocytes cultured with 10 uM insulin and 15 uM hydrocortisone, the G6PDH activity rose to 30% above the non-treated control. In the addition of both hormones and 100 uM acetaldehyde, the enzyme activity of G6PDH increased 42% over the non-treated control. Other cellular enzymes, such as lactate dehydrogenase and cytochrome P450 reductase, showed no significant affect when incubated under similar conditions. These results show that acetaldehyde may play an important role in the induction of lipogenic enzyme activity, and may indicate one of the many mechanisms of alcohol induced fatty liver. (Supported in part by NIAAA # AA06728-04A1 and NFRSF WMU)


The metabolism and tissue distribution of chlorfluazuron (CFZ) was studied in the highly susceptible Eastern spruce budworm. CFZ was not metabolized in any detectable quantities both in vivo and in vitro. 97% of the CFZ was recovered virtually unchanged. A radiolaabelled unknown was retained at the origin during TLC analysis. In the tissue distribution experiment, CFZ was retained, and only slowly eliminated by the spruce budworm. CFZ/wet wt was concentrated in the internal organs and the integument (target tissue), at a similar mean level, with a similar pattern over time that peaked at 24 hours. CFZ was readily removed from hemolymph. The head tissues concentrated, and more slowly eliminated CFZ at a rate different from the other tissues, having a 48 hour peak. There was a strong correlation between tissue wt and % CFZ-equivalents recovered in the insect, in a non-linear relationship; although some concentration of CFZ in tissues did occur. This implies a generalized cellular binding of CFZ. The high toxicity of CFZ to the Eastern spruce budworm was due to a lack of metabolism and slow elimination.

Teclubenzuron [1-(3,5-dichloro-2,4-difluoro-phenyl)-(2,6difluorobenzoyl)urea] is a new acyl urea IGR, proposed for use on certain fruits and vegetables. A complete toxicology data base shows: 1) low acute toxicity by oral, dermal and inhalation routes, 2) low irritation potential to eye and skin, 3) no teratogenicity in rats or rabbits, 4) no reproductive effects in rats, 5) no mutagenicity in a battery of 5 in vitro and 4 in vivo tests, 6) no oncogenicity in rats or mice (via dietary route), except increased benign hepatocellular adenomas in male mice, 7) limited absorption from the GI tract of rats at doses above 1000-2000 ppm in diet, 8) chronic effects in livers of dogs and rats (increased liver weights, elevated serum levels of hepatic enzymes, and microscropic changes). The most sensitive species for chronic effects is the rat (NOEL = 1.1 mg/kg/day, LOEL = 5.4 mg/kg/day), based on liver effects. Evaluation of residue data from field trials and processing studies on proposed food crops provides the mean anticipated residues (AR) for human exposure assessment. Based on AR, the EPA's Dietetic Risk Evaluation System (DRES) estimates the annualized daily consumption for the entire U.S. population and 22 subgroups for comparison to the Reference Dose (RfD). Results of this assessment show that for the U.S. population, exposure is <5% of RfD and all groups are <15%. Thus, the proposed uses of teclubenzuron are toxicologically supported and offer a new, safe IGR for agricultural use.

ACUTE TOXICITY STUDY WITH A FLEA CONTROL PRODUCT CONTAINING PYRETHRINS, TETRAMETHRIN, PIPERONYL BUTOXIDE, N-OCTYLSCYCLEPENTEN DiCARBOXYLIC AND FENOXYCARB IN DOGS. F W Oehme, J A Pickrell, J S Albertson, N Alf, T G Onstott and C E Munderfield, Comparative Toxicology Laboratories, Kansas State University, Manhattan, KS and S C Johnson and Son, Inc., Racine, WI.

To assess the short-term toxicity of a new flea control product, 30 dogs were assigned to 4 groups (3 per sex per control group; 4 per sex per dose group). The material was applied at 1X, 3X, and 10X the manufacturer's recommended dosage. The dogs were observed daily for 14 days for clinical signs of toxic effect. Food consumption and body weights were recorded. Routine clinical chemistry, hematologic and urinalysis parameters were measured. No abnormal treatment-related clinical signs were observed. Food consumption was decreased following exposure in both control (vehicle exposed) and product exposed dogs. No treatment-related changes in weight gain were observed. No significant hematologic or urinalysis abnormalities were noted. Serum glucose was slightly higher in the 10X dose group when compared with the controls. These results suggest that this product is extremely safe to dogs when used at the manufacturer's recommended dose, and that it provides a wide margin of safety when applied to clinically normal dogs.


Groups of 20 CD-1 mice/sax were fed diets containing CGA-17193 (a metabolite of the insecticide isafoz of) at constant concentrations of 0, 30, 100, 300, 1000, 3500 or 7000 ppm for 90 days to determine subchronic toxicity. Clinical observations, body weight, food consumption and mortality were monitored. Complete necropsies and organ weight measurements were performed. Samples of blood were collected for clinical pathology analysis. Histopathology was evaluated on all tissues from all animals sacrificed at termination, from all animals that died and from all animals sacrificed at 28 days which appeared abnormal at necropsy. There were no differences between treated and controls in body weights, food consumption or clinical signs. There were no dose-related biologically significant effects in hematologic, clinical chemistry or organ weights after 90 days of treatment. There were no necropsy or histopathology findings suggestive of a relationship to treatment with CGA-17193 for any tissues or organs examined. This isafoz metabolite was completely devoid of cholinesterase inhibiting activity as summarized. CGA-17193 was well tolerated by CD-1 mice at 7000 ppm for 90 days.

90-DAY TOXICITY STUDY WITH CGA-184699 IN CATS. F Choe, S M MacAskill, K L Pavkov, D R Saunders, W R Campbell and D S Wyand. Environmental Health Center, Agricultural Div., CIBA-GEIGY Corp., Farmington, CT; Greensboro, NC and University of Connecticut, Storrs, CT.

Domestic shorthair cats were administered 0, 300, 900 or 1500 mg/kg/month of CGA-184699 (an experimental systemic insecticide) alone or 1500 mg/kg/month of CGA-184699 and one of the following topical insecticides: MYCODEX POWER PLUS (carbaryl); ADAMS F & T 14 DAY MIST (pyrethrin); SENDRAN LIQUID DIP (propoxur). There were no signs of toxicity. Body weights and food/water consumption also were not affected. Statistically significant increases in gamma-glutamyl transferase activity were observed in most males treated with 1500 mg/kg/month of CGA-184699 in weeks 5 and 9. Similar increases were observed in females treated with 900 or 1500 mg/kg/month of CGA-184699 alone in week 5. Serum sodium and chloride levels were decreased in several groups of treated males in week 9. Serum bilirubin levels were decreased slightly in most groups of males treated with 1500 mg/kg/month of CGA-184699 in weeks 5 and 9. Similar decreases were observed in females treated with CGA-184699 and the topical agents in week 9. The spleen weights (relative to body weights) were 72%, 67%, and 55% of controls and adrenal weights (relative to body weights) were 76%, 69%, and 67% of controls in the 300, 900 and 1500 mg/kg/month male dose groups, respectively. However, no dose-related histopathologic alterations were observed. In conclusion, CGA-184699 alone or in combination with topical insecticides was not toxic to cats even at 1500 mg/kg/month.
The objective is to determine, based on health effects data, allowable DDT, DDE, and DDD levels (DDTR for DDT residues) in soil before permitting land development from agricultural use to commercial and residential uses. DDT, DDE, and DDD are carcinogenic in laboratory animals and are considered probable human carcinogens. Cancer potency slope factors (SF) have been derived from animal bioassay data (EPA, 1990) and they are 0.34 kg/day/mg for both DDT and DDE, and 0.24 kg-day/mg for DDD. We used these data to calculate Preliminary Health Effect Levels for Soil (PHEL$_{soil}$) by using the formula PHEL$_{soil}$ = (1x10$^{-6}$) x 70 kg / SF x 0.1 g/d x 1.00 + 0.45 g/d x 0.05). The amount of dermal contact with soil (0.45 g/day) and dermal absorption for DDTR (0.05) were developed by our Program. PHEL$_{soil}$ thus calculated are: DDT = 1.7 ppm (mg/kg soil), DDE = 1.7 ppm, and DDD = 2.4 ppm. Currently there are soils containing 2 to 4 ppm DDTR in soil awaiting rezoning for land development. Because of the uniqueness of the proposed future uses, the potential exposure scenarios for humans at these sites will be different. The length and frequency of human exposure are key factors in deciding whether soil remediation is required for these sites.

O,p'-DDT INCREASES CONTRACTION FREQUENCY OF RAT UTERINE STRIPS IN VITRO. D R Juberg and R Loch-Carrara. Toxicol. Prog., Dept. Envr. & Indus. Health, The Univ. of Michigan, Ann Arbor, MI.

Exposure to organochlorine insecticides, particularly DDT, has been associated with preterm labor in animals and humans. A mechanistic basis for this association is not known, despite the prevalence of such pesticides in the environment. In this study, longitudinal uterine strips from pregnant rats were mounted in a muscle bath for isometric recording. Following equilibration, uterine strips were treated with o,p'-DDT or isopropyl alcohol (solvent control) for 3 hr, followed by 3 hr without test substance. o,p'-DDT increased the frequency of contractions in a time-dependent manner, even after removal of o,p'-DDT. Compared to controls, there were no significant differences in contraction frequency at 10$^{-5}$ M o,p'-DDT; however, 5x10$^{-3}$ M significantly increased contractility during the post-exposure period only, while 10$^{-4}$ M significantly increased contractility throughout the 6-hr test period. A dose effect was clearly observed during the post-exposure period: the mean contraction frequency of uterine strips treated with 10$^{-4}$ M o,p'-DDT was significantly greater than that of 5x10$^{-3}$ M, which was greater than that of 10$^{-5}$ M. Overall, 10$^{-4}$ M, 5x10$^{-3}$ M and 10$^{-5}$ M o,p'-DDT increased contraction frequency by 84%, 44% and 4%, respectively, compared to controls. These data show that o,p'-DDT directly stimulated isometric contractions in rat uterine strips. Administration of uterine contractility may be relevant to preterm birth, since uterine contraction frequency is greater in women who deliver prematurely compared to women who deliver at term.

CATALYTIC POTENTIAL OF LIPOXGENASE IN XENOBIOTIC METABOLISM: EPOXIDATION OF ALDRIN. A K Naidu A K Naidu and A P Kulkarni. Toxicology Program, College of Public Health, University of South Florida, Tampa, FL.

Epoxidation of aldrin was investigated using highly purified soybean lipoxgenase in the presence of linoleic acid. The amount of dieldrin produced was dependent on concentration of linoleic acid, aldrin and enzyme. The epoxidation was linear with respect to time and exhibited pH optimum of 7.4. The optimal conditions to observe maximum enzyme velocity included the presence of 0.25 mM linoleic acid, 200 uM aldrin, 20 nM enzyme. Dieldrin, the primary stable reaction product, was quantified by Electron Capture Gas Chromatography. Catalytic potential of lipoxynase as expressed in terms of specific activity, was 4.0 nmole/ min/mg lipoxynase. Linoleic acid can be effectively substituted by H$_2$O$_2$ to mediate aldrin epoxidation. Lipoxynase inhibitors 1-Phenyl-3-Pyrrolidone (Phenindone), nordihydroguaiaretic acid (NDGA), 5,8,11-eicosatriynoic acid (ETI), 5,8,11,14-eicosatetraenoic acid (ETYA) significantly inhibited epoxidation in a dose dependent manner. These results suggest that lipoxynases, which are widely distributed in plants and animals, may play an important role in oxidative metabolism of pesticides.

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ASSESSMENT OF PENTACHLOROPHENOL EXPOSURE IN HUMANS USING THE CLEARANCE CONCEPT. B G Reigner, F Y Bois and T N Tozer. Department of Pharmacy, University of California (UC) San Francisco and School of Public Health, UC Berkeley, CA. Sponsor: P R Ortiz de Montellano.

Pentachlorophenol (PCP), a widely used wood preservative, is a ubiquitous compound which has been found recently to be carcinogenic in mice. The objective of this study is to assess the daily intake of PCP in groups of individuals who are not specifically exposed to PCP (group I), residents of homes made of PCP-treated logs (group II), and occupationally exposed to PCP (group III). The average daily intake was calculated using a basic pharmacokinetic principle, the clearance (CL) concept: daily intake equals CL (in L/day) times the average steady-state concentration of PCP in plasma (C$_{ss}$). C$_{ss}$ values reported in the literature (22 publications) and a CL of 0.4 L/day (estimate from 11 publications) were used for the calculations. In group I, daily intake estimated in 8 countries varied from 0.06 to 35 μg/kg/day in Nigeria. Exposure was between 25 and 148 μg/kg/day in group II. In group III, daily intake varied widely (from 35 to 23,000 μg/kg/day depending on the type of work). Daily intakes estimated in the general population of continental USA (from 12 to 20 μg/kg/day) are similar to those obtained previously using other approaches, namely, an environmental partitioning model (intake estimate = 16 μg/kg/day) and a kinetic method based on the knowledge of body burden (intake estimate = 23 μg/kg/day). Of the three approaches, the use of the clearance concept to assess exposure is the most readily accomplished. It may be applied to other toxicants for which a clearance value is known. Supported by NIH Grant ES04705 and the U.C. Toxic Substances Program.
HEALTH EFFECTS AND ENVIRONMENTAL CHARACTERIZATION OF OCTACHLORODIBENZO-P-DIOXIN (OCDD): IMPACT ON RISK ASSESSMENT OF FORMER WOOD TREATMENT SITES. T Copeland, M Harris, B Finley and D Faustenbach. ChemRisk, division of McLaren/Hart, Irvine, CA.

Recently the USEPA revised the 1987 Toxic Equivalent Factors (TEFs) for PCDDs and PCDFs. While many of the changes to the 1987 TEFs did affect the risk assessment process for PCDDs/PCDFs, the increase in the TEF from zero to 0.001 for OCDD/OCDF significantly affects the possible hazards associated with pentachlorophenol contaminated sites. The primary PCDD/PCDF contaminants of pentachlorophenol sites are the hepta- and octa- chlorinated dibenzo-p-dioxins and dibenzofurans such as OCDD. This paper presents a comprehensive review of the literature dealing with the health effects and behavior in the environment of OCDD. New guidelines are suggested pertaining to the risk assessment of pentachlorophenol sites. These guidelines include such elements as selection of indicator chemicals and the most appropriate soil bioavailability factors.

ISSUES SURROUNDING THE QUANTITATIVE CANCER RISK ASSESSMENT OF PENTACHLOROPHENOL. V J Cogliano, S Irene, R Rubenstein, D V Singh, S Segal, and B Allen. U.S. Environmental Protection Agency and Clement Assoc., Inc., Washington, DC.

The U.S. Environmental Protection Agency has classified pentachlorophenol as a probable human carcinogen, in Group B2. In a National Toxicology Program study using B6C3F1 mice, pentachlorophenol caused liver tumors and pheochromocytomas in both males and females, and hemangiosarcomas in females only.

With positive results at three sites, in two sexes and with two different preparations of pentachlorophenol, there are many alternative ways of calculating a quantitative risk estimate, raising many interesting issues concerning the selection of an appropriate data set. There were large increased incidences of liver tumors and pheochromocytomas, but these tumors were predominantly benign. The increased incidence of hemangiosarcomas was more modest, but these tumors were malignant and constituted the tumor type of greatest concern. Only female mice developed hemangiosarcomas; although female mice developed all three tumor types, male mice developed more tumors overall. The appropriateness of using liver tumors and pheochromocytomas for estimating human cancer risks has been questioned by some. The resolution of these and other issues provokes discussion about the integration of biological and statistical concerns and suggests some revisions to EPA's current practices.

PHENOXY-HERBICIDES EFFECT GLUTATHIONE LEVELS IN 3T3 MOUSE FIBROBLASTS. C D Andry, W Li and JN Chou. Deps. of Microbiology and Pathology, Boston Univ. School of Medicine, Boston, MA.

In quiescent 3T3 cells, the herbicides 2,4-dichloro-(2,4'-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) alter the organization of microtubules and microfilaments, two major components of the cytoskeleton. The mechanism that leads to this derangement is unclear. The role of glutathione (GSH) as a reducing and detoxifying agent has led us to examine its status in herbicide-treated cells. GSH content was determined by both enzymatic and HPLC analysis. Exposure of 3T3 cells to 2.5 mM of either 2,4-D or 2,4,5-T resulted in significant increases in total cellular GSH, which was maximized at 5 h and subsequently plateaued over the next 17.5 h. HPLC analysis confirmed the enhancing effects of 2,4,5-T (2.5 mM for 5 h) on cellular GSH, and an increase in cystine, cysteine and glutamate was also observed. To evaluate if new GSH synthesis might account for its observed rise, we pre-incubated cells for 5 h with buthionine-L-R-sulfoximine (BSO), a potent inhibitor of GSH synthesis. Cells treated for 5 h with BSO, followed by a 17.5 h incubation in the absence of the inhibitor were depleted of GSH by 85%. Addition of 2,4-D or 2,4,5-T for 17.5 h post BSO-treatment did not result in an increase in GSH levels. These results suggest that the elevated GSH in 2,4-D or 2,4,5-T-treated cells results from de novo synthesis of GSH. Phenoxy-herbicides clearly have a marked effect on the content of non-protein thiols in 3T3 cells. The mechanisms by which 2,4-D and 2,4,5-T treatment enhance cellular GSH are currently under investigation.
DEVELOPMENTAL STUDIES ON A SERIES OF 2,4-D SALTS AND ESTERS IN THE RAT. T. R. Hanley, Jr.1, R. E. Schroeder2, and W. J. Breslin1.  
1The Toxicology Research Laboratory, The Dow Chemical Company, Midland, MI, and 2Bio/dynamics, Inc., East Millisone, NJ.

Pregnant Sprague-Dawley rats were administered 2,4-D butoxyethyl ester (BEE), or 2,4-D isopropylamine (IPA) or trisopropanolamine (TIPA) salts by gavage on days 6-15 of gestation at dose levels of 0, 80, and 230 (all), and 575 (2,4-D BEE), 680 (2,4-D IPA) or 790 (2,4-D TIPA) µmol/kg/day. At 575 µmol/kg/day of 2,4-D BEE, maternal toxicity and slight alterations in skeletal ossification were noted. At 680 µmol/kg/day of 2,4-D IPA, maternal toxicity but no developmental effects were observed. At 790 µmol/kg/day of 2,4-D TIPA, severe maternal toxicity with 13% mortality, weight loss, decreased feed consumption, and clinical effects were noted. A variety of developmental effects considered secondary to severe maternal toxicity and/or stress were also observed at 790 µmol 2,4-D TIPA. Dose-related though less severe effects on maternal weight gain and feed consumption were also seen at 80 and 230 µmol/kg/day, 2,4-D TIPA, but no developmental effects were noted. In conclusion, the developmental no-observed-effect levels (NOELS) for 2,4-D BEE, IPA, and TIPA were ≤ 230 µmol; the maternal NOEL was ≤ 230 µmol. Thus, 2,4-D BEE, IPA and TIPA are generally less toxic to the embryo/fetus than to the dam in rats.

EXPOSURE OF HOMEOWNERS, PROFESSIONAL APPLICATORS AND BYSTANDERS TO 2,4-DICHLOROPHENOXACYCETIC ACID (2,4-D). S. A. Harris, FR. Solomon Canadian Centre for Toxicology, 645 Gordon Street, Guelph, Ont., Canada. Sponsor: I. C. Munro

Total body dose received in home gardeners, professional applicators applying 2,4-D and bystanders living within the household but not applying the pesticide was measured. Levels of 2,4-D were monitored in air samples both inside the home and downwind of the application site. Homeowners were divided into protective and non-protective apparel groups and applied both a granular and liquid formulation of 2,4-D on two separate dates. Analyses of urine collected from homeowners for up to 96 hours following applications found total body doses ranging from non detectable to 0.671 mg/kg of body weight. The highest exposures occurred in the non protected group and were consistently associated with spills of the liquid concentrate or excessive contact with the dilute mixture on the hands or forearms. No detectable 2,4-D was found in urine samples supplied by bystanders to home applicators. Residues of 2,4-D were detected in 5 of the 76 air samples taken during the home applications. Two of these air samples occurred with detectable applicator exposure but it is unlikely that this was a major route of exposure to the applicator. Professional applicators who provided urine samples over a 14 day period received average exposures ranging from 0.0017 to 0.0035 mg/kg/day with an average of 0.0033 mg/kg/day. Total exposure was poorly correlated with total amount of active ingredient (A.I.) applied. Personal work habits and hygiene appear to be the major factors affecting exposure. No detectable 2,4-D was found in air samples or urine samples supplied by 7 bystanders who had a professional application of 2,4-D.

A SIMPLE METHOD FOR ANALYSIS OF DIQUAT IN TISSUES AND BIOLOGICAL FLUIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY. Z. Gregus, C Madhu and C D Haasen. Univ Kansas Med Ctr. Kansas City, KS

Diquat is a widely used non-selective herbicide. Recently we observed a marked inter-animal difference in rats to diquat-induced oxidative stress and thought that the variation might be due to differences in diquat disposition. Therefore, in the present study an HPLC method was developed for quantitation of diquat in various tissues and fluids of rats. An HPLC system with a µ-Bondapack C18 column and UV detector (315 nm) was used. The mobile phase consisted of H2O (900 ml), orthophosphoric acid (13.5 ml), diethylamine (10.3 ml), octanesulfonic acid (850 mg), acetonitrile (75 ml) and tetrahydrofuran (40 ml). The flow rates of the eluent was 1 ml/min. With this system, diquat eluted at 6.7 min. A high degree of correlation (r=0.996) was observed between the amount of diquat injected (25-500 pmol) and peak areas obtained. Diquat (0, 10, 30, 100 and 300 pmol/g or ml) was added to blood, bile, urine, liver and kidney and extracted with 70% methanol containing 21 mM perchloric acid. Twenty µl of each sample was injected onto the column. The blank samples had no interfering peaks with diquat. Recovery of diquat was 96-105% for blood, bile, urine, liver and kidney. In conclusion, the present HPLC method is a simple and sensitive method for quantitating diquat in tissues and biological fluids. Additionally, this method may also be applicable for other structurally similar compounds such as paraquat. (Supported by NIH Grant ES-03192).


Absorption of paraquat was studied using isolated mucosa from the gastrointestinal tract of rats. Tissues were maintained in flux chambers by bathing both serosal and luminal membranes. Paraquat absorption, transmucosal potential difference (PD) and permeability were studied. Paraquat (100mg/ml) was preferentially absorbed across the small intestine. Descending order of absorption (% per cm² mucosa) was jejunum (17.6 ± 0.8%), ileum (10.0 ± 1.7%), colon (5.7 ± 3.2%), duodenum (5.5 ± 1.3%), stomach (2.0 ± 0.8%) and oesophagus (0.5 ± 0.7%). Mucosal uptake of paraquat (2-200mg/ml) in the ileum was non-linear. Three phases to paraquat absorption were identified in the ileum: (i) a rate faster than diffusion (2-20mg/ml paraquat), (ii) a rate slower than diffusion which obeyed saturation kinetics (50-150mg/ml), and (iii) a rate similar to that of diffusion at 200mg/ml. Paraquat absorption at 200mg/ml was also associated with an increase in mucosal permeability and reduction in PD. Inhibition of tissue metabolism abolished the saturable uptake of paraquat (2-200mg/ml). It is suggested that paraquat absorption in the rat occurs principally in the small intestine and by a mechanism which consists of facilitated, saturable and diffusional components. Knowledge of the mechanism by which paraquat is absorbed may offer new approaches to the development of safer formulations of the herbicide.

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Glyphosate is a broad-spectrum post-emergence translocated herbicide. Its interactions with skin and potential systemic availability through percutaneous absorption was studied by skin binding, skin absorption, residual tissue distribution, and skin decontamination. Glyphosate in a final formulation, (Roundup®), undiluted and diluted with water 1:20 and 1:32, would not penetrate into powdered human stratum corneum (≤1%). In vitro percutaneous absorption through human skin into human plasma as receptor fluid was no more than 2% over a concentration range of 0.5 - 154 μg/cm² and a topical volume range of 0.014-0.14 m/v/cm². Disposition of glyphosate following iv. administration of 93 μg and 9 μg doses to rhesus monkeys was mainly through urine excretion, 95 ± 8 and 99 ± 4% in 7 days, respectively. Percutaneous absorption in vivo in rhesus monkey was 0.8 ± 0.5% for the low dose (25 μg/cm²) and 2.2 ± 0.8% for the high dose (270 μg/cm²). No residual 14C was found in organs for the monkeys sacrificed 7 days after the topical application. Washing the skin application site with soap and water removed 90 ± 4% of applied dose, and washing with water only removed 84 ± 3% of applied dose. Both soap and water and water-only were equal in ability to remove glyphosate from skin over a 24 hr skin application period. About 50% of the initially applied dose could be recovered after 24 hours. Glyphosate is very soluble in water and insoluble in most organic solvents (log P ~ -1.70) and therefore not compatible with the lipid laden stratum corneum. This is consistent with the low skin binding and skin absorption, and also consistent with the efficient removal from skin with soap and water or water-only wash.

COVALENT BINDING OF CHICK EMBRYO PROTEINS BY THE ALKYLTHIOCARbamate MOLiNATE. M. D. Falmi, N. Chu, B. W Hart and P A Kitos, Dept. Pharmaco. and Toxicol., and Dept. Biochem., University of Kansas, Lawrence, KS

Organophosphate (OP) and alkylcarbamate (AC) insecticides or their metabolic derivatives are potent acetylcholinesterase (AChE) inhibitors. They form covalent adducts with their target proteins. In avian eggs many OP and AC compounds are embryotoxic and/or teratogenic because they react with AChE and other serine esterases. Alkylthiocarbamates (ATC) are structural analogs of the ACs but are used as herbicides rather than insecticides. Studies were carried out with molinate (M), an extensively used ATC herbicide, to determine whether it forms covalent conjugates with DFP-reactive proteins of embryos. M (3.6 pmole) was administered to four day old chicken eggs and the embryos were harvested at day 10. Cell-free extracts of the torsos and brains were prepared and incubated at 0°C with [3HIDP. The protein constituents were resolved by SDS PAGE and the radioactive proteins visualized by autoradiography and quantified by gel slicing and scintillation spectrometry. Three major DFP-labeled proteins were identified in these tissues (Mr 94, 83 & 72 KD). DFP binding to all three proteins was suppressed, but especially to the 72 KD protein. As M was incubated in vitro with extracts from control embryos, DFP binding was not diminished. However, if molinate sulfoxide, a metabolite of M, was used instead of M, DFP binding to the three proteins was suppressed in the same pattern as when M was administered to the embryo. The data suggest that some DFP-binding proteins of avian embryos are inhibited by metabolically derived products of M, but not by M itself. (Supported by Wesley Foundation grant #8612007 and NIIA 05377)
EFFECT OF HERBICIDES ON INDICATORS OF XENOBIOTIC-METABOLISING ENZYME INDUCTION AND/OR PEROXISOME PROLIFERATION.

DE Moody, BA Narloch, LR Shull, and BD Hammock. Deps. of Entomology and Environmental Toxicology, University of California, Davis, CA.

The mammalian toxicity of herbicides is less well described than for other classes of pesticides. We have now tested a number of diverse herbicides for hepatotoxic responses as indicated by induction of enzyme activities associated with xenobiotic-metabolism and/or peroxisome proliferation. The herbicides tested below were administered (i.p., 3 days) to male Swiss Webster mice at 250 mg/kg (2,4-DB & MCPP at 100 mg/kg). Liver fractions were prepared from mice killed 24 h after the last injection and used to assay for microsomal mixed-function oxidase (MFO), microsomal and cytosolic epoxide hydrolase (mEH & cEH), cytosolic glutathione S-transferase (GST) and large particle carnitine acetyltransferases (CAT) activities. Alachlor, chlorfluorecol, propanil & trifuralin were generalized inducers, causing increases in P450 and/or MFO, mEH and selected GST activities. Benthionac & molinate increased mEH and certain GST activities. MCP and 2,4-DB caused increases in cEH and CAT activities, suggesting that they had weak peroxisome proliferating activity as seen with other chlorophenoxy herbicides. Atrazine had no significant effect on any of the activities monitored, while dicamba appeared to be hepatotoxic at the dose used, as it resulted in decreases in the activities of many enzymes. A number of the herbicides tested were inducers of xenobiotic-metabolizing enzymes at high doses over a short time. This may prove more significant if the same effect is seen with lower doses over prolonged periods.

INDUCTION OF CYTOCHROME P450 IVA1 IN MICE BY THE HERBICIDE SYNERGIST TRIDIPHANE.

P.E. Levy and E. Rodriguez. Department of Toxicology, North Carolina State University, Raleigh, NC.

The herbicide synergist tridiphane, which had been shown by this laboratory to selectively inhibit certain P450 isozymes in vitro, has been examined for its ability to induce P450 in vivo. Liver weight and P450 content were increased after tridiphane treatment. This increase was accompanied by a significant elevation in microsomal lauric acid hydroxylase activity. No increase, however, was observed in either benzphetamine N-demethylase or ethoxyresorufin O-deethylase activities. Western blot analysis using an antibody specific for P450 IVA1 showed induction of a protein corresponding to purified P450 IVA1. A number of chemically diverse compounds including certain hypolipidemic drugs, phthalate ester plasticizers, and phenoxyacetic herbicides induce P450 IVA1 and also cause significant proliferation of peroxisomes. The increase in ω-hydroxylase activity associated with the induction of P450 IVA1 is thought to be the initiating event in peroxisome proliferation. Although these chemicals are nonmutagenic, some peroxisome proliferators induce liver tumors in rodents following chronic administration, and may form a novel class of carcinogens.

DEVELOPMENTAL TOXICITY OF PICLORAM POTASSIUM (K) AND TRISOPROPANOLAMINE (TIPA) SALTS IN THE RAT. W J Breslin, R E Schroeder and T R Hanley. Toxicology Research Laboratory, The Dow Chemical Company, Midland, MI and Bio/dynamics Inc., East Millstone, NJ.

The objective of this study was to evaluate the maternal and developmental toxicity of Picloram K and TIPA salts in rats. Pregnant Sprague-Dawley rats were gavaged with 0, 100, 500 or 1000 mg/kg/day of Picloram K or TIPA salt on days 6 through 15 of gestation. Maternal observations included changes in behavior and demeanor, feed consumption, body weight gain, gross pathologic alterations, liver and kidney weights and various reproductive parameters. On day 20 of gestation, fetuses were removed following cesarean section, weighed and examined for external, visceral and skeletal alterations. Maternal toxicity was noted in high dose females administered Picloram TIPA salt. Dams given 1000 mg/kg/day of Picloram TIPA salt had decreased feed consumption and body weight gain during the exposure period. No adverse maternal effects were observed with Picloram K salt and neither Picloram K nor Picloram TIPA salts were embryotoxic or teratogenic at any dose level. Thus, the developmental no-observed-effect-levels for Picloram K and TIPA salts were 1000 mg/kg/day.


Groups of 4 dogs of each sex were administered capsules containing fluxofenim (CONCEP III, a seed safener) to provide doses of 0, 0.4, 4, 20 or 40 mg/kg/day for 3 months to determine general toxicity and a no-observable-effect level (NOEL). Clinical signs, body weights, food consumptions, and mortality were monitored throughout the study. Clinical laboratory tests and necropsies were conducted at study termination. Administration of up to 40 mg/kg/day of fluxofenim did not produce mortality or significant changes in body weight, food consumption, clinical observations or clinical chemistry parameters. Hematological parameters were significantly changed at the high dose in both sexes. Mean corpuscular hemoglobin concentration was decreased in male dogs at 40 mg/kg/day. The red blood cell count was decreased while the platelet count was increased in female dogs at 40 mg/kg/day. The slope of the RCC osmotic fragility curve was significantly reduced at 14 weeks in male dogs treated at 40 mg/kg/day and at 9 weeks in female dogs treated at 40 mg/kg/day. Thyroid weights (absolute, relative to body and to brain), liver weights (relative to body and to brain) and kidney weights (relative to brain) were increased in female dogs at 40 mg/kg/day. Gross lesions observed at necropsy and histopathologic findings were unrelated to treatment with fluxofenim. The NOEL for this study was 20 mg/kg/day.
POSSIBLE MECHANISMS OF BENOMYL-INDUCED LIVER TUMORS IN CD-1 MICE. C S Van Pelt, S R Frame, H S Bogdanoff, J C Cook, and C A Nebus. The Du Pont Co., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE.

In a previous 2-year feeding study in CD-1 mice, benomyl induced an increased incidence of hepatocellular adenomas and increased multiplicity of adenomas, but did not cause an increase in malignant hepatocellular tumors. In the present study, CD-1 mice were fed benomyl for 28 days at dietary concentrations of 0, 100, 500, 3750, and 7500 ppm with a 2-week interim sacrifice. Multiple end points were utilized to assess possible mechanisms of benomyl-induced mouse liver tumors. Results indicate that benomyl, at dietary concentrations of 3750 ppm or greater for 2- or 4-weeks, induces adaptive responses characterized by increased liver weight, centrilobular hypertrophy, induction of hepatic smooth endoplasmic reticulum and hepatic cytosome F-440, and increased hepatocellular proliferation. Benomyl did not induce hepatic peroxisome proliferation as measured by peroxisome beta-oxidation and ultrastructural examination. The results of this study are consistent with the hypothesis that benomyl causes induction of a normal adaptive response that results in an increase in cell proliferation and, thus, acts indirectly by modulating a high spontaneous incidence of mouse hepatic tumors through physiological mechanisms and not as a direct acting carcinogen.


Analysis of benomyl, a broad-spectrum fungicide, in food commodities has traditionally been accomplished by solvent extraction and thin-layer detection by HPLC. The use of IA with antibody to the metabolite methyl 2-benzimidazolcarbamate (MBC) has the advantage of more rapid analysis. We compared the detection of benomyl residues in field treated crops using both HPLC and IA. Benomyl was applied to artichoke, currant, lyccees, mint, and sorghum. After harvest, the samples were stored frozen at -20°C until analysis. For HPLC analysis, the samples were extracted in methanol/ethyl acetate. Benomyl as MBC was partitioned into ethyl acetate and later dissolved in phosphoric acid for analysis by HPLC using a Zipax SCX column. For the immunosassay, the samples were extracted in methanol and diluted in water. Residues were quantified using the Benomyl Res-1-Quant® kit (Immunosystems, Inc.). The residue levels in the crops were less than 10 ppm. Of the crops analyzed by IA, lyccees contained the highest level of background. After background subtraction, there was good correlation (r2 = 0.61 to 0.95) of residue levels detected by HPLC and IA. (funded by USDA 88-34108-3263 IR-4, and E.I. Du Pont de Nemours)

COMPARISON OF THE NEUROBEHAVIORAL EFFECTS OF TRIADIMEFON (TDM) AND BUTERANTOL (BTN) IN RATS. R C Allen and R C MacPhail. University of North Carolina, Chapel Hill, NC and Neurotoxicology Division, U.S. EPA, RTP, NC.

The triazole fungicide TDM has been shown to increase motor activity, and increase response rates and disrupt patterns of responding in rats (e.g., Moser & MacPhail, Neurotoxicol Teratol. 13:286, 1991). These results raised questions of whether other triazoles produced similar effects. This study therefore determined the effect of another triazole, BTN, on motor activity and on operant performance maintained by fixed-interval (FI) reinforcement. Adult male LE rats (N=4) were trained to respond under fixed-interval (FI) schedules of reinforcement. Once performances stabilized, BTN (10-300 mg/kg) or vehicle (2 ml/kg) was administered i.p. 60 min prior to testing. Low to intermediate dosages increased rates of responding while larger dosages produced either smaller increases or decreases in responding. Relatively small disruptions in the patterns of FI responding were produced by BTN. Additional rats (N=6/group) were used to determine BTN effects on motor activity (MA). BTN (10-300 mg/kg, i.p., 45 min pre-session) did not increase MA over levels seen in control rats. These data suggest rate-increasing effects may be characteristic of triazole fungicides, and that effects on operant performance may not be predicted from effects on MA.


The acaricide chlordimeform (CDF) appears to interfere with adrenergic mechanisms of neurotransmission. The present study examined CDF effects on the brain-pituitary-adrenal axis, a system known to involve central d-adrenergic receptors. Male Long-Evans rats were injected i.p. with 20 or 50 mg/kg CDF and killed after 1, 4, 8 or 24 hrs. Both non-injected controls and saline-injected (SAL) controls were included. Dosing was structured so that trunk blood could be collected during the morning nadir of circulating corticosterone (CORT). Assays for plasma adrenocorticotropic hormone (ACTH), CORT and prolactin (PRL) showed that with 50 mg/kg all 3 hormones rose sharply by 1 hr. CORT increased in a dose-dependent fashion and declined over the ensuing 8 hrs. Other rats were treated with the d-adrenergic antagonist phentolamine (PBZ, 20 mg/kg) or the d-agonist clonidine (CLON, 0.6 mg/kg) 40 min before and killed 1 hr after CDF (25 mg/kg) injection. Using the following pre-treatment and treatment combinations, SAL-SAL, SAL-CDF, PBZ-SAL, PBZ-CDF, CLON-SAL, or CLON-CDF, CLON was found to suppress completely the CDF-induced rise in CORT, while PBZ enhanced the CORT response to CDF. These data indicate that the effect of the pesticide is likely via a blockade of central d-adrenergic receptors that normally function to suppress ACTH and CORT secretion.

The carboxylic chloridemform (CDF) has been shown to reduce serum gonadotropins and testosterone (T) levels in male rats within 24 h after two ip injections (50 mg/kg, 2 x d) (Goldman et al., 1990, TAP 104:25). With no further treatment, these CDF-induced endocrine changes had returned to control levels by 96 h. The current study was conducted to determine the effects of longer CDF exposure on luteinizing hormone (LH) synthesis and secretion in serum T. Male Long-Evans hooded rats were injected twice daily with CDF (25 mg/kg, ip) for 5 d. Tail blood was taken for LH and T measurements 8, 24, 32, 48, and 56 h following the initial dose. Serum LH and T were reduced at 24 h in CDF-treated rats but returned to control levels by 48 h, despite the continuous exposure to CDF. Pituitary LH concentrations were unchanged when the animals were killed at 120 h. To evaluate whether the transient CDF-induced reduction in serum LH was due to an alteration in LH synthesis, rats were killed at various times and mRNA levels of the a and LH-B subunits in the pituitary were assayed by Northern blot hybridization. At 48 h, a-subunit mRNA was reduced to 6% of the control while LH-B was not altered. These data show that CDF causes significant changes in serum LH and T levels which return to control values within 2-3 days after treatment is initiated and that changes in pituitary hormone levels in the blood appear to be associated with CDF-induced alterations in gonadotropin gene expression.


DACT (a metabolite of atrazine) was fed to groups of 15 female rats at dietary concentrations of 0, 10, 100, 250, or 500 ppm for 90 days. Vaginal smears were prepared and examined on study days 14-28, 42-56 and 70-85. The cell types and their relative proportionality were recorded and the stage of estrus determined. Blood samples were collected and levels of estradiol, progesterone, prolactin and corticosterone determined. On days 14-28, the incidence of rats exhibiting 5-day cycles and/or cycles with persistent diestrus was increased at 500 ppm. Effects on days 42-56 were more severe and apparent at >100 ppm. Effects included an increased incidence of cycles that were variable, indeterminate and < 4 or > 5 days in length. Several 250- and 500-ppm treated females exhibited cycles with persistent estrus. On days 70-85, further progression of these effects was observed at 250 and 500 ppm including a higher incidence of females exhibiting cycles that were variable, indeterminate and < 4 or > 5 days in length (500 ppm), prolonged or persistent estrus (250 and 500 ppm) and persistent diestrus (500 ppm). Serum hormone levels appeared to be unaffected. In conclusion, DACT caused changes in the estrous cycle at dietary levels ≥ 100 ppm.
ACUTE AND SUBCHRONIC INHALATION TOXICITY OF PHOSPHINE, P E Newton, Bio/Dynamics, Inc., East Millstone, NJ; J B Sullivan, DEGESCH AMERICA, Meyers Cave, VA; and W M Busey, D A Banas, EPL, Herndon, VA.

Phosphine is lethal in rats at a cumulative concentration-time product of about 160 ppm·hours. For daily 6-hour exposures, the median lethal times were 3 days at 10 ppm and 4 days at 7.5 ppm. Females were more sensitive than males. Repeated exposures to 5 ppm were not lethal. Decreased erythrocytes, lung congestion and increased kidney weights with coagulative necrosis of the tubular epithelium of the outer cortex were seen in the 10 ppm rats only. The effects were more severe in the females than in the males. Exposures to 0.4, 1 and 3 ppm for 13 weeks followed by a 4-week recovery period produced a dose related decrease in body weight gain at 1 ppm and 3 ppm and decreased food consumption in all groups including a transient decrease in the 0.4 ppm group. Decreased erythrocytes, hemoglobin and hematocrit were seen in the 3 ppm group after 13 weeks of exposure. The females were again more sensitive. All effects seen in the subchronic study were completely reversible either during the 13 weeks of exposure or the 4-week recovery period.

IMMUNE SUPPRESSION IN FISCHER 344 RATS BY ORAL EXPOSURE TO GLYCOL ETHERS. R Sniawskiz, M Riddle, W Williams, C Copeland, D Andrews, and R Loseke. U.S. EPA, Research Triangle Park, NC.

Oral dosing of adult male F344 rats with the glycol ether 2-methoxyethanol (ME) or its' principal metabolite 2-methoxyacetic acid (MAA) results in the suppression of the primary plaque-forming cell (PFC) response to trinitrophenyl lipopolysaccharide (TNP-LPS). In the present study, the PFC response to TNP-LPS was used to evaluate the immuno-toxic potential of ethylene glycol (EG) as well as the glycol ethers 2-ethoxyethanol (EE), 2-ethoxyethyl acetate (EEA), 2-butoxyethanol (BE), and 2-methoxyethyl acetate (MEA) relative to ME and MAA. Rats were immunized with TNP-LPS and then exposed 2 and 26 hours later to 50, 100, 200 or 400 mg/kg of each glycol ether or EG. Three days following immunization the PFC response to TNP-LPS was determined. In addition to ME and MAA, only MEA, which was as effective as ME, suppressed the PFC response to TNP-LPS. Concomitant administration of the alcohol dehydrogenase inhibitor 4-methylpyrazole with ME or MEA prevented suppression of the PFC response by these glycol ethers. These results indicate that the chemicals tested only ME, MEA and MAA are immunosuppressive, and that oxidative metabolism via alcohol dehydrogenase is necessary for ME and MEA suppression of the response to TNP-LPS. (This abstract does not necessarily reflect EPA policy.)

BENZENE (BZ)-INDUCED PHAGOCYTE ACTIVATION ALTERS MOUSE BONE MARROW (BM) PROGENITOR CELL DEVELOPMENT. L MacEachern, R Snyder, and D Laskin. Joint Grad. Prog. in Toxicology, Rutgers Univ., Piscataway, NJ.

Treatment of mice with BZ results in morphologic and functional activation of BM phagocytes. In the present studies we analyzed the effects of BZ-induced BM phagocyte activation on the development of BM progenitor cells. Male Balb/c mice were injected i.p. with BZ (660 mg/kg) or control once per day for 3 days. BM cells were harvested from the lower leg bones of the mice and cultured with and without GM-CSF. Cell growth was quantified by [3H]-UDP incorporation. We found that BM cell growth was significantly (p<0.02) reduced following BZ treatment of mice. Incubation of BM cells with endotoxin, a known phagocyte activator, further decreased (p<0.02) growth of the BM cells. Cells from BZ treated mice were more sensitive to the effects of endotoxin than were cells from control mice. The addition of GM-CSF to the BM cultures completely abrogated the growth inhibition observed in cells from BZ treated mice suggesting that one mechanism of BZ-induced toxicity is decreased GM-CSF production in the bone marrow. These results support our hypothesis that benzene treatment induces activation of BM phagocytes which modulate toxicity. Supported by AMOCO Corp.
THE EFFECT OF ACETOXY-DIMETHYLNITROSAMINE (ACDMN), ACROLEIN (ACR) and 2,3,7,8-tetrachloro-p-dioxin (TCDD) ON MURINE AND HUMAN LYMPHOCYTES
Steven C Wood, James G Karras, and M. P. Holisapie. MCV/VCU, RICHMOND, VA.

The focus of these studies was to determine if human lymphocytes could be used as an immunotoxicological model. Human lymphocytes are very difficult to immunize in vitro, but they can be driven by pokeweed mitogen (PWM) which is a macrophage and T cell dependent B cell mitogen. The approach taken in these investigations was to examine the effect of these xenobiotics upon the anti-SRBC response, PWM induced proliferation and the IgM response in murine splenocytes (SPLC). These experiments were then repeated with human tonsilar lymphocytes (HTL) where the end points were PWM induced proliferation and Ig(M+A+G) secretion. ACDMN inhibited the anti-SRBC response, PWM induced proliferation and IgM responses as measured by the reverse plaque assay in SPLC between 0.1 to 100 uM. In HTL, ACDMN inhibited proliferation and Ig(M+A+G) response over a comparable concentration. ACR inhibited the anti-SRBC response between 10 and 100 uM in SPLC as well as the proliferative and IgM and Ig(M+A+G) responses of SPLC and HTL. TCDD inhibited the anti-SRBC response from 0.3 and 100 nM in SPLC. However, TCDD had no effect on PWM-induced proliferation or IgM or Ig(M+A+G) responses in either murine SPLC or HTL. These studies suggest that human lymphocytes can be utilized for evaluating the immunotoxic potential of certain xenobiotics. This work was supported by NIH training grant ES03520 and NIH Grant ES07087.

IN VITRO EVALUATION OF CYTOKINES AS POTENTIAL MARKERS OF DRUG-INDUCED TOXIC EFFECTS ON THE IMMUNE SYSTEM.
C. Blot 1,2, H. Lebre 1,2, G. R. Burleson 3, C. Bohou 1,2, and M. Pallardy 1,2, 1 Laboratoire de Toxicologie, Faculté de Pharmacie Paris XI, Chatenay-Malabry, France. 2 Département de Biologie Clinique, Institut G. Roussy, Villejuif, France. 3 Health Effects Research Laboratory, USEPA, Research Triangle Park, U.S.A.

Cytokines are believed to represent the biologic response modifiers that dictate the course of an immunologic response. The purpose of this work is to evaluate the usefulness of several cytokines as potential markers in immunotoxicology using an in vitro lymphocyte activation model. Some types of drugs were utilized: drugs without any known effects on the immune system (furosemide, indomethacin, immunosuppressive drugs: hydrocortisone, dexamethasone, cicleson, azathioprin) and drugs known to induce immunopathological effects (hydralazine, procainamide, amoxicillin). Interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6) and interferon-gamma (IFN-γ) production were measured in splenocytes from conscious A. (ConA) activated lymphocytes exposed for 24 hr to different drugs. For the drugs without any known effects on the immune system, furosemide significantly augmented IL-2 production (+25% at 10^{-5} M) and indomethacin significantly inhibited IL-6 production (-57% at 10^{-6} M). Cicleson significantly decreased IL-2 and IFN-γ production. Azathioprin did not modify the secretion of any of the cytokines assessed. IL-1, IL-2, IL-6 and IFN-γ synthesis were profoundly affected by dexamethasone and hydrocortisone. Among the drugs known to induce immunopathological disorders, only amoxicillin had an effect in these tests (augmentation of IL-2 production). In conclusion, this approach is of utility to try to select a molecular marker of drug-induced immunotoxic injury. (This abstract does not necessarily reflect EPA policy)


Altered immune functions result from subchronic dimethyltrimethoxamine (DMN) exposure in vivo. These studies were conducted to determine whether serum borne inflammatory cytokines and/or the expression of acute reactive proteins by hepatocytes could be used to predict subsequent changes in macrophage and T cell functions. Serum samples obtained from individual mice exposed to either vehicle (PBS) or DMN (1.3; 3.0; and 5.0 mg/kg) every 4 hr were analyzed for albumin, immunoglobulin (lg), complement products (C3 and C3b), IL-1β, IL-3, IL-6, CSF-1, GM-CSF and TNFα at either 0, 1, 2, 3, 6, 12, 24, 48, 72, 120 and 192 hr dosing times. Livers were obtained and RNA was isolated for Northern blot analyses. No differences were observed in CSF-1, IL-3 nor Ig serum concentrations between vehicle and DMN exposed groups. Acute differences (by day 3 of treatment) were seen for IL-1β, TNFα, IL-6, C3 and albumin serum levels. Differences in IL-1β (100 U/ml), IL-6 (1.3 U/ml) and TNFα (150 pg/ml) were observed within 2 hr of exposure and returned to control levels by day 3 even though DMN exposure was maintained. Chronic differences (occurring after day 3) were observed for GM-CSF levels (>100 CFU/ml). Time and dose of cytokine activity following DMN exposure varied between animals. However, acute and chronic patterns did not vary. Northern blot analyses of total cellular liver RNA was not adequately sensitive to detect differences in cytokine transcripts. GM-CSF transcripts, however, were detected using PCR amplification. Current experiments are utilizing quantitative PCR techniques to detect cytokine gene expression (Supported by ES04348).

REVERSAL OF ANTIBODY-FORMING-CELL RESPONSE SUPPRESSED BY 2',3'-DIDEOXYADENOSINE (DDA). W Cao, Y C Han, and A. E. Munson. Department of Pharmacology and Toxicology, Medical College of Virginia/VCU, Richmond, VA.

DDA is a nucleoside analogue with inhibitory effects on HIV viruses. Previous studies showed that exposure to DDA in B/C3F1 female mice induced a suppression of antibody-forming-cell (AFC) response of splenocytes against sheep red blood cells (SRBCs), which was selectively inhibitory to B lymphocytes. The purpose of this study was to further define the subcellular site(s) of action of DDA on antibody production. DDA (350 mg/kg) or 0.5% methylcellulose as vehicle (VM) were administered to mice daily for 15 days by the oral route. Splenocytes from VM- or DDA-treated mice (VM-cells or DDA-cells) were cultured for 5 days. Supernatants (SN) from VM- or DDA-cell cultures incubated for 24 hr were exchanged. Day 5 IgM plaque-forming-cell (PFC) assays showed that neither 24 nor 72 hr DDA-cell SN affected the AFC response of VM-cell cultures; 24 hr VM-cell SN did not reverse the suppressed AFC response when added into DDA-cell cultures at 24 hr; 3 hr 72 hr VM-cell SN reversed the suppressed AFC response of DDA-cells when added into DDA-cell cultures after 72 hr. Interleukin 2, 4, or 6, or the extracts prepared from the SNs of PHA-conditioned splenocyte culture (PHA-SN) did not reverse the PFC response DDA cultures. Factors from LPS-SN reverse the suppressed AFC response of DDA-cells when added at 24 hr of culture, even in the presence of LPS antagonist, polymyxin B. Since T cells and macrophages have been shown to be intact in DDA-treated mice, the SN from VM cultures and the extracts from LPS-SN has undefined factor(s) in normal AFC response which may be deficient in the spleen cells from DDA-treated mice. (Supported by ES 55094).
Previous studies have indicated that gallium arsenide (GaAs) suppresses the in vivo-generated primary antibody response to sheep red cells (SRBC). Data from atomic absorption (AA) analysis performed on tissues of mice exposed to a single intratracheal dose of 200 mg/kg GaAs revealed As concentrations in the spleen peak at 24 hours at 1294 ng/mg. Ga concentrations in the spleen time-dependently increased to 11.5 mg/ml at 14 days. To determine whether the action of GaAs on the IgM AFC response was direct or indirect, splenocyte cultures were treated with GaAs (1-100 μM). Between 6 and 50 μM, GaAs suppressed the AFC response dose-dependently with no significant cytotoxicity. Time of addition studies showed GaAs (50 μM) must be added within the first 36 hours following immunization to be suppressive. Indicating GaAs acts early in the response. Studies conducted to evaluate which component of GaAs, Ga or As, was responsible for the immunosuppression showed a primary role for As as the immunosuppressive metal. Meso-2,3-dimercaptosuccinic acid (DMASA: 1-100 μM) was found to block the in vitro suppression of the AFC response in a dose-dependently when given at the time of immunization with antigen. Additional studies in which DMASA was added to GaAs-treated cultures at various times after immunization, showed complete reversal of GaAs-induced suppression at all time points except 96 hours. Supernatants from GaAs-treated cultures were removed at 24, 36, and 48 hours and replaced with conditioned media from VH cultures at the same time points. The conditioned media reverses GaAs suppression 80%, 40%, and 0% at 24, 36, and 48 hours, respectively. These data support the hypothesis that the As component exerts a suppressive effect early in the in vitro AFC response and the result may be the loss of production of, or, alternatively, production of an abnormal protein required for the response. Supported by EIS55904 and NIEHS 00787.

2,3,7-tetrachlorodibenzo-p-dioxin (TCDD) is a thymotoxic compound that induces atrophy of the cortex at dosages at which there is no general toxicity. The mechanism of this induction is a controversial issue: a direct action on lymphocytes and the epithelial microenvironment has been claimed. We addressed this question by electron microscopy of the thymus 4 or 10 days after instillation of rats with TCDD (50 or 150 mg/kg body weight). For comparison, the thymus after instillation with bis(tri-n-butyltin) oxide (TBTO, 30 or 90 mg/kg) was studied. TBTO is known for its direct action on thymocytes. After high dose TCDD treatment, the thymus cortex shows epithelial cell clusters. There is a relative increase in dark epithelial cells judged by their nuclear and cytoplasmic electron density. This increase reflects a differentiation from electron-lucent pale cells into a more degenerative state. This shift in epithelial subtypes was not observed after low dose TCDD treatment and not after TBTO treatment. Increased vacuolization of epithelial subtypes was observed after both TCDD and TBTO treatment at high dose. There was no necrosis or apoptosis of epithelial cells after treatment with these compounds.

Previously we showed that topical application of polyaromatic hydrocarbons, such as benzo(a)pyrene and DMBA, to the skin of C57/HeN mice resulted in the development of a cell-mediated contact hypersensitivity (CHS) response. This study evaluated the genetic basis for this phenomenon in contrast to Ah receptor positive C57/HeN and A/J strains which exhibited a significant CHS response. Ah receptor negative mice (DBA/2, AKR/J and SJL/J) failed to do so indicating that Ah receptor binding was necessary for induction of CHS. In order to evaluate the role of the murine major histocompatibility complex (MHC) in this type of reaction, Ah receptor positive, MHC congenic mice were treated with DMBA and compared for their capacity to mount a CHS response. DMBA induced CHS could be generated in H-2b (C3H, B10.BR) and H-2d (A/J, B10.A) strains but not in H-2a (C57 B1/6) or H-2c (B10.A, C3H.SW) strains. Ah receptor positive BALB/C (H-2d) and C57 B1/6 (H-2b) also failed to develop a DMBA induced CHS response. DMBA CHS was also compared in C3H/HeN and C3H/HeJ mice, whose only known genetic difference is at the LPS locus. The magnitude of the CHS response in C3H/HeJ mice (LPS non-responsive) was only 50% of that in C3H/HeN mice (LPS responsive). These results indicate that the magnitude of the cell mediated immune response to topically applied DMBA is governed by Ah receptor, MHC, and LPS genetic loci.
INHIBITION OF MURINE SPLENIC B LYMPHOCYTE ACTIVATION FOLLOWING ORAL EXPOSURE TO 7,12-DIMETHYLBENZ(A)ANTHRACENE (DMBA). D.P. Davis, S.W. Burchiel, R.M. Montano, and L.C. Seamer*. The University of New Mexico Toxicology Program, College of Pharmacy, and the University of New Mexico Medical Center Flow Cytometry Core Lab, Albuquerque, NM 87131

Previous results from this laboratory have demonstrated that oral exposure of B6C3F1 mice to DMBA inhibited mitogen-stimulated lymphocyte activation in cells recovered from several lymphoid organs. These studies showed that both LPS and PHA-stimulated lymphocyte proliferation and PHA-induced Ca²⁺ mobilization were significantly inhibited by DMBA exposure, supporting the hypothesis that DMBA inhibits early events associated with lymphocyte activation. The purpose of the current studies was to test this hypothesis directly for B cell activation. B6C3F1 mice were treated with 0, 1.0, or 10 mg/kg/day doses of DMBA for 14 days (total cumulative doses of 0, 14, or 140 mg/kg). B lymphocyte populations were then selected on the flow cytometer by direct positive staining of spleen cells with phycoerythrin-labeled anti-Ly5 (B lymphocyte marker) antibodies. Ca²⁺ mobilization studies were performed using affinity-purified goat anti-mouse IgG antibodies as the stimulant and Indo-1 as the intracellular Ca²⁺ indicator. Cell proliferation studies were also performed using 3H-thymidine and insoluble anti-IgD antibodies. Anti-IgD-stimulated Ca²⁺ mobilization was significantly reduced at the 140 mg/kg dose of DMBA. A statistically significant decrease in anti-IgD stimulated B lymphocyte proliferation at the 14 mg/kg and 140 mg/kg doses of DMBA was found. These results suggest that B lymphocytes may be important targets for DMBA-mediated immunosuppression.


High-level lead exposure can have serious effects on the intellectual and behavioral development of young children. There has been much controversy in the last decade concerning the possible impact of low-level lead exposure upon the neurobehavioral and psychomotor development of children. Five longitudinal studies (Boston, Cincinnati, Cleveland, Port Pirie and Sydney) examining lead effects on child development were initiated in the early 1980s. These studies share multiple design features and include data on blood lead and neurobehavioral measurements from birth, six months, or annual intervals to seven years. All the studies use multivariate analysis to take into account possible confounding covariates with outcome measures. The studies tend to have varying results based on the covariates used and type of subject population. An analysis of the results of the five studies with regard to effects associated with prenatal and postnatal lead exposure and pregnancy outcome has been carried out and reveals inconsistencies in the onset, stability, and nature of neurobehavioral effects correlated with different indices of lead exposure. Combined with considerations of study sensitivity, this analysis suggests that data on the possible effects of low-level lead exposure are not concordant with the health effects being cited as justification for impending public health initiatives.
Artillerymen are exposed by inhalation to lead aerosols during weapons firing. To evaluate effects of career duration, measurements on artillerymen (A) and military intelligence (MI) controls with 5 to 15 years of service were made for blood lead (PbB), bone lead, free erythrocyte protoporphyrin (FEP), conduction velocity of 3 motor and 3 sensory nerves (NCV), and blood pressure (BP). PbB, NCV, and BP values did not differ between artillerymen (n = 22) and MI controls (n = 19) (mean ± SD): PbB (ug/dL) for A was 3.9 ± 1.0 and for MI was 4.2 ± 2.7; mean NCV values (m/sec) for A/M were 57.9/58.6, 60.7/61.3, 49.6/49.8, 59.1/59.2, 60.8/60.9, and 38.4/36.3 for the median motor, ulnar motor, peroneal motor, median sensory, ulnar sensory, and sural sensory nerves, respectively; BP (mm Hg) for A was 124 ± 7 (systolic) and 77 ± 8 (diastolic), and for MI was 125 ± 7 (systolic), and 79 ± 7 (diastolic). Spectra from bone lead measurements obtained by x-ray fluorescence are being analyzed. All results will be evaluated for the effects of increasing career duration to determine whether differences exist between the long-term artillery vs. military intelligence populations. Work supported by the U.S. Army under an Interagency agreement with Haslo, U.S. Army Med. R&D Command, Project Order 68P6821.

Bone is the major reservoir of the body burden of Pb; and we have shown that measurements of bone Pb content by LXRFL in Pb-toxic children are predictive of CaNa3EDTA (EDTA) test outcomes, the current standard that determines the need for chelation therapy in mild to moderate Pb poisoning. A low energy x-ray generator with a silver anode was used to measure Pb LαXRF from tibial cortical bone of 65 untreated Pb-toxic children. Using partially polarized photons directed at the mid-tibia, the reproducibility of the LXRFL technique was ±2 ppm (95% confidence limits). 28 African-American and 37 Hispanic children, previously untreated, 1-7 years old, with blood Pb and EP levels of 25-55 ug/dl and >35 ug/dl, respectively, had bone Pb measurements carried out during the week prior to the EDTA test. Results: Mean LXRFL counts were 228±154 and 137±84 for the African-American and white children, respectively, (t = 2.83, p<0.007-corrected for unequal variance). Despite higher bone Pb content in African-American children, the following variables were not statistically different between the groups: age, sex, standardized height, blood Pb, EP, EDTA test outcomes, ferritin or hematocrit. In summary, African-American untreated Pb-toxic have higher average bone Pb values compared to their white peers. Because adult African-Americans evidence increased bone density and turnover rates, larger skeletal Pb stores may contribute, in part, to the higher blood Pb levels observed in this population.

Lead affects fertility in both males and females at relatively high dose. At lower doses, concerns have focussed on neurodevelopmental effects of lead mediated through maternal exposure. We exposed male and female Sprague-Dawley rats to lead (25 or 250 ppm PbAcetate in drinking water) for 5 weeks prior to mating, through mating and gestation. This exposure did not affect growth of adults. Blood lead levels were as follows: Controls - 1-4 mg/dl; 25 ppm - 17-23 mg/dl; 250 ppm - 29-60 mg/dl. Results are as follows: (male:female, n) C:C, 4-100% fertility; 25C, 6-75% fertility; 250C, 8-12.5% fertility; C25, 6-100% fertility; C250, 6-50% fertility; 25:25, 8-83.3% fertility; 25:250, 8-66.7% fertility; 250:25, 6-25% fertility; 250:250, 8-12.5% fertility. There is a clear male-mediated effect upon fertility. All males appeared capable of mating. Lead may have caused azospermia or some sperm-mediated defect may have interfered with fertilization, implantation, or intrauterine development. In addition, offspring born to treated males and control females, there is evidence of reduced neuronic development of hippocampal pyramidal cells. These results may support the identification of lead as a male reproductice toxicant at low dose. Research supported by the Agency for Toxic Substances and Disease Registry, USPHS.

To evaluate the potential reproductive effects of lead in developing animals, offspring of female Sprague Dawley rats administered 1% lead acetate during lactation, were serially sacrificed on postnatal days 9, 12, 15, 18, and 21. Uterine and ovarian weights, plasma estrogen and progesterone concentrations, uterine estrogen cytosolic and nuclear receptor levels and affinity were compared to age-matched controls. Age-related increases in uterine and ovarian weight and plasma estrogen and progesterone concentrations were demonstrated, but, were unchanged by lead treatment. Cytosolic and total estrogen receptor levels were significantly increased on postnatal day 18 and 21 in lead treated offspring. Additionally, there was a consistent elevation in cytosolic receptor Kd. Nuclear receptor levels and Kd were unchanged. These data indicate that postnatal lead exposure is associated with an alteration in the number and affinity of the estrogen receptor in the postnatal rat uterus.

LEAD EXPOSURE AND EARLY ENDOCHONDRAL BONE GROWTH. J D Hamilton and E J O'Flaherty. UNIVERSITY OF CINCINNATI, CINCINNATI, OH.

Lead exposure adversely affects skeletal development in young children and in experimental animals. This project was designed to investigate the effects of lead in drinking water on skeletal development in the rat. Increased epiphyseal growth plate widths and altered endochondral bone growth of the proximal tibiae were observed in weanlings which were exposed to lead both prenatally and postnatally. An ectopic endochondral bone induction system showed that (i) lead applied locally to the induction matrix will alter endochondral mineralization by co-mineralizing with calcium, independently of ectopic bone cellular activity and (ii) lead will inhibit chondrogenesis as demonstrated by lead-related decreases in plaque alkaline phosphatase and glycosaminoglycans. The findings are consistent with the hypothesis that lead alters cartilage matrix development, thereby disrupting endochondral bone growth primarily through its interference with chondrogenic, not osteogenic, activity in growing bone.

LEAD PERTURBS EPIDERMAL GROWTH FACTOR (EGF) MODULATION OF INTRACELLULAR CALCIUM METABOLISM IN CLONAL RAT OSTEOSTEOBLASTIC (ROS 17/2.8) CELLS. G J Long and J F Rosen. Albert Einstein College of Medicine, Bronx, NY.

EGF, a single chain polypeptide growth factor important for many cellular functions including glycosylation and protein phosphorylation, is known to modulate calcium metabolism in several cell systems. It has been shown that EGF causes an increase in intracellular calcium influx and accumulation of inositol triphosphate, and probably exhibits many, if not all, of its effects via the calcium messenger system. Lead is known to interact with and perturb normal calcium signalling pathways; hence, the purpose of this work was to determine if lead perturbs EGF modulation of calcium metabolism in ROS 17/2.8 cells and if cell functions controlled by EGF were impaired. Cells were labelled with 45Ca (1.87 mCi Ca) for 20 hr in the presence of 5 µl Pb, 50 ng/ml EGF or 5 µl Pb and 50 ng/ml EGF. Following an EGTA rinse, kinetic parameters were determined from 45Ca efflux curves. Three kinetic compartments described the intracellular metabolism of 45Ca. 5 µg/ml Pb significantly altered the effect of EGF on intracellular calcium metabolism. Calcium distribution was shifted from the fast exchanging, quantitatively small calcium pools S1 and S2 to the slow exchanging, quantitatively large S3. There was also a 50% increase in total cell calcium in cells treated with 5 µl Pb and 50 ng/ml EGF over cells treated with 50 ng/ml EGF alone. There was also a 25% decrease in the half-time for calcium exchange across the plasma membrane. The half-time for calcium exchange from S1 to S2 was also decreased. These data show that Pb impairs the normal modulation of intracellular calcium homeostasis by EGF and may therefore perturb functions that are modulated by EGF via the calcium messenger system. (This work supported by NIH grant ES01000 and Human Developmental Biology grant 9-526-6865).

LEAD-ZINC INTERACTIONS IN THE PRODUCTION OF OSTEOCALCIN BY ROS 17/2.8 OSTEOBLASTIC BONE CELLS. J Pounds. Institute of Clinical Toxicology, Wayne State University. Detroit MI.

The serum level of osteocalcin, a bone specific protein produced by osteoblasts and used clinically as a marker of osteoblast activity, is decreased in lead intoxicated children. Previous studies suggest that the reduced osteocalcin production appears to be the result of impaired transcriptional regulation of this 1,25-dihydroxyvitamin D3 gene product, and not translation. As part of a study to investigate the potential interaction of Pb** with Zn**, and with the zinc fingers of the vitamin D receptor, ROS cells were treated with 0, 5, 10, or 25 µl Pb acetate for 24 hr, in the presence of 10, 30, or 50 µg Zn followed by an additional 24 hr treatment with lead 1,25-dihydroxyvitamin D3 (100 pg/ml media). At the end of this period a radioimmunoassay was conducted to determine the amount of osteocalcin in the cells and secreted in the media. 1,25-dihydroxyvitamin D3 caused an increase in osteocalcin secreted into the media in cultures containing 0 µl lead, but this increase was inhibited by lead in a concentration dependent manner, so that osteocalcin secretion in 10 or 25 µl lead treated groups was less than cultures without 1,25-dihydroxyvitamin D3 treatment. This inhibitory effect of lead was blocked by increasing the medium zinc concentration to 50 µM. Increasing medium Pb** concentrations decreased the amount of **Zn taken up by cells by <3%, which was nullified by increasing the medium Zn. These results suggest that lead produces a localized and specific Zn deficiency in the vitamin D receptor zinc finger, and perhaps other zinc metalloproteins, and that these effects of lead are not mediated through general effects on RNA or protein synthesis. (Supported by NIH grant ES04040)
Lead, an immunomodulator and potential human carcinogen, is a major airborne pollutant in industrial environments which poses a serious threat to human health. Despite the widespread nature of respirable lead particles in the workplace and the potential health risks associated with exposure, little is known about the effects of inhaled particulate lead on the lung. For this study, we examined the effects of inhaled lead oxide (PbO) on pulmonary macrophages functional and biochemical activities important for lung defense. Rabbits exposed to PbO, at 30 μg/m³ for 4 d (3 h/d), were sacrificed and their lungs lavaged immediately (t₀), 24 (t₂₄) and 72 (t₇₂) h after the final exposure. Lactate dehydrogenase (LDH) and total protein, measured in lavage fluid, increased ~150% at t₇₂ and decreased ~30% at t₂₄, respectively. Inhalation of PbO increased neutrophil influx ~4-fold, only at t₂₄, and had no effects on macrophage number or viability. Effects on macrophage activity were as follows. Phagocytic activity was depressed at t₂₄ and even greater at t₇₂. Spontaneous and zymosan-stimulated H₂O₂ production was unaffected at t₀, significantly increased at t₂₄ and spontaneous production still significantly elevated at t₇₂. Superoxide anion radical (O₂⁻) production by resting Mac was depressed ~30% at t₀, at t₂₄, spontaneous production increased ~3-fold and recovered at t₇₂. Zymosan-stimulated O₂⁻ production was altered only after 72 h. LPS-stimulated TNF production was depressed ~75%, immediately following exposure and enhanced ~2-fold, at t₇₂. This study provides the first evidence that repeated inhalation of respirable lead particles, at an occupationally relevant level, alters macrophage functions critical for lung defense against infectious agents and cancer and that effects are time-dependent. Supported by NIEHS Grant No. ES 04627.


Groups of Fisher 344 rats were exposed 4 h/day 3 days/week for 4 weeks to 3 concentrations of an air pollutant mixture and to purified air. High (Hi) concentration was 0.6 ppm O₃, 0.4 ppm NO₂, 0.2 mg/m³ NH₃, 0.12 mg/m³ cocaine particles, and 0.1 mg/m³ NO. Medium (Med) and low (Lo) concentrations were respectively 1/2 and 1/4 of Hi. Rapid-shallow irritant breathing pattern responses were present at first exposure in Med and Hi groups and, with successive exposures, showed diminished response in Med and exacerbated response in Hi groups. At end-exposure, rats exposed to Hi showed lung parenchymal lesions typical of oxidant injury, reduced pulmonary macrophage Fc receptor binding, and increased macrophage acid phosphatase activity. Both Hi and Med groups showed depressed macrophage phagocytic activity, and increased secretory activity in tracheal epithelia. The results indicate that breathing pattern responses to Hi and Med mixtures of pulmonary irritants show a concentration-dependent differential adaptive response to repeated episodic exposure. Persistent and progressive breathing pattern responses to repeated exposures were associated with lung tissue injury and decreased macrophage function. Attenuating or "adaptive" breathing pattern responses occurred in the presence of a smaller compromise of macrophage function. Supported by Calif. Air Resources Board AB33-104.


In a previous comparative inhalation study, Fischer 344 rats were exposed separately to 100 mg/m³ of Titanium Dioxide (TiO₂) and 100 mg/m³ of synthetic and natural graphite (G) dusts for 4 h/day for 4 days resulting in a mild reversible inflammatory response. Even though there were greater bronchioalveolar lavage (BAL) changes in the G exposed rats, all three materials met the criteria of a "nuisance dust" established by the American Conference of Governmental Industrial Hygienists (ACGIH). In this experiment, Fischer 344 rats were exposed to an equal mixture of G and TiO₂ accounting to the same format of the previous study to determine if the combined effects of two "nuisance dusts" is more deleterious than exposure to a single dust. At 24 h, 4 days, and 3 mos post-exposure, exposed and control rats were evaluated for physiological, BAL, and histopathological changes. Initial BAL parameters indicated there were greater changes from exposure to the mixture than from either dust alone.

EFFECTS OF INHALED COAL FLY ASH ON LUNG BIOCHEMISTRY AND FUNCTION IN GUINEA PIGS. TA Kimmel, LC Chan, MM Ryan, I Gordon, and MO Amud. Institute on Environ. Medicine, NYU Medical Center, Tuxedo, NY 10987

The ultrafine fraction of particles produced during the combustion of coal are the most difficult to remove with control devices and are retained longest in the atmosphere. Combustion of a high-sulfur coal, such as Illinois No. 6, produces a significant quantity of sulfuric acid, most of which is absorbed to the surface of those particles smaller than 1 μm in diameter. Particles smaller than 0.05 μm in diameter, moreover, consist largely of sulfuric acid; thus these particles penetrate to the deepest regions of the lung, exposure to coal fly ash can result in the administration of large doses of acid to the alveolar tissues. Using a combustion system that generates coal fly ash similar to that collected in flue gas, guinea pigs were exposed for 2 h to aerosols produced from Illinois No. 6 (mean aerodynamic diameter 0.2 μm) at concentrations of 5 and 20 mg/m³. The animals were lavaged at 24 h post-exposure and levels of lactate dehydrogenase (LDH), glutaromidase (8-GC), and protein were compared to those of control animals. After 24 h, no changes in levels of LDH and 8-GC were seen in the lavage fluid from both high-dose and low-dose animals. Slight, but statistically significant elevations in protein concentration were measured in the high-dose exposure group. The total cell number in the lavage fluid was also found to be unchanged following both exposures. It was previously found that exposure to 5 mg/m³ of Illinois No. 6 fly ash results in immediate reductions in pulmonary diffusing capacity (DLco), total lung capacity (TLC), and vital capacity, and that both DLco and TLC values are not completely restored to normal 96 h post-exposure. These results suggest that the alterations in pulmonary function resulting from exposure to acidic coal fly ash are not accompanied by major inflammatory changes in lavage fluid. Supported by NIEHS Grants P01-ES2429-10.
1NIEHS, RTP, NC. 2Battelle Northwest, Richland, WA.

Gallium arsenide (GaAs) toxicity in F344 rats and B6C3F1 mice was investigated in 14- and 90-day inhalation studies. Exposure concentrations ranging from 1-150 mg/m^3 (14-d) and 0.1-75 mg/m^3 (90-d) were utilized. Mortality and reduction in rate of weight gain were not observed in the 14-d studies. One female mouse (75 mg/m^3) died; male mice (75 mg/m^3) appeared to gain less weight than controls in the 90-d studies. Clinical signs were not observed in either study. Histologically, exposure of rats and mice for 14-days was associated with inflammation, metaplasia, and necrosis of the laryngeal epithelium and with an increased secretion of surfactant, an accumulation of macrophages, and hyperplasia in the pulmonary alveoli. Similar lesions, but with greater severity and higher incidence, were observed in the 90-d studies. Ninety-day exposure to GaAs had significant effects on the reproductive system of male rats and mice as evidenced by the reduction in caudal, epididymal, and testicular weights with accompanying decreases in sperm count and motility. Histologically, the atrophied testicular epithelium was characterized by decreased seminiferous tubular cellularity with loss of spermatids, spermatocytes, and to a lesser extent spermatogonia. Hypospermatia of the epididymis accompanied the testicular atrophy. A dose-related increase in estrual cycle was seen in mice. There was increased hemosiderin in the liver and spleen of mice. Hyperplasia of femoral bone marrow was observed in male rats.

251 THE ACUTE TOXICITY OF INHALED BERYLLIUM METAL IN RATS. P J Haley, G L Finch, M D Hoover and R G Cuddihy. Inhalation Toxicology Research Institute, Albuquerque, NM.

We exposed rats once to beryllium metal by nose only to achieve an initial lung burden of 625 μg and to characterize the acute toxic effects within the lung. Histological changes within the lung and enzyme changes within bronchoalveolar lavage (BAL) fluid were evaluated at 3, 7, 10, 14, 31, 59, 115 and 171 days post exposure (dpe). Beryllium metal-exposed rats developed acute, necrotizing pneumonitis and intra-alveolar fibrosis that peaked at 34 dpe and was replaced by minimal interstitial and intra-alveolar fibrosis by 31 dpe. Necrotizing inflammation was observed again at 59 dpe which progressed to chronic-active inflammation by 115 dpe. This inflammation worsened progressively, becoming severe at 171 dpe. Low numbers of lymphocytes were also present, but they were not associated with granulomas. Neutrophils, but not lymphocytes, were elevated in BAL samples after exposure. Lactate dehydrogenase (LDH), beta-glucuronidase, and protein levels were elevated in BAL fluid from 3 through 14 dpe, but returned to near normal levels by 31 dpe. LDH increased once again at 59 dpe and remained elevated at 171 dpe. Beta-glucuronidase and protein levels were slightly but not significantly elevated from 31 through 171 dpe. Beryllium-induced lung lesions in rats appear to be due to direct chemical toxicity and foreign-body type reactions, whereas chronic beryllium lung disease in man is an immunologically mediated granulomatous lung disease. (Research supported by DOE/OTHER under contract No. DE-AC04-76EV01013.)


The subchronic inhalation toxicity of CdO (the most widely used form of the metal cadmium) was assessed in F344 rats and B6C3F1 mice exposed to 0, 0.025, 0.05, 0.10, 0.25, and 1.0 mg/m^3 cadmium oxide, 6 hr/dy, 5 dy/wk for 13 wk. Body weight gain and terminal body weights were not affected in either rats or mice; however, lung weights and lung to body weight ratios were significantly increased in both species at 0.10 mg/m^3 and above. Lung weights were increased for the lower exposure groups, but did not achieve statistical significance. The concentration of testicular spermatozoa was reduced at 1.0 mg/m^3 in rats and the average estrus cycle was lengthened. Reproductive parameters in mice were not affected. Significant decreases in numbers of total leukocytes and lymphocytes were found in the 1.0 mg/m^3 group in both sexes of rats on study day 24; however, these effects were not apparent at terminal sacrifice. No treatment-related effects on bone marrow cellularity were noted. Respiratory tract lesions were observed in both species and included degeneration of olfactory epithelium; pulmonary inflammation, type II cell hyperplasia, fibrosis; and hyperplasia of bronchial lymph nodes. Mice appeared to be more sensitive to the effects of CdO than rats, and the pulmonary lesions in both species were considered to be the most life-threatening in a long term study. Both species exhibited lesions in the respiratory tract when exposed to the current TLV - 0.05 mg/m^3 CdO. The no-observed effect level (NOEL) for pulmonary changes in rats was 0.025 mg/m^3, but was not achieved in mice.

252 SUBCHRONIC EXPOSURE TO AEROSOLIZED GENERIC CUTTING OIL FORMULATION. V Dalbey, C Kominek, T Roy, K Bodnar, and J Yang. Toxicology Div., Mobil Oil Corp., Princeton, NJ.

A subchronic inhalation study was performed with an aerosol of a generic formulation of noncorrosive straight cutting oil (85X 100° SUS solvent-refined paraffinic neutral oil and 3 additives, including 25% chlorinated wax) to obtain data on long-term exposure for this class of materials. Sprague-Dawley rats were exposed to 0, 0.05, 0.15, or 0.50 mg/L for 6 hr/day, 5 days/week, for 13 weeks. The lowest dose was selected at 10 times the TLV for mineral oil mists. MMAD was ~1.2 μm. At necropsy, 15 rats/group were sacrificed. Serum chemistry and epididymal sperm were not altered by exposure. Hematologically, at 0.50 mg/L the percent neutrophils increased and percent lymphocytes decreased, a shift possibly related to a slight increase in granulopoiesis in sternal bone marrow. Among 15 organs, only lung weight increased in a concentration-related manner. Microscopic concentration-related changes in lung included aggregates of foamy macrophages in alveoli (particularly close to alveolar ducts), neutrophils, thick alveolar walls, and a few lymphocytes; quasistatic pulmonary pressure-volume curves in 10 additional males/group were not affected; pulmonary hydroxyproline was elevated by 40X with 0.50 mg/L. The NOEL, based on cellular changes in lung, was 0.05 mg/L. Analysis of base oil remaining in lung suggested removal mechanisms other than tracheal clearance.
Male Fisher 344 rats were exposed 6 hrs/day, 5 days/week, for 104 weeks by nose-only inhalation to a fiber aerosol of 30 mg/m³ - approx. 250 fibers/cc (asbestos 10 mg/m³ - approx. 5,000 fibers/cc). The fibers tested were RCF1, RCF2, RCF3, RCF4 (after service RCF), and chrysotile asbestos. On the day of sacrifice at 104 weeks, tracheal airflow and airway pressure were measured under ventilation at 90 cycles/min with air/O₂ (40/60% v/v). Pulmonary resistance (Rₚ) and compliance (Cₚ) were computed using a PMS 300 Pulmonary Monitoring System (UMMC LTD, UK). A significant decrease in Cₚ was detected for asbestos (54% decrease), RCF1, 2, 3 (27-31%), and RCF4 (17%), as compared to the air control group. Asbestos produced a 1% increase in Rₚ, and a slight increase (5%) was seen also in RCF1, 2, and 3 treated groups. There was no change in RCF4 compared with the air control. The histopathological evaluation revealed that the decrease in Cₚ and the increase in Rₚ observed in the present study were associated with altered pulmonary structure.

In a previous study, kaolin refractory ceramic fibers (RCF) were shown to induce pulmonary fibrosis and pleural mesotheliomas in Syrian golden hamsters. The present study was initiated to assess the potential for different compositions of RCF to induce lung toxicity and tumors after chronic inhalation exposure of Fisher 344 rats. Rats were exposed in nose-only inhalation chambers, 6 hrs/day, 5 days/week, for 24 months to 30 mg/m³ (approx. 250 f/cc) of four different RCFs (ave. dia. = 1 μm, ave. length = 22 μm), 10 mg/m³ of chrysotile asbestos (positive control; ave. diameter = 0.1μm, ave. length = 3 μm), or to filtered air (negative control). Interim sacrifices were scheduled at 3 or 6 month intervals to monitor the progression of pulmonary changes during the study. The lungs of sacrificed animals were perfused with Karnovsky’s fixative, embedded in paraffin, sectioned, and stained for pathomorphological examination. After 9 months of exposure, the lungs of all RCF groups revealed moderate collagen deposition (fibrosis) and epithelialization, microgranulomas at the bronchiol-alveolar junction, and macrophages in bronchioli and alveoli. Similar changes were observed in positive control lungs. Little progression of these lesions were observed throughout the remainder of the exposure. The final sacrifice will occur in November 1990 and tumor data will be presented at the time of the meeting.

Male and female Fischer 344 rats (80/sex/group) were exposed to CSM Fiber 6 hrs/day, 5 days/week at target exposure levels of 0, 1, 5, or 25 mg/m³ for 24 months. At 3 & 12 months, 10 rats/sex/group were killed. At 18 and 24 months 5 rats/sex/group were killed. In addition, 5 rats/sex/group were removed from exposure at 18 months and maintained for a 6 month recovery period. All surviving animals were sacrificed at 29 months. Clinical lab examinations were performed on 10 animals/sex/group at 3, 12 and 24 months. Body weight and survival did not appear to be affected by treatment. There were no biologically significant effects on clinical parameters. The only compound-related change in organ weights was a dose-related increase in lung weight during the exposure period which was generally reversible during the recovery periods. No increase in tumors (benign or malignant) were observed in this study. Microscopic changes considered reflective of an irritant response were observed in the nasal turbinates especially at the 5 and 25 mg/m³ levels. Histological changes were also observed in the lungs at the 5 and 25 mg/m³ levels. The low exposure level of 1 mg/m³ was considered to be a no-observed-effect-level (NOEL).
THE RESPIRATORY EFFECTS OF n-AMYL AND n-BUTYL ACETATE IN MICE. H. D. Burleigh-Flarley\textsuperscript{a}, D. E. Dode\textsuperscript{b}, J. C. Walk\textsuperscript{b}, R. A. Jennings\textsuperscript{b}, A. T. Mosberg\textsuperscript{c}, and M. W. Ots\textsuperscript{b}

Bushy Run Research Center/Union Carbide Chemicals and Plastics Company Inc., Export, PA\textsuperscript{d} and R. J. Reynolds Tobacco Company, Winston-Salem, NC\textsuperscript{d}.

The respiratory effects, including the sensory irritation potential, of n-amyl acetate (AA) and n-butyl acetate (BA) in male Swiss Webster mice were investigated. Groups of sixteen mice were exposed to four logarithmically spaced concentrations of each acetate. Respiratory rate (l), tidal volume (VT), time of inspiration (TI), and time of expiration (TE) were monitored during a 10-minute preexposure period; a 30-minute exposure period, and a 10-minute recovery period. Sensory irritation was assessed by the decrease in respiratory rate which resulted during exposure. Concentrations of n-amyl acetate and n-butyl acetate which produced a 50 percent decrease in respiratory rate (RD50) were 1438 and 735 ppm, respectively. Little or no change in VT occurred during exposure to either acetate except at the highest n-amyl acetate concentration. The parameter showing the greatest percentage change was TE which increased with increasing exposure concentrations of either acetate. TI also increased with increasing concentrations of n-butyl and n-amyl acetate. Thus, the respiratory effects of both acetates were similar although n-butyl acetate was determined to be a more potent sensory irritant than n-amyl acetate. In conclusion, comparison of the RD50 values for n-butyl and n-amyl acetate and a previously published RD50 value for ethyl acetate indicates the sensory irritation potential of acetates increases with decreasing chain length.

Role of Humidity in the Acute Inhalation Toxicity of Hydrogen Fluoride (HF). G. T. Makova\textsuperscript{a}, D. T. Cline and B. Valentine. Du Pont, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE.

The role of humidity in the acute inhalation toxicity of HF was studied in male CrI:CBA\textsuperscript{a} rats. Groups of 4 rats were exposed nose-only for 1 hour to HF mixed with either low (10X) or high (60X) relative humidity (RH) air. HF was measured using Teflon\textsuperscript{a}-lined, midget impingers containing 0.1 N NaOH and a fluoride selective ion electrode. Following exposure, clinical signs and mortality data were obtained over a 14-day period. In addition, respiratory tract pathology was evaluated in 2 groups of 4 rats exposed to approximately 1800 ppm HF at low and high RH. Measured LC50 values for HF at low and high RH were similar at 2240 and 2340 ppm, respectively. Deaths typically occurred 1-7 days post exposure after prolonged weight losses. Clinical signs were indicative of respiratory tract, ocular and dental injury. Pathologic examination of rats killed 1 day after exposure revealed microscopic lesions only in the nose; no injury was found in the trachea or lungs. The nasal lesions involved extensive necrosis and inflammation of the respiratory epithelium primarily in the anterior nose. No difference in the severity or distribution of tissue injury was noted between the two groups. By 14 days post exposure, evidence of epithelial regeneration and repair was found in all rats. These data indicate that the acute lethality and respiratory tract toxicity of HF is not dependent upon RH.
In order to evaluate the toxicity of UCAFLNK® XL-20 in different solvent systems, Fisher 344 male and females rats were exposed to air, n-butyl acetate (NBA), or UCAFLNK® XL-20 in other NBA, methanol (PMA), or a 50/50 mixture of NBA/PMA. Animals were exposed for 6 hours/day, 5 days/week for 4 weeks to a target UCAFLNK® XL-20 aerosol concentration of 200 mg/m³. Male and females rats in all the UCAFLNK® XL-20 groups had decreased body weights and increased lung and adrenal weights. Female brain weights were also decreased in these groups. Elevated erythrocyte counts, total hemoglobin, and hematocrit values were observed in males and females of all UCAFLNK® XL-20 groups with the females also having a decreased platelet count. In the nasal mucosa of UCAFLNK® XL-20 exposed animals, hyperplasia of goblet cells and squamous metaplasia of epithelium were observed. The lung had indicators (eosinophonic intranuclear inclusions and perivascular infiltration by lymphocytes) of an inflammatory reaction in all animals exposed to UCAFLNK® XL-20. There were no differences observed between the air- and NBA-exposed animals. In conclusion, the toxic effects observed upon exposure to this UCAFLNK® XL-20 aerosol concentration did not differ with these solvent systems.

The subchronic inhalation toxicity of 4-CN was assessed in F344/N rats and B6C3F1 mice exposed to 0, 1.5, 3.0, 6.0, 12, and 24 ppm 4-CN 6 hr/day, 5 dy/wk for 13 wk. No significant differences in body weight or clinical observations were observed in either species. Evaluations of reproductive parameters in male rats at 0, 6.0, and 24 ppm revealed depression of caudal epididymal and testicular weights, epididymal sperm count, and total number of spermatids at 24 ppm. Vaginal cytology studies indicated altered estrous cycle length in all exposed groups of rats examined, and mice at 24 ppm. Spleen and liver weights increased with exposure concentration in both species. Histopathologically, splenic congestion, hemosiderosis and increased hematopoiesis were noted in both species. Hepatic hemosiderosis was observed in rats, while in mice hepatocellular basophilias and hemosiderosis in Kupffer cells were observed at 24 ppm. In rats, proximal tubular degeneration in males, renal hemosiderosis, and hyperplasia of mediastinal lymph nodes and bone marrow in both sexes were also seen. In mice at 24 ppm, forestomach epithelial hyperplasia was observed in females and increased red cell fragments in peripheral blood and bone marrow were seen in both sexes. In rats, increased methemoglobin production was progressive with time and resulted in a high incidence of Heinz body inclusions in erythrocytes. Anemia was also present, considered to be the result of a shortened red cell lifespan. A no-observed-effect level (NOEL) was not achieved for histopathology in rats. The NOEL in mice was 6.0 ppm for splenic lesions and 12 ppm for other organs.
Four groups of 15 Sprague-Dawley rats per sex were exposed to vapors of IPA at mean analytical concentrations of 0, 0.1, 0.5, or 1.35 mg/l in air. There were 20, 6-hr exposures (5 days/week) over a 1-month period. Significant depression in body weights in high level animals occurred throughout the exposure interval. Body weights were decreased in mid level females during the last 2 weeks of exposure. Clinical signs - nasal eczema, sneezing, and ocular opacity - noted mostly in high level animals during the study indicated that IPA was irritating to the eyes and nasal tissue. At the end of exposures, decreases in numbers of lymphocytes were noted in high level males. Changes in serum chemistries and eye lesions (gross) were also present in high level animals. Other changes included microscopic ocular and nasal lesions in mid and high level animals that indicated inflammatory and degenerative changes. A "no-observable-effect level" for IPA vapor in this 1-month inhalation study is considered to be 0.1 mg/l in air.

We have recently reported that xylazine causes pulmonary edema (PE) and pleural effusion in rats (Amouzadeh et al., 1990). This study characterizes some of biochemical and ultrastructural alterations in xylazine-induced PE. Sprague-Dawley rats were given 42 mg/kg xylazine intramuscularly and lungs were lavaged with phosphate-buffered saline 3, 6 and 12 hr later. Total protein, xanthine oxidase (XO) and interleukin-1 (IL-1) were measured in lavage fluid. Protein concentration and xanthine oxidase activity were significantly (p < 0.05) increased in lavage fluid from xylazine-treated rats. IL-1 level was unchanged at 3 and 6 hr, and was significantly (p < 0.05) reduced at 12 hr. Another group of rats were given 42 mg/kg xylazine intramuscularly and lungs were fixed 12 hr later for ultrastructural observations. Endothelial damage such as thinning, detachment from basement membrane and bleb formation were observed. These data indicate that xylazine-induced PE is due to increased permeability resulting from endothelial injury. While oxygen radicals are possibly involved, IL-1 does not play a role in xylazine-induced PE.

Polymorphonuclear leukocytes (PMNs) are an important component of the inflammatory response and may be involved in airway injury after O3 exposure. The influx of PMNs in trachea following 0.8 ppm O3 exposure for 3 h was studied at 4 h intervals up to 24 h post exposure to determine if PMNs are involved in permeability changes in rat airways. Tracheal PMNs were scored in sections according to their location, i.e., vascular or extravascular, and as esterase positive or negative. The data were expressed as the average PMN number per unit area. The combined total of vascular and extravascular PMNs for the control was 11.2. The total PMNs peaked at 18.8 at 12 h post exposure and then declined to 2.3 by 24 h. The PMN vascular to extravascular ratio decreased at 8 h suggesting accelerated movement of PMNs from vasculature to surrounding tissues. The ratio of esterase positive to total PMNs increased slightly at 16 h, coincident with a decrease in total PMNs. Tracheal permeability increased immediately post exposure, peaked at 8 h and declined to control value at 24 h. The increased permeability immediately after O3 exposure, but the delayed movement of PMNs, suggest that PMNs may play a role in permeability changes, but initiation is most likely due to other factors.

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271 PHARMACOKINETICS (PK) AND DISPOSITION OF LITHIUM (Li) IN THE RAT AFTER SINGLE AND MULTIPLE SUBCUTANEOUS (SC) DOSING. S A Qureshi, H S Buttler and I J McGilveray. Bureau of Drug Research, Health Protection Branch, Ottawa, Canada

Studies were done to compare the disposition and PK of the antimanic-depressive drug, Li, following single and multiple (once daily for 8 days) SC administration (2 mmol/kg as LiCl) to rats (6-8 per group). Animals were kept in individual metabolism cages while plasma Li levels and its excretion in urine were monitored up to 48 h (after the last injection in case of multiple doses). Li concentrations were determined by atomic absorption spectrophotometry. A 2 compartment open model fitted the plasma Li profiles. The peak levels (Cmax) were higher in the multiple dose regimen than with single doses (2.8±2.8 vs 2.2±4.4 mEq/L; p<.01). Other PK parameters tended to be greater with multiple dosing than with single doses, but were not significant (AUCp 15.4±5.8 vs 9.5±5.2 mEq-h/L, t1/2 0.78±0.71 vs 0.57±0.19 h, tmax 9.4±5.2 vs 6.8±1.9 h). Urinary excretion of Li was alike in both groups (78 vs 83% ). At 48 h, brain Li concentrations were similar; surprisingly kidney Li levels were greater in single dose animals than in the multiple dose group (.04±0.2 vs .02±0.1 mEq/kg; p<.05). The results show that repeated Li administration alters the kinetics and disposition of this drug in the rat.

270 dBCAMP (DIUTYRYL CYCLIC ADENOSINE MONOPHOSPHATE) AS AN ANTIDOTE FOR THE PULMONARY TOXICITY OF RICIN. l M Rosato and Y Alarie, Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA.

Ricin can be used as an agent of great specificity to induce pneumotoxicity. It has been previously shown (Toxicol. J, 41, 41, 1990) that mice exposed to various concentrations of aerosolized ricin showed a concentration dependent increase in respiratory frequency (f) which is correlated with emaciation potency. The maximum response in f and wet lung weight occurred by 5 days post-exposure. The current studies were undertaken to determine if dBCAMP, a cell permeable analog of CAMP could be used to block these effects. Groups of male, Swiss-Webster mice were exposed to an aerosol of 0.3 mg/m³ ricin (50 kD) in PBS for 10 minutes (particle size<0.85 μm). The animals were then injected with 0.72 mg/kg dBCAMP in saline thirty minutes post-exposure and f and tidal volume (Vt) were monitored. On days 1-5 post-exposure, the animals were re injected with the same dose of dBCAMP and f, VT and body weight were monitored. dBCAMP blocked the increase in f for the entire 5 days post-exposure. However the increase in wet lung weight was not completely prevented from occurring. Higher doses of dBCAMP may be therefore required to block this reaction entirely. dBCAMP therefore has the potential to be used as an antidote for ricin intoxication and may be used to explore the mechanisms of ricin pneumotoxicity. Supported under NIEHS R01-ES02747.

272 COMPARATIVE METABOLISM AND DISPOSITION OF [14C]GLYCICID FOLLOWING P.O. AND I.V. ADMINISTRATION TO MALE FISCHER 344 RATS. A Nomeir, P Markham, N Ferrara, D Silveira, M McComish, B Ghanayem and M Chadwick. Arthur D. Little, Inc., Cambridge, MA, and *NIEHS, Research Triangle Park, NC.

Glycidol has been used primarily as a stabilizer in the manufacturing of vinyl polymers. In the NTP chronic studies, glycidol was carcinogenic to rats and mice. It has been suggested that glycidol is converted in the stomach to trichlorhydrin, which is metabolized to β-chloralactic acid. This study was undertaken to further investigate the significance of this conversion by comparing the disposition and metabolism following i.v. and p.o. administration of glycidol at 37.5 and 75 mg/kg doses. Glycidol was readily absorbed from the GI tract (at least 80%). Glycidol equivalents were excreted in urine (40-48% of the dose, p.o. and i.v.), feces (4-5% i.v., 10-12% p.o.), and as 14CO2 (26-27% i.v., 32% p.o.). At 24 and 72 hr, 9-12% and 7-8% of the dose, respectively (p.o. and i.v.), remained in tissues. The highest concentration of 14C was in blood cells, thyroid, liver and kidney, and the lowest was in fat and skeletal muscle. Over the dose range studied, the extent of oral absorption, pattern and rate of excretion of 14C, relative amounts of urinary metabolites and the concentration of glycidol equivalents in most tissues were similar and linear for both p.o. and i.v. studies. Further work is in progress to investigate the possibility of conversion of glycidol to trichlorhydrin. Supported by NIEHS contract NO1-ES-65138.
273 DISTRIBUTION AND EXCRETION OF ANTHRAQUINONE IN THE MALE FISCHER-344 RAT. S M Winter, M B Steup and J G Sipes. Dept. Pharm/Tox, University of Arizona, Tucson, AZ.

Anthraquinone (AQ) has recently found application in the production of wood pulp for making paper. This has raised concern about potential environmental exposure from discharge of AQ into surface waters and sediment. To obtain data on the disposition of AQ, its excretion and tissue distribution were examined in male F-344 rats following a single oral or intravenous dose. 14C-AQ was administered i.v. at 0.35 mg/kg and p.o. at 0.35, 3.5, 35 and 350 mg/kg. Excretion of the radiolabel in the urine and feces was monitored over a period of 96 hr. The animals were then killed and tissues were sampled and analyzed for radioactivity. Cumulative excretion was similar at all dose levels studied with 26-41% and 52-63% of the dosed radioactivity appearing in the urine and feces respectively. The majority of the radiolabel was excreted within 48 hr of dose administration. Less than 7% of the administered radioactivity remained in the tissues. Highest tissue concentrations of AQ-derived radioactivity were found in the liver, kidney and blood. Within 6 hr of i.v. administration of 14C-AQ at 0.35 mg/kg, approximately 35% of the dosed radioactivity was excreted in the bile. HPLC-radiochromatographic analyses of urine and bile samples revealed little unchanged AQ but several metabolites which are currently being identified. (Supported by NTP ES-85230)


CHOX is used as a captive intermediate in the production of popcaptoplast and Nylon. Although the use of CHOX in a captive process limits the risk of occupational exposure total annual production and use of approximately one billion pounds necessitates an understanding of the fate and toxicity of this compound. The present study was designed to provide greater insight into the fate of CHOX by studying its absorption, tissue distribution, metabolism and elimination in the adult male F344 rat. After a single oral administration of 1, 10 or 50 mg/kg of 14C-CHOX, the oxime was rapidly absorbed, metabolized and cleared 24 hours after treatment. The majority of the dose (60-90%) was excreted in urine in the form of metabolites. Elimination in feces accounted for 5-10% of the dose and low levels (2-3%) were retained in the tissues. In contrast, CHOX was poorly absorbed when applied dermally (30 mg/kg), only 4-5% of the dose was recovered in urine, feces and tissues. The majority of the compound volatilized from the skin surface. However, once absorbed, the oxime was readily distributed and excreted and its metabolism was no different than that observed after oral administration. Pharmacokinetic studies showed that the oxime is rapidly cleared from plasma following i.v. administration with half lives of 6 (alpha phase) and 31 (beta phase) min. Three major urinary metabolites were detected by HPLC.

275 TISSUE DISTRIBUTION OF 1,1,1-TRICHLOROETHANE IN RATS. V Srivatsan, R Manning, S Muradlihara, CE Dallas, XM Chua, JM Gallo and IV Brucker. Departments of Pharmacology and Toxicology, and Pharmaceutics, College of Pharmacy, University of Georgia, Athens, GA.

There is a paucity of data on the tissue distribution of 1,1,1-trichloroethane (TCE), a common industrial solvent and drinking water contaminant. The objective of this study was to characterize the uptake and elimination of TRI in major tissues of the body following intratracheal (i.t.) administration. Male Sprague-Dawley rats (300-400 g) were surgically implanted with a carotid artery cannula 18 hr prior to i.t. injection of 6 mg TRI/kg bw in a 5% Emulphor® emulsion. Serial samples of blood, brain, liver, kidney, lung, heart, skeletal muscle, GI tract and adipose tissue were taken after sacrifice for up to 12 hr post dosing. The tissue samples were homogenized in ice-cold saline/isocetane, and the TRI content in an aliquot of the isocetane measured by gas chromatography headspace analysis. There was very rapid uptake of TRI by all tissues except fat, which achieved its Cmax after 30 min. Cmax and AUC values were significantly higher for well perfused (liver, kidney, and brain) than poorly perfused (muscle, GI tract) tissues. Although the AUC value for the fat was as much as 60-fold greater than AUCs for other tissues, elimination half-life values were comparable (i.e. 237-333 min) for all non-lipidic tissues, other than the heart. These tissue disposition data are being used to establish accurate input parameters (e.g. in vivo tissue-blood partition coefficients and mass transfer coefficients) for development and subsequent evaluation of predictions of tissue disposition by an improved physiologically-based pharmacokinetic model for TRI. (Supported by U.S. EPA CR-816258)


A single oral dose of 40 mg/kg (5.4 µCi/kg) of [14C acetyl]acephate was administered on day 18 of gestation to pregnant Sprague Dawley rats. Eight groups of three rats were killed after 10 min and 0.5, 1, 3, 6, 12, 24, and 48 hr. At the end of the 48 hr experimental period, a total of 22.8% of the dose was exhaled as carbon dioxide, while only 1.25% and 0.60% of the dose were eliminated in the urine and feces respectively. Trace amount (0.03% of the dose) was recovered in expired air as volatile materials. Radioactive acephate was rapidly absorbed and distributed in the tissues, with levels in most tissues reaching a peak concentration within 1 to 3 hr. The highest concentration of radioactivity was present in the maternal stomach followed by the liver. A total of 0.72% of the dose was recovered in the fetus. In another study, a single oral dose of 40 mg/kg [14C acetyl]acephate was administered to the dams right after delivery. Nursing and suckling groups were killed at intervals of 1, 3, 6, 12, 24, 36, and 48 hr after dosing. Generally, the highest concentrations of radioactivity were present in the stomach, small intestine, liver, lung, and brain. A total of 0.96% of the dose was recovered in the sucklings.
A pharmacokinetics and material balance evaluation of 14C-labeled POLYOX® Water Soluble Resin-N-10 was conducted in male and female Fischer 344 rats. The peroral 64 mg/kg dose administered was based on the dose given in prior feeding studies conducted with this polymer. The elimination in excreta was followed for 72 hr post-dosing. The overall recovery of the dose was 93.3% for the males and 103.7% for the females. Greater than 90% of the dose was excreted in the feces within the first 24 hr and another 7-12% was eliminated in the following 24-hr period. Minor amounts of 14C were also recovered in urine (0.2-0.6%), expired 14CO2 (0.1-0.2%), the tissues (0.02-0.25%), and in the carcass (0.5-1.2%). In pharmacokinetic studies no quantifiable radioactivity was found in plasma collected up to 12 hr after gavage dosing, while only minor amounts were detected between 12 and 48 hr post-administration. Plasma radioactivity was not quantifiable at 72 hr. Plasma time-course analysis was considered unjustified for these plasma data. In summary, the nearly quantitative recovery in the feces indicates that POLYOX® WSR-N-10 is probably not absorbed from the GI tract and that, subsequent to oral ingestion, it is most likely eliminated completely as an intact polymer from the gut.

Pentachlorophenol (PCA) is formed by methylation of pentachlorophenol (PCP) by algae and soil bacteria, and on occasion has been reported as a contaminant of foods. The excretion and tissue levels of radiolabel were determined following single oral (25 mg/kg) doses of PCA to Sprague-Dawley rats, New Zealand white rabbits, beagle dogs and Hormel-Hanford miniature pigs. A peak blood level of radioactivity was achieved 6 hr following the dose to rats, and the blood elimination half-life ranged from 8 to 15 hr. An average of 86.6% of the administered dose was excreted by rats in 4 days. Less than 0.1% of the dose of the ring-labeled PCA was recovered as CO2. In rabbits, a peak blood level was achieved 3-4 hr following the dose, and blood elimination half-life averaged 6 hr. An average of 97.3% of the administered dose was excreted by rabbits in 4 days. In beagle dogs, the peak blood level was achieved 2-3 hr following the dose, and the blood elimination half-life range was 36 to 48 hr. In 7-day studies, an average of 94.2% of the dose was excreted. In miniature pigs, the peak blood level occurred 48 to 72 hr following the dose, and the blood level maintained a plateau for several days. The average excretion of radiolabel from pigs in 14 days was only 60.0%. Pig tissues (blood, muscle, fat, liver) still contained an average of 30.9% of the administered radioactivity 14 days after dosing.

A PB-PK model for nicotine tissue and plasma kinetics in the Sprague-Dawley rat. D R Plowchalk and D J deBethizy. Duke University Medical Center, Durham, NC. J Reynolds Tobacco Co., Winston-Salem, NC.

A physiologically-based pharmacokinetic (PB-PK) model for nicotine that we previously developed was validated and used to predict nicotine and cotinine concentrations in plasma, as well as nicotine concentrations in various organs. Tissue:plasma partition coefficients (e.g., brain, 1.4; muscle, 1.2; liver, 5.0; and kidney, 20), cotinine volume of distribution (0.87 L/kg) and cotinine clearance (3.64 L/hr) were determined experimentally, while blood flows, organ volumes and metabolic rate constants were obtained from the literature. Using these physiologic parameters, this model successfully predicted plasma nicotine and cotinine concentrations that were experimentally determined in six male Sprague-Dawley rats which received 0.1 mg/kg of (S)-[5-3H]nicotine intra-arterially. Furthermore, a time-course (15 min - 8 hr) of nicotine tissue concentrations was also modelled in eight organs of 21 rats treated as above. Finally, this model was scaled-up to humans and accurately predicted peak plasma nicotine concentrations after controlled exposure to environmental tobacco smoke. This suggests that our current model is a useful tool for assessing nicotine tissue and plasma concentrations in the rat and allows for direct scale-up to humans.
Numerous examples of specific binding proteins for protein therapeutics have been reported. Binding proteins may have either inhibitory or stimulatory effects, may modulate efficacy at the cellular level and may also affect the pharmacokinetics and metabolism of the protein therapeutic. Furthermore, the relative importance of binding proteins may be species or disease state specific. Binding protein complexes have been shown to alter the pharmacokinetics and metabolism for growth hormone, insulin-like growth factor-I and deoxyribonuclease I and to modulate efficacy for growth hormone, insulin-like growth factor-I, tissue plasminogen activator and tissue factor. Since binding protein complex interactions are usually not covalent, it may be necessary to characterize the complexes using non-denaturing analytical methods. In addition, assays and other analytical techniques may not discriminate between bound and unbound forms of the protein; this may lead to ambiguous results regarding the disposition of the active parent protein. The problems that binding proteins present at the early stages of product development will be discussed and examples described.

Limited data exists on the disposition of xenobiotics in the channel catfish. The present study examines 1-naphthol as a model compound for phase II metabolism and dose dependent kinetics in this species. [C]-Naphthol was administered orally or intravenously at 1, 5 or 25 mg/kg. Plasma levels of naphthol after intravascular dosing were fitted to a three compartment pharmacokinetic model. There was a dose related change in several pharmacokinetic parameters but little evidence of metabolic saturation. Approximately 60% of the oral dose was renally excreted in 24 hr as glucuronide and sulfate forms. The bile also contained the glucuronide and an unidentified polar metabolite, little or no sulfate. The low bioavailability (38%) of naphthol was probably a result of first pass metabolism. The edible tissue had the lowest levels of residual compound while the liver and trunk kidney had the highest. Approximately 1% of the dose remained in the tissues at 24 hr. This study demonstrates that 1-naphthol is readily conjugated and rapidly eliminated in catfish. The dose dependency of kinetic values may have resulted from saturable tissue binding.

Male rats were exposed to methyl parathion (methyl) via oral (10 mg/kg) or i.v. (12 mg/kg) routes to determine the time course of acetylcholinesterase (AChE) inhibition in central (cerebral cortex, corpus striatum and medulla oblongata) and peripheral (skeletal and smooth muscles) tissues, and esterase inhibition in liver and plasma at 45 and 90 min. Compared with rats treated i.v., those treated orally exhibited more severe signs and higher AChE inhibition centrally (48% vs 76%) and peripherally (30% vs 74%) at 45 min. Plasma esterase inhibition was higher in the i.v.-treated rats. This may, in part, explain the greater severity of signs in gavaged rats because plasma esterase could provide greater protection from mep intoxication in i.v. treated rats. Esterases were inhibited faster in plasma than in liver. Peripheral AChE inhibition was lower and paralleled the reduced poisoning signs with time in both treatment routes. This suggests that peripheral AChE inhibition may better reflect mep poisoning signs than central AChE inhibition.

(Supported by ES04394 and ES00190).

The inhibition and recovery of cerebral cortex and medulla oblongata acetylcholinesterase (AChE) and liver and plasma esterases (AEE) were studied in female rats after i.p. administration of parathion, methyl parathion, and chlorpyrifos at 3, 10, and 70 mg/kg, respectively. Brain AChE reached peak inhibition of 69-72% at 2 hr with methyl parathion while peak inhibition for parathion, 66-69%, and chlorpyrifos, 84-86%, occurred at 24 hr. At 96 hr, AChE activity was only 35-50% inhibited with both methyl parathion and parathion, whereas it was still 65-75% inhibited with chlorpyrifos. Chlorpyrifos resulted in massive inhibition of both liver and plasma AEE (above 96%) through 96 hrs. Plasma AEE activity recovered faster than liver esterase activity with both methyl parathion and parathion. AEE appear to function as protective mechanisms against AChE inhibition; the more rapid inhibition of brain AChE with methyl parathion than parathion and chlorpyrifos in vivo may be the result of the lower in vitro affinity of methyl parathion for AEE. The persistence of esterase inhibition following chlorpyrifos suggests some temporary storage and gradual release of this insecticide. (Supported by ES04394 and ES00190).

Cystamine has been reported to be taken up by the lung slices and metabolized to taurine via hypotaurine through enzymatic processes. The objectives of this study was to determine whether intact isolated, ventilated and perfused rat and rabbit lungs also possess similar characteristics. The lungs were isolated from male New Zealand white rabbits and S-D rats and perfused with 20 μM [14C] cystamine (Sp. Act. 16.4 mCi/mmol) for 60 min and 30 min, respectively. Cystamine and its metabolites in lung as well as in perfusate were separated by TLC and quantitated using scintillation spectrometry. Similar experiments were also conducted with 20 μM taurine to investigate its fate in perfused lungs. Significant pulmonary uptake of cystamine and taurine occurred during perfusion. Cystamine was metabolized to [14C] hypotaurine and [14C] taurine. No further metabolism of taurine was evident in rat or rabbit lungs. Inclusion of 1 mM GSH did not significantly alter the ability of lungs to sequester cystamine, but the metabolism of hypotaurine to taurine was decreased. It was evident that cystamine was metabolized to taurine by perfused lungs in the same way as in lung slices. (Supported by HL-20622.)

286 EFFECT OF MICROSOMAL ENZYME INDUCERS ON UPTAKE OF THYROXINE BY ISOLATED HEPATOCYTES. J O Akpan, J Liu, Y P Liu and C D Klaassen. Univ Kansas Med Ctr, Kansas City, KS

Microsomal enzyme inducers have been shown to decrease plasma thyroxine levels by an extrathyroidal process. One possible mechanism for this phenomenon is an increase in carrier-mediated transport of thyroxine from plasma into liver. To test this proposed mechanism, rats were placed on diets containing phenobarbital (PB; 1200 ppm), 3-methylcholanthrene (3MC; 250 ppm), pregnenolone-16a-carbonitrile (PCN; 1000 ppm), or polychlorinated biphenyls (PCB; 250 ppm) for 14 days. Uptake of [125] I-thyroxine was determined in freshly isolated hepatocytes. Carrier transport of thyroxine was indicated in hepatocytes of control rats as its uptake was decreased by cyanide, ouabain, colchicine and cold temperature (4°C). Both the initial rate of uptake as well as the steady-state concentrations of thyroxine in hepatocytes were increased by PCN. PCB pretreatment tended to decrease the uptake of thyroxine, but had no effect on the steady-state concentration of thyroxine in hepatocytes. PB and 3MC did not have a significant effect either on the hepatic uptake or steady-state concentration of thyroxine. In conclusion, these studies demonstrate that microsomal enzyme inducers differentially affect the carrier-mediated transport of thyroxine into liver. The decrease in plasma thyroxine produced by PCN may in part be due to an increase in carrier-mediated transport of thyroxine into liver. (Supported by NIH Grant ES-03192 and an ILSI Risk Sci. Inst. Grant.)

287 DOSE-DEPENDENT DISPOSITION OF SELENIUM IN FEMALE LONG-TAILED MACAQUES (MACACA FASCICULARIS) ADMINISTERED L-SELENOMETHIONINE. DN Cox, CC Willhite, WN Choy, SA Book, WC Hawkes, ST O'neale, AF Tarantal and MJ Cukerski. ENVIRON Corp, Emeryville CA, CA Dept of Health Services, Berkeley CA, USDA and Letterman Army Inst. of Research, Presidio of San Francisco CA, and Primate Research Center, Davis CA.

The toxicity of L-Selenomethionine (L-SeMet) in relation to its disposition in pregnant and non-pregnant female M. fascicularis was studied. L-SeMet was given orally at 0, 25, 150, 300 or 600 μg Se/kg/day for up to 30 days. Se concentrations in plasma, erythrocyte (RBC), hair, urine and feces showed a dose- and time-dependent increase during the dosing period. Hair Se increased 90-fold in the 300 μg/kg/day group 13 days after treatment ended. Total tissue Se was elevated 3-13 fold in brain, kidney and skeletal muscle of animals given 600 μg/kg-day. RBC glutathione peroxidase activity showed a dose-dependent increase and remained elevated for >40 days after termination of L-SeMet administration. Pharmacokinetic parameters were determined in selected animals administered either single or multiple doses of L-SeMet. For plasma, the area under the Se concentration-time curve (AUC) and the maximum Se concentration (Cmax) increased with dose for both pregnant and non-pregnant animals. No substantial differences were observed in the uptake and distribution of L-SeMet between pregnant and non-pregnant macaques. (Supported in part by California State IMA 85-87088.)

288 THE GASTRICTESTINAL (GI) ABSORPTION OF METHYL CHLOROFORM (MC). M L Gargas, R H Reitz*, J E Murphy, and M E Andersen. Chemical Industry Institute of Toxicology, RTP, NC and *The Dow Chemical Company, Midland, MI.

The effects of vehicle volume and dose on the GI absorption of MC were examined in the female B6C3F1 mouse. Single doses of MC were delivered in Emulphor®, mineral oil, or water. Mice were dosed in groups of 4, placed in a flow-through chamber, and MC concentrations resulting from MC exhalation monitored in the chamber. Elimination data were analyzed with a physiological model that accounted for chamber dynamics and MC disposition in the mouse and assumed first-order absorption. In a corn oil volume of 2 ml/kg, 50, 250, and 500 mg MC/kg uptake was adequately modeled with a dose-independent rate constant (Kα) of 0.72 ± 0.008 hr⁻¹. With a constant dose of 250 mg MC/kg delivered in corn oil volumes of 1, 5, and 12 ml/kg, Ka values were volume dependent, 1.37 ± 0.014, 0.52 ± 0.007, and 0.56 ± 0.009 hr⁻¹, respectively. With 250 mg MC/kg dose in a volume of 2 ml Emulphor® or mineral oil, the Ka values were 1.52 ± 0.019 and 1.26 ± 0.017 hr⁻¹, respectively, and with 13.1 mg MC/kg in 12 ml/kg water Ka was 2.29 ± 0.13 hr⁻¹. Vehicle and dosing volume both affect Ka. The exhaled breath system with physiologic modeling is a convenient, noninvasive method for rapidly assessing these oral uptake rate constants for various volatile organics.
DISPOSITION AND METABOLISM OF $^{14}$C-1,2-BIS-(2,4,6-TRIBROMOPHENOXY)ETHANE (FF-680) IN MALE FISCHER 344 RATS FOLLOWING ADMINISTRATION IN THE DIET. P Markham, M Chadwick, A Mongan and A Nomier. Arthur D. Little, Inc., Cambridge, MA.

FF-680 is used as a flame-retardant in the manufacture of thermoset and thermoplastic materials that require high processing temperature. FF-680 has been nominated for chronic toxicity studies by the NTP. This study was undertaken to determine the absorption, excretion, distribution and accumulation of FF-680 in tissues following administration in the diet at 0.05, 0.5 and 1% for one day, and at 0.05% daily for ten days. At all dose levels, FF-680 equivalents were excreted almost totally in the feces (>99% of the excreted $^{14}$C), with <1% in urine. No $^{14}$CO$_2$ was in the expired air. Only 0.04% of the dose was in the bile following a p.o. dose of 200 mg/kg in corn oil. At 72 hr after one day of dosing, no $^{14}$C was in any tissue analyzed except trace levels in fat, skin and thymus. After dosing for ten days, trace levels of $^{14}$C were in all tissues analyzed. The highest concentration was in fat, kidney and skin; the lowest was in brain and testes. No FF-680 was in urine, whereas all the $^{14}$C in feces was the parent compound. Total recovery was 86-101% of the [14C]FF-680 consumed in the diet. In conclusion, FF-680 was very poorly absorbed from the GI tract, resulting in accumulation of trace amounts in tissues after ten days of administration. Supported by NIEHS contract NO1-ES-65138.

DISPOSITION OF OXYMETHOLONE IN F344 RATS. J M Sanders and H B Matthews. NIEHS, RTP, NC.

The use of oxymetholone (OXM), a synthetic anabolic steroid structurally related to testosterone, has reportedly resulted in incidences of hepatic toxicity, including tumor formation. In order to characterize further the biological fate of this suspected carcinogen, the present study has investigated the disposition of OXM in F344 rats. Concentrations of OXM-derived radioactivity peaked in blood within 4 hr following oral administration of 5 mg $^{14}$C OXM/kg to male rats, indicating rapid absorption from the GI tract. Liver contained 2-4 times the concentration of OXM-derived radioactivity in blood 4-8 hr after gavage. Within 24 hr, 18±1% of the total dose was excreted in urine and 61±1% was excreted in feces. By 72 hr, 7±1% and 80±1% of the total dose had been excreted in urine and feces, respectively. Increasing the dose to 50 mg/kg did not alter the rate or route of $^{14}$C excretion. Fecal elimination of $^{14}$C appeared to be the result of biliary excretion of OXM-derived radioactivity since approximately 35% of an iv dose of 5 mg/kg was excreted in bile over 7 hr. IV administration resulted in a 6-8 fold increase in blood concentrations of OXM-derived radioactivity 24 hr post-dosing, versus rats gavaged with a similar dose. The major portion of $^{14}$C present in blood appeared to be bound to constituents of plasma. Consecutive daily doses of 50 mg/kg administered by gavage resulted in a 5 fold increase in blood concentrations of OXM equivalents/ml within 7 days, with no increase thereafter. Data developed in this study indicate that upon absorption of OXM from the gut, OXM-derived radioactivity, with an estimated biological half-life of 12-24 hr, sequesters in blood and is eliminated primarily in feces.

DERMAL EXPOSURE IN THE PHYSIOLOGICALLY BASED SIMULATION OF TRIMETHYLPROPANE PHOSPHATE (TMPP) KINETICS. J M Garhart, J M Hamel, M R Kinker. NSF Technology Services, Corp., Dayton, OH.

Trimethylpropane phosphate (TMPP) is a neurotoxin having a higher dermal LD$_{50}$ in females than male mice. Pharmacokinetic studies in rats have shown that males have higher blood levels of TMPP than females after dermal exposure to TMPP/pyrolsate. A physiologically based pharmacokinetic (PB-PK) model was developed to describe the kinetics of this compound after dermal dosing. The in vivo tissue partitions of TMPP, the urinary excretion rate and the metabolism of TMPP were determined from studies where animals were dosed subcutaneously with TMPP/saline via osmotic pumps. The values for tissue/blood partitions, excretion and metabolism were then used in a PB-PK model to simulate dermal absorption and blood kinetics of TMPP in male and female F-344 rats after a dermal dose of TMPP (30 or 60 mg TMPP/kg body weight). Simulation of the dermal exposure data indicated that males had twice the permeability rate of females, accounting for the significantly higher blood levels of TMPP. The PB-PK model developed from experimental studies involving subcutaneous exposure provided a means of determining the rate of dermal absorption of TMPP. (Supported by DoD Contract No. F33615-85-C-0532).

COMPARATIVE DERMAL ABSORPTION OF ISOPOPHENOL (10) BY IN VITRO AND IN VIVO METHODS IN THE RAT. M F Hughes, S P Shrivastava, M R Fisher, M R Summer, B C Edwards, J M Goodwin and L L Hall. 1NSI-ES and 2US EPA, RTP, NC.

The purpose of the study was to compare the in vitro and in vivo dermal absorption of 10 in female Fischer 344 rats. The clipped dorsal side was treated with $^{14}$C-10 (4 ug/cm$^2$) and occluded. Urine and feces were collected at timed intervals up to 120 hr. At 24 hr, 53.8±2.0% (mean±SE, N=4) of the dose was excreted in the urine, <1% excreted in the feces. At 120 hr, 65.4±5.6% of the dose was excreted in the urine, 1.3±0.1% in the feces and 6.1±1.8% remained in the skin. A two compartment model was fit to the urinary excretion data. In vitro absorption was determined by both the flow-through and static systems. Clipped dorsal skin was dermatoromed to 350 um and treated with $^{14}$C-10 (4 ug/cm$^2$) and occluded. At 24 hr, 65.3±7.0% (N=9) of the dose had penetrated the skin of the flow-through system and 65.2±4.9% (N=6) in the static system. This data is in good agreement with the 24 hr urine and fecal excretion data. The use of in vitro systems may aid in the development of risk assessment for dermal exposure to chemicals. (This is an abstract of a proposed presentation and does not reflect EPA policy.)

The rate of absorption was measured for neat DEHP through rat skin and human stratum corneum in vitro and for DEHP contained as a plasticizer in PVC film through rat skin in vivo. The in vitro studies estimated the absorption rate of 14C-DEHP over a period of 32 hr using glass diffusion cells and a receptor solution containing the emulsifier Volpo-20®. The absorption rates for rat and human skin were 0.405 and 0.103 μg/cm²/hr, respectively, with a ratio of 3.93. For the in vivo studies, PVC film (15 cm²) harvested with 14C-DEHP was held against the clipped backs of two groups of male rats for 24 hr with elastic bandages. In the first study, urine and feces were collected for 7 days. The absorption rate for DEHP was 0.119 μg/cm²/hr. The second study was terminated after 24 hr and the exposure site was washed to remove residual DEHP. The absorption rate for DEHP from PVC film in this study was 0.061 μg/cm²/hr. Using the data from the second study and the interspecies ratio determined in vitro (3.93), an estimated absorption rate of 0.016 μg/cm²/hr was calculated for man from PVC film containing DEHP. (Studies sponsored by the Chemical Manufacturers Association).

Further evidence that in vitro percutaneous absorption studies can replace in vivo rat studies

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Sponsor: F.A. Lock

Current guidelines for the registration of agrochemicals in the USA often require the generation of dermal absorption data using the rat as a model for man. This is a time consuming study and requires the use of a large number of animals (at least 96 per formulation). Simpler in vitro alternatives have been suggested and shown to be acceptable. We have compared the data obtained from in vivo and in vitro absorption studies conducted in parallel with four pesticides. Measurements of the radioactivity which could be washed from the skin, associated (on/in) skin and absorbed through rat skin were made following application of radiolabelled pesticide in vivo and in vitro for 0.5, 1, 2, 4, 10, and 24 hr. At 4, 10, and 24 hr the in vitro experiments predicted the in vivo absorption within a factor of 2-3 or better. The amounts washed from and associated with skin were in even closer agreement. The in vivo data for the 0.5, 1 and 2 hr time points were very variable making correlation with the in vitro data more difficult. The results show that the in vitro method predicts with sufficient accuracy in vivo absorption. The simpler more cost effective in vitro method should therefore be more fully utilised in risk assessment.


Dissection and whole-body autoradiographic techniques were used to determine the tissue distribution profile of a leukotriene antagonist, 14C-LY170680, following nose-only inhalation exposure in the rat. Rats were exposed to a nebulized 14C-LY170680 aqueous aerosol with an average concentration of 0.23 mg/L for 1 hour. Animals were euthanized with ether at 0, 1, 2, 4, 6, or 24 hours postexposure and radiocarbon concentrations were determined in selected tissues using liquid scintillation spectrometry. Additional animals were processed for whole-body autoradiographic evaluation at 0, 1, or 8 hours postexposure. Liquid scintillation spectrometry and whole-body autoradiography indicated that highest concentrations of radiocarbon were present in stomach and small intestine at all time points. Radiocarbon reached maximum levels in stomach (2259 ng-ng/gq) and small intestine (2339 ng-ng/gq) 2 to 4 hours postexposure, respectively, and declined with time. In contrast, maximum radiocarbon concentrations in the head (146 ng-ng/gq), trachea (408 ng-ng/gq), and lung (534 ng-ng/gq) occurred at 0 hours postexposure and steadily declined with time. Low concentrations of radiocarbon were detected at the liver (<50 ng-ng/gq), kidney (<15 ng-ng/gq), and plasma (<6 ng-ng/gq). All other tissues contained only background levels of radioactivity as demonstrated by whole-body autoradiographic evaluation. Results from this study demonstrated that significant amounts of radiocarbon associated with 14C-LY170680 were deposited in the head and within the lung following inhalation exposure. However, higher levels of radiocarbon present in the stomach and small intestine suggested significant nasal deposition followed by rapid clearance and ingestion of inhaled radioactive material. Distribution of radiocarbon limited to the respiratory and gastrointestinal tracts demonstrated minimal systemic absorption and exposure over the time course of this study.

Castration increases elimination of perfluorooctanoic acid (PFOA) in male rats. J.W. Davis, N. J.P. Vanden Heuvel, B.I. Kuslikis and R.E. Peterson. Environmental Toxicology Center and School of Pharmacy, University of Wisconsin, Madison, WI.

There is a marked sex difference in the whole body elimination of PFOA in rats with females (t1/2<1 day) excreting the perfluorinated acid much more rapidly than males (t1/2~15 days). We were interested in determining if the levels of circulating androgens or estrogens modulate PFOA elimination. That is, if castrated males pretreated with estradiol exhibit a female pattern of PFOA elimination and if ovarioctomized females pretreated with testosterone exhibit the male pattern. Castration of males greatly increased the elimination of [14C]PFOA (9.4 μmol/kg, ip) into both urine and feces, but castration plus estradiol produced no further augmentation. In female rats, neither ovarioectomy nor ovarioectomy plus testosterone affected the elimination of PFOA. We conclude that a factor produced by the testis, most likely androgens, modulate the elimination of PFOA in the male rat. (Supported by NIH grant GM 41131).
URINARY pH EFFECTS ON THE RENAL CLEARANCE OF LIDOCAINE AND PHENYLButAZone IN EXERCISING HORSES. D F Gerken, R A Sams, K McKeever, K Hinchcliff, and S Ashcraft. The Ohio State University, Columbus, OH. Sponsor: V L Carter

Renal clearance of certain acidic and basic drugs are strongly affected by urine pH. The urine of resting horses ranges from pH 7.5 to 8.5 because of their herbivorous diet. However, the effects of exercise on urine pH and renal clearance of drugs in horses has not been studied.

Eight female horses were administered either 60 mg lidocaine subcutaneously or 2.2 mg phenylbutazone kg BW intravenously. Blood and urine samples were collected from each horse confined in a stall at rest or exercised in increasing speed increments to attain a maximum heart rate. Lidocaine concentrations were determined by gas chromatography/mass spectrometry. Phenylbutazone concentrations were determined by high performance liquid chromatography.

Urinary pH decreased to between 5.0 to 7.0 after exercise but returned to pre-exercise values within one hour post exercise. Urinary lidocaine excretion rates increased approximately 1000-fold after exercise. Urinary phenylbutazone excretion rates decreased 20 to 50-fold after exercise when compared with pre-exercise rates or with rates in resting horses. It was concluded that a maximal exercise period in horses caused a significant change in urinary pH resulting in changes in urinary excretion rates of certain drugs.

EFFECTS OF DOSE, SPECIES, AND DOSING VEHICLE ON THE DISPOSITION OF METHACRYLONITRILE (MAN) IN MALE RATS. I M Sanchez AND B I Ghanayem, National Toxicology Program, NIEHS, RTP, NC

MAN is structurally similar to the known carcinogen acrylonitrile (AN), with both nitriles having similar industrial uses. Current studies were designed to investigate the biological rate of 2-14C-MAN in rats. After gavage administration of 115, 11.5 or 1.15 mg MAN/kg in water, F344 male rats were placed in glass metabolism cages and urine, expired air and feces were collected. Rats were sacrificed at various times and the concentration of MAN-derived radioactivity in tissues was determined. MAN was rapidly absorbed from the GI tract and distributed to all major tissues. Sixty percent of the low and medium doses were exhaled as 14CO2 in 72 hr compared to 25% of the highest dose. While 40% of the highest dose was expelled as organic volatiles in 72 hr, only 9-12% of the lower doses were exhaled as such. Urinary excretion accounted for 20-30% of all doses within 72 hr after dosing. Comparison of MAN disposition in Sprague-Dawley (SD) and F344 rats at 115 mg/kg revealed that SD rats excreted a greater % of the dose as 14CO2 and in the urine than did F344 rats. Administration of 115 mg MAN/kg to SD male rats in safflower oil resulted in increased elimination of MAN-derived radioactivity as CO2, volatiles, and in the urine over that observed when administered in water. These results suggest that: 1) saturation of MAN metabolism occurs at high doses; 2) MAN metabolism and disposition differ with the strain of rats studied; 3) MAN disposition may vary with the dosing vehicle used; and 4) MAN metabolism and disposition are apparently different from that reported on AN.

EVALUATION OF A PHYSIOLOGICAL CHAMBER TO MEASURE ELIMINATION OF PENTACHLOROPHENOL BY RAINBOW TROUT (Oncorhynchus mykiss). W C Black, Zoology Department, Oklahoma State University, Stillwater, OK. Sponsor: G E Burrows.

A physiological chamber designed to separate xenobiotic excretion by the skin, gills, urine, and feces was used to study metabolism and elimination of pentachlorophenol (PCP) by adult rainbow trout. Trout were injected with [14C]-PCP via an i. p. cannula. Excretion of PCP and metabolites was monitored over 48h. The gill was the primary elimination route during the first 4h; substantial increases occurred in urinary, skin, and fecal excretion during the remaining 44h. At 48h fecal excretion was the major elimination pathway. Sulfate and glucuronide conjugates of PCP were both detected in bile at 48h; however, only the glucuronide conjugate was detected in urine and feces, suggesting that the sulfate conjugate was reabsorbed, hydrolyzed, and ultimately eliminated as the glucuronide conjugate. This interpretation also explains the delayed appearance of PCP metabolites in the feces. This study demonstrates that the physiological chamber is a useful apparatus for studying the pharmacodynamics of xenobiotic elimination by fish.

CHEMBSORPTION OF AFLATOXIN M1 FROM MILK BY HYDRATED SODIUM CALCIUM ALUMINOSILICATE. J A Ellis, R H Bailey, B A Clement, and T D Phillips Department of Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX.

Hydrated sodium calcium aluminosilicate (HSCAS) has been reported to prevent aflatoxicosis in poultry and swine and to markedly reduce aflatoxin M1 (AFM1) residues in milk following its addition to the diets of dairy cows. The mechanism of action apparently involves aflatoxin sequestration and chemisorption (tight binding) by HSCAS in the gastrointestinal tract of animals, resulting in a major reduction in aflatoxin bioavailability (and toxicity). The objectives of this study were to evaluate the ability of HSCAS to bind AFM1 in water and milk, and to determine various parameters associated with this interaction. AFM1 binding was determined by HPLC analysis of organic extracts from water and AFM1 contaminated milk. An index of chemisorption to HSCAS was determined from AFM1 bound, AFM1 desorbed and initial AFM1. Desorption was assessed by sequential washing of the pellet with methanol, chloroform, and hexane. Significant reductions in AFM1 (from water and naturally contaminated milk samples) were noted as a result of treatment with HSCAS at levels as low as 0.02% (w/w). Proximate analysis of treated milk samples demonstrated that HSCAS did not interfere with total milk solids, lactose and protein. These findings suggest that aluminosilicates (e.g., HSCAS) might be useful in the clarification/remediation of aflatoxin-contaminated milk (Supported by Texas A&T Project 3842, USAID Project 02-5030-2 and TAES H6215).

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MOLECULAR MECHANISM OF AFLATOXIN B, CHEMISORPTION BY HYDRATED SODIUM CALCIUM ALUMINOSILICATE. A B Sarr, B A Clement, and T D Phillips. Department of Veterinary Anatomy and Public Health, Texas A&M University College Station, TX.

Hydrated sodium calcium aluminosilicate (HSCAS) chemisorbs aflatoxin B, (AFB1) and prevents aflatoxicosis and aflatoxin residues in animals. Compounds with one or more of the functional groups in common with AFB1 were reacted with HSCAS in vitro in an attempt to elucidate the mechanism of chemisorption. The β-dicarbonyl system was found to be essential for chemisorption by HSCAS. Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) was used to examine sorbed pellets of AFB1-HSCAS. DRIFTS clearly showed dramatic shifts of the C=O absorptions which were identical to those known to arise from metal-acetylacetonates. These findings indicate that the mechanism of AFB1 chemisorption involves the chelation of the β-dicarbonyl moiety in aflatoxin with incompletely coordinated metal ions present in HSCAS. A chemisorption index (C) was developed allowing direct comparison of various phyllosilicates with HSCAS. Cs were determined by HPLC analysis of extracts of the supernatants and sorbed pellets (extracted with methanol, chloroform, and hexane). Comparison of the Cs of various classes of compounds with spectral data (DRIFTS) supported the proposed mechanism of chemisorption. With knowledge of the mechanism involved, it has been possible to chemically activate various clays, increasing C, from < 0.5 to > 0.9 (Supported by TAES Project H6215).

THE ROLE OF CALCIUM IN ACETAMINOPHEN-INDUCED HEPATOTOXICITY. K Greweal and W J Racz. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada. Sponsor: J Breau.

When consumed in large quantities acetaminophen (A) can produce fatal hepatotoxicity. One hypothesis as to the mechanism of this toxic response postulates that the reactive metabolite of A binds to protein thiolis critical to calcium transport and sequestration in the ER, mitochondrial and plasma membrane. Calcium is then released from these organelles into the cytosol, with the cell unable to extrude it via the plasma membrane. This results in a sustained increase in cytosolic [Ca2+] above physiological levels (0.1-0.2 μM) leading to activation of degradative enzymes with eventual cell death. Isolated hepatocytes were prepared from mice and treated with a toxic dose of A. The cells were washed at 1.5 h and resuspended in fresh media containing either N-acetylcysteine (NAC) or dithiothreitol (DTT). The cytosolic [Ca2+] was determined using fura 2. It was shown that the cytosolic [Ca2+] in NAC-treated cells increased to above 0.2 μM and correlated with loss of cell viability. In contrast, in cells treated with DTT there was no increase in cytosolic [Ca2+] above physiological values and no significant loss of viability as compared to control. Also, DTT in contrast to NAC reverses the protein thiol depletion that occurs with acetaminophen. This supports the hypothesis of a role of these thiol groups critical for calcium maintenance in acetaminophen toxicity.

ACETAMINOPHEN HEPATOTOXICITY DOES NOT INVOLVE A SUSTAINED INCREASE IN CYTOSOLIC FREE CALCIUM. A W Harman, S O Mahar, P C Burcham and B W Madsen. The University of Western Australia, Nedlands, Australia. Sponsor: J L Farber.

It has been suggested that elevated cytosolic free calcium (Ca2+) plays a role in the pathogenesis of cell killing by a number of toxic agents, including acetaminophen (Moore et al, 1985). Many of these studies have relied on using phosphorylase a activation as an indirect measure of an increase in Ca2+. In the present study we examined the effect of a toxic concentration of acetaminophen on Ca2+, in single mouse hepatocytes using the dye fura-2 and video imaging fluorescence microscopy. Ca2+ was calculated from the ratio of emitted fluorescence at 510 nm produced by excitation at 340 and 380 nm using a double-intensified silicon target camera and digital image processing. During a 2 h exposure to 5 mM acetaminophen Ca2+ varied from 195 ± 14 nM at t=0 to 250 ± 22 nM at 2 h and was no different to controls (ANOVA, P>0.05). However, when such cells were exposed to the same concentration of acetaminophen, there was a 2.5-fold increase in phosphorylase a activity within 2 h. It seems that phosphorylase a activation is not a reliable measure of an increase in Ca2+. The data suggest that there is no sustained change in Ca2+ in acetaminophen injury either before or during the time when irreversible toxic events occur in the hepatocyte.

CALCIUM-DEPENDENT INHIBITION OF MITOCHONDRIAL RESPIRATION BY DOXORUBICIN. L E Solem and K B Wallace. Department of Pharmacology, School of Medicine, University of Minnesota, Duluth, MN.

The cumulative cardiomyopathy associated with doxorubicin antineoplastic therapy severely limits the clinical utility of the drug. Among the various cytotoxic effects of the drug, doxorubicin also inhibits mitochondrial respiration and enhances the accumulation of Ca2+ in the mitochondrial matrix. Mitochondrial Ca2+ accumulation is dependent on the electron transport system and competes with oxidative phosphorylation. The objective of this investigation was to determine whether the inhibition of respiration by doxorubicin is influenced by mitochondrial Ca2+ transport mechanisms. Respiration of isolated rat heart mitochondria was measured polarographically using a YSI model 53 oxygen monitor fitted with a Clark type oxygen electrode. Doxorubicin inhibited mitochondrial state 3 respiration and decreased the ADP/O ratio in the presence of Ca2+. Both of these effects were attenuated by inhibiting the mitochondrial Ca2+ uniport with ruthenium red. The data suggest that the effects of doxorubicin on mitochondrial respiration and Ca2+ accumulation may be interdependent. Inhibition of state 3 respiration may be a consequence of the stimulation of the mitochondrial Ca2+ uniport by the drug.

IMPLICATIONS FOR DOXORUBICIN-INDUCED OXIDATIVE STRESS BY MITOCHONDRIAL CALCIUM CYCLING. E Chacon, D Acosta, and R G Ulrich. The University of Texas, Austin, TX. and The Upjohn Company, Kalamazoo, MI.

Previous studies have suggested that doxorubicin (DOX) may produce intracellular reactive oxygen species (ROS) by affecting mitochondrial Ca++-mitoCa transport. Mito-Ca uptake is driven by the membrane potential established through respiration. Concomitant with the energy dependent uptake of Ca++ is its electron neutral release that provides a continuous cycling of the cation across the membrane. This cycling may then act to dissipate the membrane potential. A method has been employed which allows cytotoxic and mitochondrial calcium to be monitored by using digitized fluorescence imaging (DFI). DOX-induced Mito-Ca overload was observed by DFI of fura 2 entrapped in the mitochondria after selective permeabilization of the plasma membrane to release the cytotoxic fura 2. Upon permeabilization of the plasma membrane, Mito-Ca flux was blocked by EGTA and ruthenium red to prevent Ca++ uptake and by diltiazem to block Ca++ release. Elimination of diltiazem from the permeabilizing solution revealed the mitochondria to be low in Ca++, suggesting that DOX was also stimulating Ca++ release. This apparent Ca++ cycling was concomitant with the formation of intracellular ROS, as well as a dissipation in the mitochondrial membrane potential observed by DFI of cytochrome oxidase loaded mito-Ca. These findings suggest that DOX may be inducing a futile cycling of Ca++ across the mitochondrial membrane. (Supported by grants from Procter and Gamble, The Upjohn Co., and Burroughs Wellcome.)

INVOVLEMENT OF CALCIUM IN DITHRANOL-INDUCED CYTOXICITY IN PRIMARY CULTURES OF RAT EPIDERMAL KERATINOCYTES. D Acosta and G C Hsieh. Department of Pharmacology & Toxicology, University of Texas, Austin, TX.

Previous studies have shown that dithranol exerts oxidative injury in cultured keratinocytes and that mitochondria may be the site of cytotoxicity. This study examined the possible role of Ca++ in the mechanism of cell killing by this compound. Epidermal cells prepared from the skin of neonatal rats by a trypsin flotation method and cultured in a serum-free medium containing 0.1 mM Ca++ were exposed to the test compound (10-100 uM). Cytotoxicity was evaluated by cytosolic lactate dehydrogenase leakage and mitochondrial MTT reduction. Increasing the extracellular Ca++ from 0.1 to 1.2 mM resulted in progressive increases in dithranol-induced cell injury. The Ca++-potentiated toxicity was prevented by EGTA (Ca++-chelating agent) or chlorpromazine (Ca++-dependent phospholipase A2 inhibitor). Calcium ionophore A23187 caused a marked increase in dithranol toxicity. Ruthenium red, which blocks mitochondrial Ca++ uptake, decreased toxic injury. Production of superoxide occurred before and concomitant with cell injury. Dithranol also caused rapid stimulation of C-++-dependent phosphorylase a activity and appearance of plasma membrane blebs. Pretreatment of cells with Quin2-AM to buffer cytosolic Ca++ delayed the loss of cell viability. These results suggest that a culture system of rat keratinocytes may be prove useful in evaluating the Ca++-mediated mechanism of toxicity of dermatotoxicants. (Supported by Colgate-Palmolive Post-Doctoral Fellowship and a grant from Johnson & Johnson)

ALTERATION IN MITOCHONDRIAL FUNCTION AS A MECHANISM OF CELL INJURY BY DITHRANOL. G C Hsieh, E Chacon, and D Acosta. Department of Pharmacology & Toxicology, Univ. of Texas, Austin, TX.

Dithranol is a widely-used antipsoriasis drug with tumor-promoting and skin-irritating properties. Impairment of mitochondrial respiration is suggested as the key cellular event leading to cell injury. In this study mitochondrial function was monitored by measuring oxygen consumption, respiratory control ratios (RCR), ADP/O ratios, and oxidative phosphorylation rates in isolated rat liver mitochondria. Dithranol (5-50 uM) caused significant inhibition of state 3 respiration with succinate or glutamate/malate. State 4 oxygen consumption was not affected. The RC ratios of mitochondria, respiring on either succinate or glutamate/malate, exposed to dithranol were depressed by concentrations as low as 5 uM. ADP/O ratios remained constant. Dithranol also reduced succinate or glutamate/malate-supported oxidative phosphorylation rates at 10 and 25 uM, respectively. Uncoupler (CCCP)-stimulated respiration was diminished by dithranol, indicating inhibition of electron transport chain. Cyanide-insensitive oxygen consumption was increased in NADHlinked respiration, suggesting formation of reactive oxygen species. Exposure of cultured rat hepatocytes and skin keratinocytes to dithranol produced time- and dose-related toxic responses. Mitochondrial metabolic function (MTT reduction) was found to be a more sensitive endpoint of cytotoxicity, showing toxic effects at 2 hr; while changes in plasma membrane integrity (lactate dehydrogenase leakage) did not result until 4 hr. These data suggest that mitochondrial damage may be involved in the mechanism of toxicity of dithranol. (Supported by Colgate-Palmolive Post-Doctoral Fellowship and a grant from Johnson & Johnson.)

PRODUCTION OF REACTIVE OXYGEN SPECIES (ROS) BY RAT HEART MITOCHONDRIA: EFFECT OF CALCIUM AND ISCHEMIA. T. Paradaithanah, H de Groot and J.P. Keizer. Div. of Pharmacology & Toxicology, College of Pharmacy, The Univ. of Texas at Austin, Austin, TX and 2Klinische Forschegruppe Lebererschädigung, Inst. Physiologische Chemie I, Heinrich-Heine Universität, Düsseldorf, FRG.

Mitochondria are intimately involved with cellular oxygen utilization, and can produce ROS during state 4 respiration. This organelle can also accumulate calcium in response to various insults. The formation of ROS by normal and ischemic rat heart mitochondria was studied in the presence and absence of extramitochondrial calcium. Ischemic mitochondria were isolated from rat hearts and incubated at 37°C for 15, 30 or 60 min in glucose-free anoxic Krebs-Henseleit buffer. The production of ROS, as determined by luminescence in the presence of 238 uM luminol and 5 units horseradish peroxidase, was increased by 5 uM succinate. This luminescence response was blocked by amytal or rotenone and was not evident during state 3 respiration. Succinate-supported ROS production was unchanged after 15 min ischemia, but was greatly diminished after 30 or 60 min ischemia. However, in the presence of 1 mM calcium and 0.3 uM antimycin A, ROS production was increased to similar levels in both normal and ischemic mitochondria. Mitochondria were also incubated with 15 uM 2,3-dichlorofluorescin diacetate (DCFDA) which can be hydrolyzed then oxidized to the fluorescent compound dichlorofluorescein. When extramitochondrial DCFDA was left in the incubation medium a 100% increase in ROS production (compared to state 1) was evident upon addition of succinate, and a further 40% increase occurred with the addition of 40 uM calcium. In contrast, there was no evidence of intramitochondrial ROS production using mitochondria washed to remove extramitochondrial DCFDA. This may be due to endogenous antioxidants defense systems. There was also no evidence of ROS production in the presence of 1.25 mM malate and 12.4 mM glutamate unless antimycin A and calcium were also present. These data suggest that increases in intracellular calcium during or after ischemic episodes may enhance ROS production by cardiac mitochondria and that NADH as well as succinate-linked substrates can support ROS production. (Supported by NIH grant HL40695 and the Deutsche Forschungsgemeinschaft.)
309 CALCIUM- AND PHOSPHATE-DEPENDENT RELEASE AND UPTAKE OF GLUTATHIONE BY LIVER MITOCHONDRIA. M K Savage, D P Jones* and D J Reed. Environmental Health Sciences Center, Oregon State University, Corvallis, OR and *Department of Biochemistry and Winship Cancer Center, Emory University, Atlanta, GA.

The mechanism of glutathione (GSH) transport was studied in isolated rat liver mitochondria under conditions which induce a permeability transition. This transition, which is inhibited by cyclosporin A (CyA), requires the presence of Ca²⁺ and an inducing agent such as physiological levels (3 mM) of inorganic phosphate (Pi). It is characterized by increased inner membrane permeability to some low molecular weight solutes and large amplitude swelling. Addition of Ca²⁺ and 3 mM Pi to mitochondria resulted in mitochondrial swelling and a significant release of GSH which was quantitatively recovered in the extramitochondrial medium as GSH. Both swelling and efflux of mitochondrial GSH was prevented by CyA. Incubation of mitochondria in the presence of Ca²⁺, Pi and GSH followed by addition of CyA provided a mechanism to lead mitochondria with exogenous GSH that was greater than the rate of uptake by untreated mitochondria. Thus, GSH efflux from mitochondria may occur under toxicological and pathophysiological conditions in which mitochondria are exposed to elevated Ca²⁺ in the presence of physiological concentrations of Pi through a nonspecific pore. Cyclical opening and closing of the pore could also provide a mechanism for uptake of GSH by mitochondria, but it is unclear whether this occurs under physiological conditions.

311 EFFECT OF CYCLOSPORIN A ON HEPTACELLULAR TOXICITY CAUSED BY T-BUTYLHYDROPEROXIDE. L C Davo, K M Broekemeier, D J Read and D R Pfeiffer. Dept. of Biochemistry and Biophysics, Oregon State Univ., Corvallis, OR and Univ. of Minnesota, Hormel Institute, Austin, MN.

In the presence of Ca²⁺ and t-butylhydroperoxide (TB), mitochondria undergo a permeability transition which allows normally impermeant solutes to cross the inner membrane. Cyclosporin A (CyA) is a potent inhibitor of this transition. We investigate the possibility that this mitochondrial permeability transition participates in the mechanism whereby TB causes cell death, isolated rat hepatocytes were pretreated with CyA (0.5 μM) and incubated with TB (0.8 mM) for 4 hr. CyA significantly inhibited LDH leakage induced by TB when the extracellular medium contained 10 μM Ca²⁺ but not when the medium contained 2.5 mM Ca²⁺. In the presence or absence of CyA, lipid peroxidation was 4-5X greater in cells incubated with TB and 2.5 mM Ca²⁺ than in cells treated with TB and 10 mM Ca²⁺. These data suggest that when peroxidation induced by TB is attenuated by high extracellular Ca²⁺, cell death occurs via a CyA-inhibitable mechanism. Further work is underway to determine if this mechanism involves a permeability transition of the mitochondrial inner membrane.

310 GLUTATHIONE DYNAMICS IN ISOLATED TUMOR CELL MITOCHONDRIA. A E Brodie and D J Reed. Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR.

Mitochondria (MT) were isolated from rat ascites hepatoma cells (AS-30D) with digitonin to permeabilize the cells (Moresmith, R.; Fiskum, G. (1984) Anal. Biochem. 137, 360). Treatment of the MT with t-butyl hydroperoxide (tBuOOH, 0.5-5 mM) decreased the total amount of glutathione and the amount of reduced glutathione (GSH) and increased the concentration of oxidized GSH (GSGG). Some GSH was found in the medium, but no GSG. The amount of protein-SG mixed disulfide was low in all cases. When the MT were coupled by the addition of succinate and ADP, some recovery occurred: GSGG decreased and GSH increased in 30 min (1 and 5 mM tBuOOH). As GSSG is not effluxed and GSH is not synthesized in MT, the dynamics of GSH influx and efflux implies a specific pore or permeability. Slightly more calcium, potassium, and cyclosporin A (CyA) were required for tumor MT swelling than found in rat MT (Broekemeier, K.M.; Dempsey, E.; Pfeiffer, D.R. (1989) J. Biol. Chem. 264, 7826). GSH efflux was correlated with induced swelling and was inhibited by CyA. Studies utilizing 35S GSH indicate that GSH uptake is sensitive to the presence of different substrates. (ACS grant CH-109 aided in this research.)


Histologic evidence from cell cultures exposed to cyclosporin A (CsA), including vacuolation and multiple nuclei indicate a possible involvement of the cytoskeleton. CsA (1-5 μM, 30 min) inhibits cytokeratin intermediate filament phosphorylation by 75%. Phosphorylation of these filaments can be rescued by addition of 100 nM 12-O-tetradecanoylphorbol-13-acetate (TPA), but not 50 μΜ forskolin, indicating that CsA alters TPA and CsA mediated phosphorylation in vitro. Phosphorylation impacts cytokeratin filament assembly/disassembly. Monomeric cytokeratin proteins exist at 9.5 M urea and will spontaneously reassemble into tetrameric forms at lower urea concentrations. CsA (5 μM) does not interfere with in vitro assembly of cytokeratin tetrameric units, but interferes with in vitro disassembly of tetrameric units. Regulation of cytokeratin protein phosphorylation for assembly and disassembly of intermediate filaments is important, and may indicate a site of CsA interaction that is toxic to the cell. (Supported by U of A Small Grant Award and the Arizona Kidney Foundation).
313 EFFECTS OF CA CHANNEL ANTAGONIST DRUGS ON CA UPTAKE AND CELL VIABILITY IN ISOLATED ENTEROCYTES. G M Hart, C W Jones, P T Jain and J T Peato, Department of Pharmacodynamics and Toxicology, University of Oklahoma, Health Sciences Center, Oklahoma City, OK. Sponsor: C P Robinson.

In a previous study, it was established that the Ca channel antagonist drug verapamil reduced Ca transport and tissue uptake in isolated segments of rat intestine (J. T. Peato and M. E. Johnson, Pharmacology 22:343-349, 1983). The focus of the present study was to examine the effects of various Ca channel antagonist drugs on Ca uptake and cell viability at the cellular level in isolated enterocytes. Intestinal enterocytes were isolated from a 10-15 cm segment of duodenal-jejunal intestine immediately distal to the pylorus. Viable cell number was determined by hemocytometer using the trypan blue exclusion method and the isolated cells were found to be 80-90% viable. The enterocytes were used immediately following isolation and incubated in a 10 mM HEPES balanced salt buffer containing 2 mM CaCl₂, 0.67 μM Ca/mℓ and Ca channel antagonist drug ranging in concentration from 0.05 to 1 mM. At various time intervals between 2 to 20 minutes triplicate incubation aliquots were filtered and cells collected and washed on 5 μm SM Millipore filters. The cell-associated Ca retained on the filters was determined by liquid scintillation spectrometry. The results indicated that verapamil and diltiazem at a concentration of 0.15 to 1 mM produced a significant reduction in 45Ca uptake into isolated cells while nifedipine did not alter 45Ca uptake. However, all three Ca channel antagonist drugs significantly reduced cell viability over the 20 minute incubation period. In conclusion, the results of this study indicated that Ca channel antagonist drugs inhibit the Ca transport process and the viability of isolated enterocytes and suggest that chronic use of these drugs may damage intestinal tissue and alter calcium homeostasis in vivo. (This study was supported in part by a grant from the Presbyterian Health Foundation, OKC, OK).

315 THIOLE DEPLETION ALTERS Ca²⁺ HOMEOSTASIS, PROMOTES LIPID PEROXIDATION AND INDUCES CELL INJURY IN CULTURED CARDIOMYOCYTES. C M Dhanabhoo and J R Babson, Dep. of Pharmacology and Toxicology, College of Pharmacy, University of Rhode Island, Kingston, RI. Sponsor: Z A Shalik.

Ethylenic acid-induced depletion of glutathione and protein thiols preceded a rise in intracellular free Ca²⁺ and ultimately resulted in lipid peroxidation and cell death in cultured rat cardiomyocytes. Two experimental approaches were undertaken to determine whether the cell death observed was due to peroxidative damage or to elevated intracellular free Ca²⁺. First, the antioxidant DPPD was used to determine the contribution of lipid peroxidation to cell injury. DPPD prevented the ethylenic acid-induced lipid peroxidation and cell injury but had no effect on elevated intracellular Ca²⁺, as monitored by Ca²⁺-dependent phosphorylase a activation. Second, the intracellular Ca²⁺ chelators, Quin-2-acetoxy- methyl ester and EGTA-acetoxy methyl ester, were used to assess the importance of the ethylenic acid-induced Ca²⁺ increase. Surprisingly, both intracellular Ca²⁺ chelators markedly reduced lipid peroxidation and cell injury but failed to reduce the magnitude of the intracellular free Ca²⁺ increase. These data suggest that while cardiomyocyte intracellular thiol status may be linked to intracellular Ca²⁺ homeostasis, the primary cause of cell injury following ethylenic acid-induced thiol depletion is peroxidative damage.

(Supported by NIH grant GM41496.)

314 NEOMYCIN INHIBITS Ca²⁺-STIMULATED PHOSPHATIDYLINOSITOL HYDROLYSIS AND PROTECTS CULTURED RAT CARDIOMYOCYTES FROM Ca²⁺-DEPENDENT CELL INJURY. J R Babson and J M Dougherty, Dep. of Pharmacology and Toxicology, College of Pharmacy, University of Rhode Island, Kingston, RI. Sponsor: Z A Shalik.

Exposure of cultured rat cardiomyocytes to ionomycin and extracellular Ca²⁺ leads to a rapid, sustained increase in intracellular free Ca²⁺ as monitored by Ca²⁺-dependent phosphorylase a activation and to a subsequent loss of cardiomyocyte viability as determined by lactate dehydrogenase (LDH) leakage. The intracellular free Ca²⁺ increase coincided with a rapid hydrolysis of phosphatidylinositol that preceded cell death. Phosphatidylinositol hydrolysis was monitored by the release of radiolabeled phosphoinositides from cardiomyocytes prelabeled with [2-³²P]-myo-inositol. Neomycin, a known inhibitor of phospholipase C, inhibited the phosphotidylinositol hydrolysis and markedly reduced the extent of cell injury. Inhibitors of other Ca²⁺-activated processes, including intracellular proteases and phospholipase A₂, had no effect on ionomycin-mediated cell injury. These data suggest that ionomycin-inhibited Ca²⁺-dependent cell injury in cultured cardiomyocytes may be due in part to the stimulation of phosphatidylinositol hydrolysis, presumably catalysed by a Ca²⁺-dependent phospholipase C.

(Supported by NIH grant GM41496.)


Amiodarone (AM), a potent antiarrhythmic drug, is known to cause several side effects including neurotoxicity. Since our previous studies indicated that AM alters Ca²⁺ regulated events, we have studied the effects of AM and its major metabolite, desethylamiodarone (DEA) on the compartmentation of free Ca²⁺ in synaptic membranes to understand their mechanism of neurotoxicity. Intact synaptosomes were prepared from male Sprague-Dawley rats. AM and DEA produced a concentration dependent increase in synaptosomal Ca²⁺ levels. The increase was dependent on external Ca²⁺. AM- and DEA-induced increase in synaptosomal Ca²⁺ was blocked only by MK-801 but not by verapamil, or with tetradoxin indicating that AM and DEA induced rise Ca²⁺ is through receptor mediated channel. AM and DEA inhibited N-methyl-D-aspartic acid receptor binding in synaptic membranes. Ca²⁺ accumulation into brain microsomes and mitochondria was significantly inhibited by AM and DEA, but without any effect on the Ca²⁺ release from these organelles. These results clearly indicate that AM and DEA increase synaptosomal Ca²⁺ by an action on receptor mediated channel in plasma membrane, but not due to the release of Ca²⁺ from intracellular storage sites. This initial rise in [Ca²⁺]o, together with other changes in Ca²⁺ homeostasis, might be responsible for AM and DEA-induced neurotoxicity.
317 EFFECT OF QUIN 2 ON 2-BROMOHYDROXYTETRONE (BHQ) AND tert-BUTYLHYDROPEROXIDE (TBHP)-INDUCED RENAL PROXIMAL TUBULE (RPT) CELL DEATH. R G Schnellmann, Dept. of Physiol. & Pharmacol., Coll. Vet. Med., University of Georgia, Athens, GA.

The role of cytosolic free calcium (Ca_2^+) in cell death has been a subject of much debate. This study determined the effect of the intracellular calcium chelator Quin 2 on BHQ- and TBHP-induced rabbit RPT cell death. After 0.25, 1 and 4 hr exposures, 0.2 mM BHQ decreased tubular glutathione 86% and oxygen consumption (QO_2) 31%, and increased LDH release from RPT suspensions from 6% to 44%, respectively. Pretreatment of RPT with 50 µM Quin 2/am for 15 min had no effect on BHQ-induced decreases in tubular glutathione or QO_2, but decreased cell death by 50% at 4 hr. After 1 and 4 hr exposures, 0.5 mM TBHP increased tubular malondialdehyde (MDA) levels, and increased LDH release from 7 to 41%, respectively. Pretreatment of RPT with 50 µM Quin 2/am for 15 min completely prevented TBHP-induced increases in MDA levels and LDH release. Quin 2/am concentrations of 5 µM and 1 µM resulted in LDH releases of 4% and 15% after a 4 hr TBHP exposure. Likewise, 5 µM Quin 2/am decreased TBHP-induced MDA levels to control values. Since TBHP induced lipid peroxidation and cell death are iron-dependent in this model, these results suggest that Quin 2 inhibits oxidant-induced cell death by chelating iron. In contrast, BHQ toxicity is iron-independent which suggests that Quin 2 may decrease BHQ-induced cell death by inhibiting a rise in Ca_2^+. (Supported by NIH ES-04410).

319 THE ROLE OF CYTOSOLIC IONIZED CALCIUM DEREGULATION IN CELL DEATH OR CELL SURVIVAL CAUSED BY EXOGENOUS OXIDATIVE INJURY. J D Swann, M W Smith, P C Phelps, I K Berezeyzky and B F Trump. Department of Pathology, University of Maryland, and MIESS, Baltimore, MD. Sponsor: Thomas W. Jones

Continuous exposure of cultured adult rat proximal tubular epithelium to various concentrations (0-25 mM/l) of extracellular xanthine/xanthine oxidase (X/XOD) results in cell death in a graded time- and dose-dependent fashion. As we have previously shown, significantly increased release of lactate dehydrogenase (LDH) occurs 2 hours after exposure to the highest concentration (25 mM/l) of X/XOD. Moreover, continuous exposure to X/XOD (0-25 mM/l) results in a striking time- and dose-dependent increase in the concentration of cytosolic ionized calcium ([Ca_2^+]_i), as measured by digital imaging fluorescence microscopy. However, exposure to 25 mM/l X/XOD for 1 minute followed by buffer leads to a brief and reversible increase in [Ca_2^+]_i. In addition, cell survival at 1, 2 and 4 hours after a 1 minute exposure to X/XOD (0-25 mM/l) is evident by the lack of significant LDH release, well-preserved cellular morphology and minimal trypan blue staining after 4 hours. This evidence indicates that a large magnitude, sustained increase in [Ca_2^+]_i, is associated with subsequent cell death while a brief, reversible increase in [Ca_2^+]_i, is followed by cell survival. Thus, the magnitude and duration of [Ca_2^+]_i deregulation may determine the outcome of eventual cell death or survival. (Supported by Nav N0014-89-K-0427 and NIH DK15440.)

318 Ca_2^+ IS INVOLVED IN CYSTEINE CONJUGATE CYTOTOXICITY. O Chen and JL Stevens. W. Alton Jones Cell Science Center, Lake Placid, NY.

The mechanism of S-(1,2-dichlorovinyl)-L-cysteine (DCVC) cytotoxicity involves covalent binding, thiol depletion and lipid peroxidation (LPO) in LLC-PK1 cells (Toxicol. 10,471). However, the endogenous source of oxidative stress and the role of Ca_2^+ in cytotoxicity of DCVC is unknown. Altering extracellular Ca_2^+ had no effect on DCVC toxicity, but intracellular Ca_2^+ chelators Quin 2 and EGTA ester and mitochondria Ca_2^+ uptake blocker ruthenium red inhibited DCVC toxicity. Preloading cells with cytosolic Ca_2^+ by ionomycin potentiated DCVC toxicity. Measurement of intracellular Ca_2^+ indicated that cytosolic Ca_2^+ increased soon after the treatment with DCVC. The increase was prevented by AOA, an inhibitor of DCVC metabolism, but not by antioxidants, which inhibited DCVC toxicity by preventing LPO. Quin 2, EGTA ester and ruthenium red blocked LPO in the absence of an effect on cellular nonprotein thiols. The data suggest that increased cytosolic Ca_2^+ plays a role in DCVC cytotoxicity by facilitating mitochondrial Ca_2^+ recycling and the generation of endogenous oxidants. The loss of cellular nonprotein thiols allows the accumulation of oxidants resulting LPO and cell death.

320 THE ROLE OF CYTOSOLIC CALCIUM AND OXIDATIVE STRESS IN THE ISOLATED RENAL EPITHELIAL CELL (IREC) TOXICITY OF S-(1,2,3,4-PENTACHLOROBUTADIENYL)-L-CYSTEINE (PCBC). P C Brown and T W Jones. University of Maryland Toxicology Program and Department of Pathology, University of Maryland School of Medicine, Baltimore MD.

Treatment of IREC with 25 µM PCBC results in an elevation of cytosolic Ca_2^+ which precedes cell death. Pretreatment of IREC with a cell permeant Ca_2^+ chelator, EDTA AM (50 µM), decreased the IREC toxicity of PCBC supporting a critical role for cytosolic Ca_2^+ deregulation in this model of renal cell injury. In addition IREC were protected from PCBC-induced cell death by the antioxidant N,N-diphenyl-p-phenyldiamine (DPPD) (20 µM). Suggesting a possible oxidative component in PCBC-mediated cell injury. Neither of these protective agents affected the covalent binding of [35S]PCBC in this model. Further indication of oxidative injury was provided by the PCBC-mediated production of malondialdehyde (MDA), an indicator of lipid peroxidation. As expected, pretreatment of cells with DPPD (5 prevented the production of MDA. Interestingly, treatment of cells with EDTA AM was also effective at preventing lipid peroxidation in this model. However, EDTA AM did not prevent the MDA production associated with exposure of IREC to 3 mM tert-butylhydroperoxide, indicating that EDTA AM does not act as a non-specific antioxidant. Taken together, these results suggest that intracellular Ca_2^+ deregulation contributes to the oxidative damage associated with PCBC-induced IREC toxicity. (Supported by NIH ROI ES05436)
Glutathione and cysteine conjugates of a variety of haloalkanes are nephrotoxic through a mechanism involving metabolism by the enzyme, cysteine conjugate θ-lyase (ζL). ζL metabolism has been shown to result in the generation of mutagenic species when tested in prokaryotic systems and DNA single strand breaks (SSB) in eukaryotic systems. The purpose of the present study was to investigate the mechanism involved in the genotoxicity of this class of compounds in the critical target cell type. Primary renal proximal tubule cells were cultured from Fischer 344 rats and treated with the nephrotoxic cysteine conjugate, ζ-(1,2,3,4,4-pentachlorobutadienyl)-L-cysteine (PCBC), from 0.5-100 μM for up to 20 hr. Lactate dehydrogenase (LDH) release was determined as an indicator of cell death. Alkaline elution was used to evaluate SSB. The results indicate that PCBC exposure caused SSB in a time and dose-dependent manner. However, the extent of DNA damage showed a strong correlation with the degree of cytotoxicity (R² = 0.961, p < 0.001). Aminooxycetic acid, an inhibitor of ζL, was found to protect against PCBC-induced cytotoxicity and SSB. In addition, N,N'-diphenyl-p-phenylenediamine, a potent antioxidant that does not affect ζL metabolism of PCBC, also protected against both cytotoxicity and SSB. These results indicate that ζL-mediated, cysteine conjugate genotoxicity in proximal tubule cells is directly related to the process of cytotoxicity.

DNA fragmentation and increased nuclear Ca²⁺ precede dimethylnitrosamine-induced hepatic necrosis in mice. GB Corcoran, SD Rey, CL Sorge, LM Kamendulis. Toxicology Program, Univ. New Mexico College of Pharmacy, Albuquerque, NM.

Hepatotoxins impair Ca²⁺ regulation and produce necrosis in an apparent cause-and-effect manner, yet vital targets of unregulated Ca²⁺ remain unidentified. During cell death by apoptosis, DNA appears to be key target of Ca²⁺. The present studies explore whether nuclear Ca²⁺ regulation is lost early during dimethylnitrosamine (DMN) induced necrosis, and whether Ca²⁺ stimulation attack on DNA by endonucleases. Male ICR mice were treated with 100 mg/kg DMN ip, livers were preserved in N₂, and blood was collected for aminotransferase activity (ALT). Total Ca²⁺ was analyzed in nuclei and DNA fragmentation assessed by differential sedimentation and diphenylamine reaction. Liver was digested prior to genomic DNA analysis by agarose gel electrophoresis. At 4-6 hr, DMN produced a 30-40% rise in total nuclear Ca²⁺, a 200% increase in DNA fragmentation, substantial loss of large genomic DNA from gels, and appearance of a fragmentation “ladder.” Larger Ca²⁺ and fragmentation increases accompanied massive ALT release at 12-24 hr. In vivo, 3-aminobenzamide and theophylline, agents thought to permit Ca²⁺-endonuclease activation by inhibiting poly (ADP-ribose) polymerase, potentiated ALT increases 2-fold. Aurintricarboxylic acid, an endonuclease inhibitor, decreased ALT 50%. These results support the possibility that early DNA damage plays a role in DMN-induced hepatic necrosis. (Supported in part by NIH GM-41564)
325 CHRONIC TOXICITY OF HYDROCHLOROFLUOROCARBON HCFC-123 IN RATS. L Angeline Mailey, M C Carakostas, J F Hennessy, M 2 Trachtman, and G W Rusch. The Du Pont Co., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE., and Allied-Signal Co., Morristown, NJ.

As part of an ongoing 2-yr inhalation study, 80 rats/sex/group were exposed to vapors of 0, 300, 1000, or 5000 ppm HCFC-123 6 hr/day, 5 days/wk. Ten rats/sex/group were sacrificed at 1 yr for morphologic evaluation of tissues. Body weight was measured biweekly. Clinical pathology evaluations occurred at 6 and 12 months. Body weight and weight gain were decreased in males and females at 5000, and in females at 1000 ppm. Triglycerides were decreased at 300, 1000, and 5000 ppm in fasted males, fasted and nonfasted females; and were decreased in nonfasted males at 5000 ppm. Cholesterol was decreased in fasted and nonfasted females at 300, 1000 and 5000, and in fasted males at 5000 ppm. Urinary fluoride was increased in both sexes at all concentrations. Males and females at 5000 ppm had higher relative liver weight. No compound-related pathological changes were detected at any concentration at the 1 yr sacrifice. Increased hepatic B-oxidation activity was observed in males at 300, 1000, and 5000 and in females at 1000 and 5000 ppm. No differences were observed in the rate of hepatic cell proliferation at any exposure concentration. (Sponsored by the Program for Alternative Fluorocarbon Toxicity Testing)

326 EPA'S PRELIMINARY CRITERIA FOR DETERMINING "SAFE" ALTERNATIVES TO OZONE-DEPLETING SUBSTANCES. R Rubenstein, B Haemisger, and S Segal. U.S. Environmental Protection Agency and Clement International Corp., Washington, D.C. Sponsor: Edward V. Chianale

Congress is currently working towards passage of the proposed Clean Air Act (CAA) Amendments, which will mandate the implementation of a program to protect stratospheric ozone. One of the cornerstone activities of this program will require EPA to identify which substitute chemicals, or alternative manufacturing processes are safe, (i.e., will reduce the risks to human health and the environment). In this paper, we present EPA's preliminary criteria for identifying "safe" alternatives. While the toxicity of the substitutes will be a critical parameter, the risk balancing that EPA must perform necessitates consideration of other factors (e.g., flammability, the ozone depletion potential of the substitute chemicals, energy impacts, technical feasibility). We examine these issues in detail, indicating which decisions are critical, especially the interpretation of toxicological data. We then present a case study demonstrating the application of these preliminary criteria to one of the substitute chemicals. We conclude with an assessment of the next steps for EPA's safe alternatives program, as well as activities underway both domestically and internationally that will enhance the database for this program.


Production of the currently used fire-fighting agents, halons 1211, 1301 and 2402, is to be progressively reduced with eventual phase-out by the year 2000 as a result of environmental concerns. An intensive search is underway to find alternatives which combine the outstanding fire extinguishing properties of the halons with acceptably benign toxicological and environmental properties. Clearly, fire-fighting agents require the usual toxicity testing to define the safe handling procedures to be used during the charging and filling of fire-fighting systems. However, the manner of use of these materials on fires introduces special consideration. In some fire-fighting applications, no, or incidental low level, human exposures to the gaseous or volatile agent may occur; but, in others, very high levels of exposure may be unavoidable (typical extinguishing concentrations in laboratory tests are 3-5%). Thus the effects of short-term, very high levels of exposure have to be investigated and, if the halon alternative is a halogenated compound, factors such as anesthetic effects and potential cardiac sensitization have to be considered. In addition to the intrinsic toxicity of the fire-fighting agent, the smoke over the extinguished agents regarded as possible halon replacements. In studying the properties of these by-products, the first stage is to capture and analysis of inorganic and organic materials generated in laboratory tests of fire extinguishment. These procedures have now been developed and validated. Finding ideal halon replacements is proving difficult and thus a toxicity testing program which allows early screening of candidates and which grows in complexity in parallel with the aspects of the development of the chemical has been devised.

328 SUBCHRONIC INHALATION TOXICITY STUDY WITH HCFC-225 ISOMER MIXTURE IN RATS. M E Varhegi, MC Carakostas, and S Frame. Du Pont, Haskell Lab., Newark, DE.

HCFC-225 Isomer mixture is a potential candidate replacement for chlorofluorocarbon (CFC) solvents. HCFC-225 Isomer mixture has very low toxicity in acute inhalation studies (1531 ppm). This study was carried out to determine the toxic effects of repeated inhalation. Four groups of 10 male CD® rats were exposed 6 hrs/day, 5 days/wk for 2 wks to 0, 500, 5,000, and 13,000 ppm. At the end of the exposure period and following a 14-day recovery period, clinical and pathologic evaluations were carried out. Mean absolute and relative liver weights were increased in all treated males following a 2-week exposure. These changes were correlative to the microscopic finding of hepatocellular hypertrophy which was also present at all exposure concentrations. Hepatocellular hypertrophy was diffuse and was characterized by slight enlargement of hepatocytes and granular eosinophilia of hepatocyte cytoplasm. Eosinophilic granularity correlated Ultrastructurally with increased numbers of peroxisomes in hepatocytes. Clinical chemical effects included decreased mean serum cholesterol, triglycerides and globulin at all exposure levels, and increased serum albumin and urinary fluoride at all exposure levels. All of these effects were reversible. Plasma fluoride concs were increased in all treatment groups following the recovery period. Based upon the histopathologic and clinical chemical parameters measured, a no-effect exposure concentration could not be determined.

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SUBCHRONIC INHALATION TOXICITY STUDY WITH HCFC-132c IN RATS. SR Frame, MC Carakostas, and DB Vachelt. Du Pont, Haskell Lab., Newark, DE.

HCFC-132c is a potential candidate replacement for chlorofluorocarbon (CFC) solvents. HCFC-132c has very low toxicity in acute inhalation studies (ALC=26,000 ppm). This study was carried out to determine the toxic effects of repeated inhalation. Four groups of 10 male CD® rats were exposed 6 hrs/day, 5 days/wk for 2 wks to 0, 400, 2,000, and 10,000 ppm. At the end of the exposure period and following a 14-day recovery period, clinical and pathologic evaluations were carried out. Male rats exposed to 10,000 ppm had mild to moderate degeneration and necrosis of germinal epithelium in the seminiferous tubules. This corresponded to decreased numbers of sperm and increased numbers of degenerated epithelial cells in the epididymides. Following recovery, most seminiferous tubules were either atrophic or undergoing apparent regeneration. Less severe changes were also present in the testes and epididymides of animals exposed to 2,000 but not at 400 ppm. Other dose-related pathological findings, splenic lymphoid atrophy and vacuolation of the vomeromosal organ were seen in all treated groups and were reversible. Clinical pathological findings included decreases in mean serum triglyceride concentrations for all treatment groups. Plasma and urine fluoride concentrations were increased in all treatment groups. Based upon the histopathologic and clinical chemical parameters measured, a no-effect exposure concentration could not be determined.


Perfluoroisobutylene (PFIB), a pyrolysis product of polytetrafluoroethylene (Teflon), is known to be a hazardous industrial waste product. Inhalation of PFIB can induce acute lung injury and pulmonary edema. The acute changes in blood cellular components as well as serum chemistry after exposure to a toxic dose of PFIB is still unknown. Adult, unanesthetized sheep were exposed for 10 minutes to either 333 ppm/min of PFIB (n=7) or room air (n=10). Serum samples for enzymes and electrolytes analyses, as well as EDTA-treated blood for complete blood count, were collected immediately before and after exposure, as well as at 15, 30, 60, 120, 180, and 240 min post-exposure. In sheep exposed to PFIB, only white blood cell counts showed significant decrease (p<0.05) from their baseline values and that of the control group at the end of exposure to PFIB. Our data suggest that: (1) PFIB has no systemic effect, and its toxicity is limited to the lung; (2) the leukopenia in PFIB-treated animals reflects pulmonary sequestration associated with PFIB-induced acute lung injury; and (3) hematological and serum biochemical parameters associated with PFIB exposure are non-specific and unreliable markers for PFIB toxicity.

ACUTE INHALATION OF BIS(TRIFLUOROMETHYL)DISULFIDE: CONCENTRATION-RESPONSE KINETICS DM Stavert, D Archuleta and BE Lahmard, Los Alamos National Laboratory, Los Alamos, NM.

Bis(trifluoromethyl)disulfide (TFD) is a highly toxic organ-fluoride gas that can be generated from the combustion of various fluorocarbon compounds, including Teflon. Inhalation of TFD is known to cause pulmonary edema. However, little is known about the kinetics of onset of this response relative to inhaled TFD concentrations. Another toxic organo-fluoride, perfluoroisobutylene (PFIB) has been shown to induce pulmonary edema after a latency period, the duration of which is inversely related to the concentration of inhaled PFIB, (Toxicologist 10:A809, 1990). The objective of this study was to kinetically characterize the course of development of pulmonary edema using lung gravimetric and histopathologic criteria after exposing rats to various concentrations of TFD. Fischer-344 rats were exposed to 117,137,147,170, and 177 mg/M3 TFD or air for 10 min. The rats were sacrificed 1.5,8,24, or 48 hr after the exposure for the lung studies. In contrast to post-PFIB inhalation, the onset of detectable pulmonary edema after TFD inhalation occurred within 1 hr after TFD exposure irrespective of TFD atmosphere concentration, i.e., no latency period was observed. A gradual hourly increase of lung wet weight (LWW) of approximately 4% and a gradual increase in right cranial lobes dry weight (RCLDW) of approximately 3% occurred over a 24 hr period after exposure to 137 mg/M3 TFD. In contrast, after exposure to 100 mg/M3 PFIB, increases of RCLDW increases were detectable within 8 hrs after exposure. Rapid increases in LWW and RCLDW, however, occurred between 6 and 18 hrs after PFIB exposure. The results of this study demonstrate that an increase in pulmonary edema (LWW) after exposure to TFD did not occur. The kinetics of pulmonary edema formation after exposure to TFD will be discussed in more detail.

Pulmonary Response to Polytetrafluoroethylene (PTFE) and Tetrafluoroethylene/hexafluoro-propylene (FEP) Combustion Fume and Isolated Particulate. K. P. Lee and W. C. Seidel. Du Pont, Haskell Laboratory for Toxicology & Industrial Medicine, Elkton Rd., Newark, DE 19714

Rats were exposed to the thermal degradation products of PTFE or FEP. The approximate lethal concentration (ALC) of the pyrolysis products (PP) evolved for 4 hrs from PTFE at 425°C was 0.1 mg/m³. The ALC in the National Bureau of Standards exposure chamber was 0.3 mg/m³ of FEP PP evolved for 30 min at 560°C. Numerous toxic particles were found in the PTFE or FEP fume. When the particles were aged, they agglomerated and were markedly decreased in toxicity. Fresh particulate smaller than 0.05 µm appeared to be a toxic agent. Intratracheal instillation of agglomerated PTFE particulate did not produce the generalized alveolar edema as seen in the inhalation exposure. The PTFE or FEP fume-induced pulmonary lesions were characterized by pulmonary congestion, edema, hemorrhage, and minimal airway damage. Type I pneumocytes were particularly vulnerable and showed extensive damage but the alveolar capillary endothelium was much less damaged. Pulmonary edema and hemorrhage appeared to be the result of Type I pneumocyte damage.
USE OF A ROTARY EVAPORATOR SYSTEM TO GENERATE HIGH PURITY VAPORS FROM REACTIVE LIQUIDS FOR INHALATION STUDIES. R J Weigel, M L Clark, R B Westerberg, J R Decker, and T J Goehl*. Battelle, Pacific Northwest Laboratory, Richland, WA and *NIEHS/NTP Research Triangle Park, NC. Sponsor: T J Mast

A commercial rotary evaporator apparatus has been modified to generate high purity chemical vapors for inhalation exposures of laboratory animals. The apparatus is used when one or more of the following conditions dictate: 1) the presence of components of differing vapor pressures in the bulk chemical that are not desired in the test atmosphere; 2) the potential for reactivity of the bulk chemical either by oxidation, exothermic polymerization or by formation of peroxides; and 3) the requirement to evaporate large amounts of chemical to satisfy the vapor requirements for reaching high target concentrations. The properties of a rotary evaporator apparatus are ideal for producing vapors under these constraints. The rotary action of the flask containing the bulk chemical, combined with introduction of a metered amount of nitrogen into the neck of the flask, maximizes the evaporation rate of the chemical when immersed in a heating bath at a relatively low temperature, generally near the chemical's boiling point. The chemical vapor mixed with nitrogen is carried into the condenser, whose temperature is maintained by a temperature-regulated water bath. The condenser cools the vapor to the desired temperature, condensing part of it. The refluxing action of the condenser serves two purposes: it removes higher boiling point impurities from the chemical vapor, and it limits the concentration of the vapor to the test chemical's saturation vapor pressure at the temperature of the vapor leaving the condenser. Isopropanol is an example of a chemical that has been generated by the apparatus.

INHOMOGENEITY OF REACTIVE GAS UPTAKE IN THE LUNGS. RR Mercer, S Aaljibel, JD Crape, EJ Miller, Center for Extrapolation Modeling, Duke University, Durham, N.C.

This study determined whether variations in the volume of alveoli and alveolar ducts forming single units of ventilation significantly influence the distribution and uptake of inspired reactive gases. Serial section analyses of vascular perfusion-fixed rat lungs were used to determine the dead space proximal to specific ventilatory units and the gas volume of these ventilatory units. Three reconstructions, each consisting of 26 to 71 ventilatory units distal to a specific bronchus, were carried out. The average ventilatory unit volume for the three reconstructions was 0.53 ± 0.03mm³ (Mean ± SE). The distribution of ventilatory unit volume was diverse, with 15% of the population having a volume less than 0.3mm³ and 9% of the population having a volume greater than 1.0mm³. For a gas of relatively low reactivity (e.g., oxygen) the predicted oxygen uptake per unit surface area did not vary significantly between ventilatory units. For a highly reactive gas (e.g., ozone), the predicted uptake per unit surface area in the larger and more proximal ventilatory units was four-fold the average uptake. These results suggest that focal areas of injury likely result from exposure to inhaled reactive gases. (Support provided by the US EPA and CIAR.)

GENERATION, MONITORING, AND CONCENTRATION VERIFICATION OF PBB CONCENTRATIONS OF GLUTARALDEHYDE FOR INHALATION STUDIES. A W Gieschen, B J Greenspan, R B Westerberg, T J Goehl*, and J H Roycroft*. Battelle, Pacific Northwest Laboratory, Richland, WA and *NIEHS/NTP, Research Triangle Park, NC.

Glutaraldehyde chamber concentrations of 62.5 to 1000 ppb (v/v) were generated and measured for a 13-week inhalation study. Generation of this reactive vapor, with maintenance of uniform chamber concentrations and adequate analytical accuracy and precision, required several special techniques. Glutaraldehyde vapor was generated by passing heated air through a revolving evaporation tube coated with a ~23% (w/w) aqueous glutaraldehyde solution. Incorporation of an independently monitored ~5 ppm mixing chamber into the generation system was required to produce stable exposure concentrations. A chamber recirculation system was used to maintain a consistent vapor concentration throughout the exposure chamber. Exposure chamber concentrations were continuously monitored using an on-line HP5890 gas chromatograph (GC) equipped with a gas sampling valves. Sufficient sensitivity and precision for the on-line GC was achieved by cold trapping glutaraldehyde from the 10-ml sample loop on a 95% dimethyl-5%-diphenyl polysiloxane WCOT capillary column. The on-line GC was calibrated by independent measurement of chamber grab samples by HPLC analysis of the 2,4-dinitrophenylhydrazine derivative of glutaraldehyde. Effective control of the on-line monitoring and generation methods were demonstrated by close agreement between grab concentrations, on-line monitor determined concentrations, and target concentrations. The average ratio of the on-line monitor determined concentrations to the analytical determined grab concentrations was 1.07. The average ratio of the chamber target concentration to the average grab sample concentration was also 1.07.

Potentiation of Lung Injury by Exercise Following the Inhalation of Toxic Gases: Evidence Consistent with a Postulated Mechanism. BE Lehner and DM Stavert, Los Alamos National Laboratory, Los Alamos, NM.

In a previous study (Environ. Res. 48:87-99, 1989), we found that exercise (E) performed after an acute exposure to nitrogen dioxide (NO2) substantially potentiated the expression of NO2-induced pulmonary edema (PE). As a mechanism, it is possible that such E-associated potentiation of PE is due to the imposition of physiologic factors common to E, e.g., increased cardiac output, increased pulmonary vascular flows and pressures, on an already "leaky" lung. In more recent studies, the potentiating effects of E have been examined in rats following acute exposures to the gases perfluoroisobutylene (PFIB) and bis(trifluoromethyl) disulfide (TFD). A latency period (hours) before the development of PE is normally observed following PFIB exposure, whereas, similar to NO2, no latency period for the onset of PE follows exposure to TFD. E during the latency period after PFIB exposure did not potentiate injury, while E did potentiate injury at later times when PE normally occurs. With TFD, E potentiated the expression of PE when performed immediately after exposure. The results of this study suggest that a state of pre-existing hyperpermeability is required for E to potentiate PE. Additionally, they further suggest that the potentiating effects of exercise on toxic gas-induced PE may be related to cardiovascular events common to E as opposed to an actual increase in lung damage per se. This work was supported by the USA MRDC.
The modulation of silica induced pulmonary inflammation by acute exposure to nitrogen dioxide. KJ Vettraino, JB Norris and AK Rubbard. School of Pharmacy, Univ. of Conn., Storrs, CT.

The biologic interaction between two environmental pollutants was examined by exposing mice to nitrogen dioxide (NO₂) and silica crystals (SI). Animals were exposed to NO₂ either before or after an intratracheal (IT) instillation of SI to determine if exposure to this oxidant gas modulated the acute inflammatory response induced in the lungs by inorganic particles. C57BL/6 mice were exposed for 2 hrs to NO₂ (3.5-7 ppm) or filtered air prior to an IT injection of 0.5 mg SI. Control animals received sterile saline (SAL) IT. Alternately, animals received an inhalation exposure to NO₂ immediately after or 24 hrs after SI instillation. All mice were killed 3 days after SI or SAL injection and evaluated for lavage cell type. Exposure to NO₂ prior to SI diminished the marked neutrophil (NEU) influx normally seen 3 days after SI exposure. Exposure to NO₂ 24 hrs post SI injection also ablated the NEU response whereas exposure to the gas immediately after SI injection had no modulatory effect. In no case did NO₂ modulate the SI-induced pulmonary alveolar macrophage response. These data suggest that the marked neutrophil response induced by SI can be modulated by NO₂ exposure and that the time of this oxidant gas exposure is critical to these modulatory effects. Supported by the Conn Lung Assoc. and UCSP.

Pathologic responses to inhaled HCl during periods of increased minute ventilation in pseudo-mouth breathing and nose breathing rats. D Archuleta, M Martinez, DM Stavert and BE Lehnert. Los Alamos National Laboratory, Los Alamos, NM.

Hydrogen chloride (HCl) is a potent irritant gas and a product of combustion processes. The respiratory tract lesion caused by this material has been characterized in pseudo-mouth breathing and nose breathing rats (Archuleta et al., 1990). However, little research attention has been given to the respiratory tract lesions produced by HCl during times of increased minute ventilation. The objective of this study was to characterize the upper and lower respiratory tract lesion produced by HCl inhalation by nose breathing (NB) or pseudo-mouth breathing (PMB) rats during CO₂ (5%) induced increased minute ventilation (Ve). PMB rats were placed into whole body plethysmographs for pulmonary function studies while they were exposed to air, air + 5% CO₂, 1000 ppm HCl, or 1000 ppm HCl + 5% CO₂ for a duration of 20 minutes. Groups of NB rats were likewise exposed. The animals were sacrificed 24 hr later for histopathologic analysis of the upper and lower respiratory tract and for lung gravimetric measurements. NB rats increased Ve approximately 60% during air + CO₂ exposure. HCl inhalation in the NB rats decreased Ve approximately 10% and this depression of Ve persisted during HCl exposure concurrent with 5% CO₂. However, Ve in the PMB rats, while not different from the NB during HCl inhalation alone, were increased 46% with HCl + 5% CO₂ inhalation. The Ve increase during concurrent HCl and CO₂ inhalation in the PMB rats increased mortality by 17%, and increased lung wet weight by 19% and right cranial lobe dry weight by 21% in surviving animals. This study demonstrates that: 1) CO₂ inhalation by NB rats exposed to HCl does not result in Ve increases, and 2) CO₂-induced increases in Ve in PMB rats during HCl exposure result in an increase in lung injury to the lower respiratory tract. This work was supported by the USA MRDC.

Kinetics of NO₂ interfacial transfer into pulmonary epithelial lining fluid. E M Postlethwait, S D Langford, and A Bidani. Pulmonary Research Laboratories, University of Texas Medical Branch, Galveston, TX. Sponsor M T Moslen.

Studies in intact lungs and isolated epithelial lining fluid (ELF) suggest that the steady-state uptake of inhaled NO₂ is governed by chemical reaction(s) between NO₂ and ELF solutes. These reactions exhibit NO₂ 1st order kinetics but undergo eventual [NO₂]-dependent rate saturation. We further assessed the kinetics of NO₂ interfacial transfer into biological substrates by utilizing a closed system of known geometry and initial gas phase [NO₂] to expose ELF (as bronchoalveolar lavage) and a model biochemical system (glutathione, GSH). Results: 1) NO₂ effective rate constants (kₚ) displayed [ELF] and [GSH] dependence and saturation. 2) ELF dialysis (1000 MWCO) substantially reduced both kₚ and the effect of [ELF]. 3) Interfacial transfer resistance was notably elevated in unstimred systems and was aqueous substrate-related. 4) Exposure of model GSH "thin films" resulted in similar kₚ values as larger bulk phase exposures. Conclusions: NO₂ reactive uptake is aqueous substrate-dependent and, in part, related to an small molecular weight ELF reactant. Unstimred conditions confine interfacial transfer kinetics in a dose dependent manner. The site of reactive absorption appears to be limited to the aqueous interface suggesting that primary surface interactions are critically linked to the development of subsequent pulmonary cytotoxicity. (NIH ES04952 & CIAR 90-23)

Nitrogen dioxide (NO₂) is a primary constituent of combustion atmospheres. We have characterized the lung lesion resulting from short term, high level exposure to NO₂ in the rat (Toxicologist 8:5A58, 1988). However, little attention has been given to NO₂ lung toxicity resulting from NO₂ inhalation during times of increased minute ventilation (Vₑ). The objective of this study, accordingly, was to examine the effect increased Vₑ during NO₂ exposure has on NO₂-induced acute lung injury. Lung gravimetric and histopathologic criteria were used to characterize the acute lung injury. Groups of adult Fischer-344 rats were exposed to 100 to 1000 ppm NO₂ for durations ranging from 1 to 20 minutes with and without concurrent CO₂ (2.5%-5%) inhalation. Control rats were exposed to air with and without concurrent CO₂ inhalation. Rats were exposed within plethysmographs which measured Vₑ continuously during the exposure. Rats were sacrificed 24 hrs after the exposure, and the lungs were gravimetrically measured and prepared for histopathological assessment. Inhalation of these high levels of NO₂ diminished the normal ventilatory response to CO₂. While 5% CO₂ increased Vₑ by 60% in air breathing controls, a mean Vₑ increase of only 31% was measured on animals exposed to 5% CO₂ concurrent with 100 to 1000 ppm NO₂. Increased lung wet weight (LWW) and right cranial lobe dry weight (RCLDW) values correlated well with increased Ve exposure aERICa. The results of this study demonstrate that: 1) high concentrations of NO₂ can attenuate the ventilatory response to CO₂ inhalation, and 2) increases in NO₂-induced lung injury is proportional to Vₑ. This work was supported by the USA MRDC.

The major damage to respiratory tract tissues of laboratory animals, due to inhaled gases such as ozone and nitrogen dioxide, is known to occur in the centriacinar region. The severity of this damage is observed to vary among CAR locations. To better understand this observation, a mathematical dosimetry model was developed to predict the local doses of reactive gases in mammalian lungs. The dosimetry model accounts for the fact that the reactive gases are at different distances from the trachea. Because of the completeness of data, the rat lung was chosen for the simulations. The tracheobronchial region model was based on data of Paardekooper et al. (1976) and two pulmonic region (PR) models were used for comparison. Results show that variations in CAR doses are sensitive to specific PR anatomical models. Centriacinar region doses varied by a factor of three with use of the Yeh et al. (1979) PR model while use of the PR model based on Mercer and Crapo (1987) resulted in a dose variation with a factor of 9. These simulations not only illustrate the importance of lung dimensions but also show that the inhomogeneous structure of the lung affects the distribution of absorbed reactive gases among equivalent morphological regions, which can help to explain the variations of damage severity. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

342 EFFECT OF HYDROGEN CYANIDE AND HYDROGEN CHLORIDE IN COMBINATION ON LETHALITY IN THE RAT. V G Switzer. Southwest Research Institute, San Antonio, TX.

Combustion atmospheres can consist of a variable, complex mixture of toxic gases. Determining the combined effects of these mixtures is one of the most important topics in combustion toxicology. Hydrogen cyanide (HCN) and hydrogen chloride (HCl) can be generated from combustion of nitrogen and chlorine containing materials, respectively. To investigate potential interactions for these combustion gases, despite their different mechanisms of toxicity, L(C50) values (30-minute exposure and 14-day post-exposure) for HCN in the presence of HCl were obtained for head-only exposure of Sprague-Dawley rats. The HCl levels were fixed at 0, 600, 1,000, and 1,900 ppm. A level of 600 ppm HCl can produce a 50% decrease in respiration due to its irritant properties. The 1,000 and 1,900 ppm HCl levels approximate 25% and 50%, respectively, of an HCl LC(50) value obtained in this laboratory following the same protocol. At each HCl concentration, the resultant L(C50) values for HCN were as follows: HCN LC(50) = 212 ppm (at 0 ppm HCl), 219 ppm (at 600 ppm HCl), 112 ppm (at 1,000 ppm HCl) and 70 ppm (at 1,900 ppm HCl). A statistical comparison of the L(C50) values demonstrated a significant (p < .05) difference between the values of HCN at both 1,000 and 1,900 ppm HCl compared to HCN alone. This apparent trend of decreasing HCN L(C50) values in combination with increasing HCl concentration supports the conclusion that combined exposures produce a lethality profile different than that expected from either gas alone. (Supported by NIST Grant 60ANB6D635.)

343 A DYNAMIC INHALATION CHAMBER FOR STUDYING ANTIDOTES TO ASPHYXIATING GASES. J R Mozingo, M D Dulaney Jr, and A S Hume. Dept. of Pharmacol. & Toxicol. Univ. of MS Med. Ctr., 2500 North State Street, Jackson, MS. U.S.A.

A dynamic gas exposure chamber has been developed as a model to evaluate the effectiveness of antidotes to hydrogen cyanide (HCN). This chamber features an electronic sensor system which continuously monitors the HCN concentrations in the exhaust gas with confirmation via HCN detector tubes. Hydrogen sulfide interference was eliminated using a sulfide filter. A high air flow exhaust system was developed in which a caustic scrubbing system traps exhaust cyanide. The design of this chamber allowed the test animals to be exposed to constant levels of HCN and observed for signs and symptoms of toxicity. This system is also designed to evaluate post-exposure antidotes. The efficacy of sodium nitrite (SN), sodium thiosulfate (ST), and α-ketoglutaric acid (AKG) pretreatment against gaseous cyanide was examined using this model. Male ICR mice (25-35 g) were exposed to 400-450 ppm of HCN for three minutes resulting in 80% lethality. ST or AKG alone were effective only at high doses (>500 mg/kg). Doses of SN (50 mg/kg) provided complete protection 15 minutes prior to HCN exposure but with side effects. Doses below this amount were not effective. The combination of SN (25 mg/kg) and AKG (500 mg/kg) provided complete protection. This model provides a powerful new tool to aid in the development of antidotes to asphyxiating gases such as HCN. Supported by USAMRMC Contract No. 88-C-8047.


Alpha-ketoglutarate (AKG) has previously been reported to be an effective cyanide antagonist when the cyanide is administered parenterally. This study examines the efficacy of AKG alone or in combination with the methemoglobin formers: sodium nitrite (SN) and hydroxyaminyl chloride (HAA) or the sulfur donor, sodium thiosulfate (ST) against gaseous cyanide. Male ICR mice (23-35 g) were pretreated with AKG or various methemoglobin formers 15 min prior to a three minute exposure to 400-500 ppm of HCN. AKG alone (250-1000 mg/kg) showed a dose-dependent protective effect against cyanide with the highest dose providing complete protection. The protective action of AKG was also enhanced by the addition of ST (500 mg/kg). HA alone (5-10 mg/kg, i.m.) also showed a dose-dependent protective effect against cyanide. SN (10 mg/kg) showed no protective effect against HCN. However, this dose of HA in combination with AKG (500 mg/kg) reduced lethality to 20%. This work demonstrated the value of adding AKG to cyanide treatment regimen since this non-toxic compound enhances the efficacy of the treatment. Supported by USAMRMC Contract No. 88-C-8047.
THE EFFICACY OF ALPHA-KEToglutarate (AKG), 2-AminoETHANE ThiosUlfonate (2-AETS) PRETREATMENT AND HYDROXYLAMINE (HA) POSTTREATMENT AS CYANIDE ANTIDOTE IN THE MOUSE

M. Dulaney, Jr., and A. Hume. Dept. of Pharmacol. & Toxicol., Univ. of MS Med. Ctr., Jackson, MS.

This study describes a cyanide treatment protocol which incorporates a non-toxic orally effective pretreatment and a specific post-exposure treatment. The oral pre-treatment consisted of a cyanide "binding agent", AKG and a sulfur source, 2-AETS. The treatment consists of HA. Male ICR mice were pretreated orally with AKG (500 mg/kg) and 2-AETS (50 mg/kg) 15 min prior to NaCN (7.2 mg/kg). Then, one min after cyanide, HA (10 mg/kg, i.m.) was administered. Cyanide lethality was reduced by 90%. HA alone reduced lethality by 50%. When the pretreatment was given by i.p. injection, the protection against 13.4 mg/kg of NaCN was very dramatic. AKG and 2-AETS was also effective against 400 ppm gaseous cyanide. This pretreatment combination reduced the lethality from 90% to 50%. This work demonstrated that a pretreatment-treatment regimen can be developed using AKG, 2-AETS prior to exposure and HA following exposure.

THE TOXICITY OF SMOKE FROM BURNING DOUGLAS FIR

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Mice were exposed for 30 minutes to smoke from burning Douglas fir under flaming conditions using the U.S. II apparatus for evaluation of flaming combustion/toxicity of smoke. The burning conditions were selected to provide a range from sufficient energy and oxygen to just sustain flaming above the smokes up to efficient flaming conditions of high flame height. Under inefficient burning conditions carbon monoxide evolved slowly and was found to be the principal toxicant. Under highly efficient burning conditions deaths also occurred but not due to CO. Combining all burning conditions an average LCs sub of 113 mg/L (95% confidence interval: 108 to 118 mg/L) was obtained for smoke from Douglas fir. No deaths were observed at a smoke exposure concentration below 76 mg/L for a 30 minute exposure. Lethality data were also analyzed to obtain median time to death, survival distribution function and cumulative hazard. These showed that although the smoke LCs sub was similar Lc sub for the burning conditions investigated, the time to effect was different. The cumulative hazard was highest under the most efficient flaming conditions producing low CO. Thus, the lethal effect of smoke from Douglas fir was not predicted from analysis of CO as was previously suggested. A "survivable exposure concentration" for humans exposed to smoke from Douglas fir for a 30 minute period can be set at 76 mg/L.

CARBOXYHEMOGLOBIN LEVELS IN RATS: EFFECTS OF ALTITUDE. J. J. McGrath, Dept. of Physiology, Texas Tech University HSC, Lubbock, TX.

In high altitude areas, ambient carbon monoxide (CO) concentrations are rising because of the growing number of new residents and tourists and their concomitant use of motor vehicles and heating appliances (fireplace, furnaces, stoves). These studies were conducted to assess the effects of altitude on endogenous carboxyhemoglobin (COHb) levels and on the rate of COHb build-up in rats inhaling 9 ppm CO (ambient air quality standard for CO). Male laboratory rats were exposed in barometric chambers to simulated elevations of 3300 ft (ambient), 10,000 ft, and 15,000 ft. Altitude, simulated by a system of gate valves and a vacuum pump, was measured by an altimeter. CO, from high pressure cylinders, was introduced into the air supply of each chamber through a mass flow controller and measured by an NDIR analyzer. COHb levels in animals not breathing CO and exposed to ambient, 10,000 ft, or 15,000 ft elevation, were 0.7, 1.3, and 1.7%, respectively. COHb levels in animals breathing 9 ppm CO and exposed to ambient, 10,000 ft, or 15,000 ft elevation were 1.0, 1.8, and 2.1%, respectively. These results suggest that the high altitude resident may have a greater initial body burden of COHb and will attain the COHb level associated with the National Ambient Air Quality Standard for CO more quickly than the low-altitude resident. (Supported by the Health Effects Institute)

SENSORY IRRITATION FROM THERMAL DECOMPOSITION PRODUCTS OF POLYETHYLENE. C. Pulkowski, V. Delrey and L. Bynum. Mobil Oil Corp., Princeton, NJ.

Thermal decomposition products (TDP) arise from polyethylene (PE) during extrusion of heated PE and subsequent heat-sealing of the resulting PE film to form bags and other products. Sensory irritation in factories resulting from exposure to TDP can be controlled by ventilation. The present work was conducted to help determine reasonable concentrations for potential subchronic studies with TDP. Concentrations of aerosol, gaseous acids, and gaseous aldehydes were measured as indicies of the complex components of TDP. Mice were exposed for 20 minutes to TDP from PE passing over a heater at 300°F or 700°F to simulate temperatures of extrusion and sealing. The amount of TDP produced at 300°F was not sufficient to cause irritation. TDP at 700°F caused significant depression in respiratory rate. Respiratory depression was plotted against concentration of components of TDP to estimate the concentration causing 50% depression from baseline breathing rate (RO50). The anticipated threshold of irritation in man (0.03 x RO50) was correlated to these components. For example, this threshold was correlated with TDP at 700°F to 0.79 mg of total aldehydes/m3, 0.14 mg of formaldehyde/m3, 0.08 mg of formic acid/m3, and 1.4 mg of aqresol/m3. No single component of TDP appeared to be solely responsible for its irritation although formaldehyde could be a major contributor.
RESPIRATORY RESPONSES OF MICE EXPOSED TO POLYMERS HEATED AT PROCESSING TEMPERATURES. M Schaper, R D Thompson, K Dewiler and Y Alarie. University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA.

In industry today, it is common to heat polymers for molding operations. Here, the following 4 materials were heated at 2 processing temperatures: Acrylonitrile Butadiene Styrene, Polypropylene Homopolymer and Copolymer, and Polyvinyl Chloride Compound. Each sample was placed on a mass sensor in a furnace and connected to an exposure chamber containing 4 mice. Continuous measurements of mouse respiratory frequency (f) and tidal volume (VT) were made. Each sample was heated from room temperature to the desired processing temperature and held 30 minutes isothermally. Sample mass loss was recorded and airborne particulate concentration was determined. Sensory irritation was seen in mice exposed to each heated polymer and decreases in f occurred which were proportional to 1) sample mass loaded in the furnace and 2) airborne particulate concentration. For the 4 samples, more irritation was observed at higher processing temperatures than at lower ones. Also, larger differences in potency of the 4 materials were observed at higher temperatures. This approach may be useful in identifying polymers capable of evoking irritation in the workplace. Supported by UAW-GM.


The present study was undertaken to investigate the effects of hexachlorobenzene (HCB) on the primate ovary. Cynomolgus monkeys, 4 per group were administered HCB daily for 12 weeks at 0., 0.1, 1.0 or 10 mg/kg body weight. On the menstrual period following dosing, ovulation was stimulated using pergonal and HCG. Animals were then anesthetized, ovarectomized and euthanized. One ovary was fixed in glutaraldehyde for electron microscopy (EM) and the other was processed for light microscopy (LM). Morphometric evaluation of the numbers of antral, preantral, and primordial follicles as well as the number of corpora lutea did not reveal any statistically significant differences. There was also no differences in the number of oocytes recovered or granulosa cell function. Dose-dependent ovarian lesions were observed at both the LM and EM levels in all treatment groups. These observations indicate HCB induces lesions in the primordial follicle that are not measurable in traditional tests of reproductive function.

OVARIAN TOXICITY OF CIGARETTE SMOKE IN MICE. C.G. Califoli and D.K. Culati, College of Pharmacy, Graduate Center for Toxicology, University of Kentucky, and Environmental Health Research & Testing, Inc. 2, Lexington, KY.

Epidemiological studies suggest that cigarette smoking is deleterious to female reproductive system. Our earlier studies showed that long term exposure to cigarette smoke induced alterations of the estrous cycle in mice. To determine if cigarette smoke causes ovarian toxicity, female C57Bl mice were exposed twice daily to mainstream cigarette smoke from the University of Kentucky reference cigarettes (3KI) for 50-60 weeks in a nose-only exposure system. Smoke exposure markers, viz., blood carboxyhemoglobin, pulmonary aryl hydrocarbon hydroxylase activity and urinary cotinine, were elevated in the smoke-exposed group suggesting effective exposure of animals to smoke. Serial sections of ovaries from smoke-exposed and age-matched, sham-treated, and control mice were evaluated microscopically for the number of follicles/ovary. The results showed that the number of primordial follicles in the ovaries of smoke-exposed mice was significantly reduced compared to controls. These results suggest a role of ovotoxicity in the adverse reproductive effects of cigarette smoking (supported by KTRB 5-41031).


Effects of Ketanserin (KET), a selective 5-HT, antagonist, on serum LH, FSH, prolactin (PRL) and estrogen (E2) levels and ovulation were assessed in normal cycling rats. Adult female Wistar-Ishiazuchi rats, which showed 2 or more consecutive regular 4-day cycles, were orally dosed at 10:30-11:30 on the day of proestrus at 10 mg/kg BW. Blood samples were collected by decapitation from 9:00 to 21:00, at 3 hrs interval, throughout a cycle. Number of oviducal ova was counted in the morning on each day to confirm ovulation. Significant decrease in E2 was observed shortly after KET treatment. The normal surges of LH, FSH and PRL and ovulation were blocked. The surges of LH, FSH, PRL, and E2 were shifted ca 24 hrs later. Ovulation was confirmed on 2 days after treatment and the vaginal smear remained cornified. The number of ova was not affected. It is suggested that KET has an inhibitory effect on ovulation in rats like other 5-HT, antagonists, and that E2 decrease plays an important role in KET-induced inhibition of ovulation.

The midcycle surge of luteinizing hormone (LH) from the mammalian female pituitary is required for ovulation and provides a useful biomarker in assessing toxicant effects on reproductive functions in non-primate animals. For such studies, daily LH surges can be seen in ovariectomized (OVX) female rats bearing estradiol (E2) implants. The present study evaluated the surge after treatment with melatonin (MEOH-4, 8, 16, 48 g/kg), the formamidine pesticides amitraz (AMT-7, 15, 30 mg/kg) and chloridorme (CDF-10, 25, 50 mg/kg), and the organophosphate pesticide malathion (MAL-25, 50, 100, 200 mg/kg). They were given as single intraperitoneal injections 2 days after OVX/E2-implantation and 5 hours before the expected LH peak, the sensitive period for surge disruption. Blood samples were taken at 1300, 1500, 1700, 1900 and 2100h and assayed for LH. Peak LH was affected at 1.6 g/kg MEOH and above. A partial suppression was seen with AMT at 7.5 mg/kg; a complete blockade occurred above this dose. CDF suppressed the surge at 25 and 50 mg/kg, while MAL at the levels used was ineffective. The surge reappeared on the following day. Effects of AMT and CDF are likely due to interference with α-adrenergic mechanisms of neurotransmission involved in the release from the brain of the hormonal trigger for LH. The cause of MEOH suppression is not clear, but may be associated with a known alteration by alcohol of LH release.

REPRODUCTIVE TOXICITY OF 4-VINYLCYCLOHEXENE AND 4-PHENYL-CYCLOHEXENE IN MICE. S R Hooser, L R Parola, D A Douds, P B Hoyer, and J G Stipes, Dept. of Pharm/Tox and Physiology, University of Arizona, Tucson, AZ.

4-Phenylcyclohexene (4PC), released from some latex-backed carpets, is structurally similar to the ovotoxicant, 4-vinylcyclohexene (VCH). Epoxidation of VCH by P-450 is a key in the destruction of primary follicles in mice. Because of its similarity, we wished to determine if 4PC also caused ovarian damage. B6C3F1 mice (n=15/group) were given sesame seed oil, 6 mmol/kg VCH, 6 mmol/kg 4PC (4PC-6), or 3 mmol/kg 4PC (4PC-3), ip, daily for 30 days. Daily vaginal smears determined the stage of estrus. On day 31, the mice were killed and the ovaries fixed and stained. Primary and secondary follicles in every 20th section were counted. The numbers of primary and secondary follicles (X ± s.d.) were reduced dramatically in VCH-treated mice (22 ± 10 and 26 ± 7), but not in controls (28 ± 63 and 115 ± 32), 4PC-6 (265 ± 53 and 110 ± 22), or 4PC-3 (278 ± 72 and 123 ± 20). The number of estrous cycles/30 days was significantly reduced in VCH (3.2 ± 1.0) and 4PC-6 (2.6 ± 0.7) compared to controls (4.8 ± 1.5) or 4PC-3 (4.2 ± 1.0). The mechanism of VCH ovotoxicity is unknown. Although substitution of a phenyl group (4PC) for a vinyl group (VCH) eliminates follicular loss, the number of estrous cycles is reduced for both VCH and 4PC-6, possibly indicating an adverse reproductive effect for 4PC. (Supported by March of Dimes grant #15-165).

rHRx is a recombinant human protein that is currently being developed as a cervical ripening agent. Single-dose clinical trials are now complete with rHRx administered intravaginally (IVAG) to healthy volunteers. Since topical routes of administration restrict dosage volume, 2 alternate formulations of 5 times higher concentration were developed for dose escalation studies. In order to determine the relevance of prior preclinical safety data generated with the initial formulation, it was necessary to examine the relative bioequivalence of the 3 formulations in rabbits based on irritation potential and bioavailability. Nonpregnant female rabbits were dosed IVAG with 0.55 mg (0.15 mg/kg) or 1.0 mg (0.61 to 0.63 mg/kg) rHRx once daily for 5 consecutive days. Animals were necropsied, and vaginal and cervical tissues were harvested and processed for histopathological evaluation 24 hr after the last dose. There were no test material-related overt clinical signs of toxicity. Macro- and microscopic evaluations did not reveal any evidence of irritation that could be attributed to the test formulations. The bioavailability of rHRx in these 3 formulations were determined in rabbits following a single IVAG dose. The serum concentrations of rHRx in blood samples collected over 48 hr were measured by ELISA. The bioavailability of rHRx in the 3 formulations was low (<5%). Based on these data, the 3 formulations were considered to be biologically similar, and the higher concentrations in the alternate formulations were determined to be suitable for clinical use.
DIRECT ASSESSMENT OF OOCYTE CHROMOSOMES AND PRE-IMPLANTATION EMBRYOS IN HAMSTERS EXPOSED TO METHYL BENZIMIDAZOLE CARBAMATE (MBC) DURING MEIOSIS. S Perreault, S Jeffay, R Barbee and B Libbus. US EPAMERL, NSI, and Genetic Research, Inc. RTP, NC. Sponsor: L E Gray, Jr.

A single oral dose of the microtubule poison, MBC, timed to coincide with meiosis I, causes infertility and reduced litter size with both pre- and early post-implantation loss (Toxicologist 10: 210, 1990). In this report, groups of female hamsters (n=10) were given a single high dose of MBC (1000 mg/kg) or corn oil during meiosis I (proestrus, PM) and either killed shortly after ovulation (d.1) to recover oocytes, or bred and killed on d.1-5 of pregnancy to recover embryos. Chromosome analysis in unfertilized oocytes revealed an MBC-induced increase in aneuploidy (37% vs. 13% in controls). When animals were bred after dosing, MBC had no effect on ovulation or the % eggs fertilized (d.1), but decreased the mean % cells per embryo (15% vs. 22% on d. 4), the % embryos reaching the blastocyst stage (53% vs. 87% on d. 4), and the rate of implantation on d.4 and 5. Such simple direct assessments are helpful in elucidating mechanisms (chromosome non-disjunction) of early pregnancy loss, and characterizing subsequent pre-implantation effects in the hamster animal model.


In vitro techniques were used to evaluate changes in ovarian steroidogenesis produced by in vivo toxicant exposure in both cycling and pregnant rats. The current effort has focused on identifying variables which indicate changes in the normal steroidogenic pattern and characterize a toxicant response using discriminant analysis. These variables include progesterone (P₄), estradiol (E₂), and testosterone (T) in both serum and ovarian culture. Ovarian steroidogenic profiles were obtained from minced ovaries in untreated, cycling and pregnant rats, and from bis(2-dicyethylxy) phthalate (DEHP) treated cycling or dibutyl phthalate (DBP) treated pregnant rats. A basal (without hCG) culture was used to profile one hour production of P₄, E₂, and T, and correctly identified 90% of the stages in untreated rats. Profiles following hCG stimulation for one hour improved stage identification to better than 95%. In DEHP or DBP treated rats, profiles from cultures identified treated animals and provided information on the site of toxicant action. In general, the culture hormone profile was more useful than the serum hormone profile in identifying normal and toxicant treated animals.

ASSESSMENT OF A SHORT-TERM REPRODUCTIVE AND DEVELOPMENTAL TOXICITY SCREEN. M W Harris, R E Chapin, A C Lockhart, J A Allen, E A Haskins, NTP/NIEHS, Research Triangle Park, NC.

Short-term tests for reproductive and developmental toxicity are needed to provide preliminary data on the toxicity of chemicals about which little or no data exist. An ideal design would test all aspects of reproduction and identify the target process in a short time period. One potential design has been evaluated using 4 chemicals of varying reproductive/developmental toxicity. Swiss mice were mated for 3 days prior to chemical exposure to produce timed-mated females for gestational exposure and to ascertain fertility of the untreated males. The group of time-mated females was treated during GD 8-14 and allowed to litter for postnatal (pu) observations through day 4. Endpoints observed include pup number and body weights on pnd 0,1, and 4 and number of implantation sites. A second group of females was dosed daily for 19 days. After 7d, these females (n=10/group) were cohabited with the male mice who had been treated for 5 d. prior to this second mating. Daily chemical dosing continued during the 5-day cohabitation. This second group of females was killed after 19 days of treatment and the number of live and dead fetuses and implantation sites was recorded. After 17 d. dosing, male mice were killed and the reproductive system evaluated by organ weights, total epididymal sperm counts and motility, and testicular histology. The 4 chemicals tested, Boric Acid, Ethylene Glycol, Ethylene Glycol Monomethyl Ether and Theophylline, impaired reproduction when tested in the NTP Continuous Breeding protocol. This short-term (24d) design correctly identified 3 of these 4 chemicals as reproductive toxicants. More chemicals will be tested to examine further the sensitivity of this design.

Weight loss is a common result of toxicant exposure that complicates data interpretation. To assess the effects of measured weight loss on reproductive endpoints, groups of 20 male and female SD rats were maintained at 90%, 80%, and 70% of control body weight (CBW) for up to 15 wks. During wks 7-8 and 14-15, males were mated with untreated females, and restricted females were subject to vaginal lavage to evaluate estrous cycle length. At wk 15, females were mated for 1 week with un-mated males, killed 14 d. after start of mating, and uterine contents examined. Diet restriction had no effect on male reproductive success at either time. Similarly, at necropsy, testis and epididymal weights and sperm morphology were unaffected. In all restricted groups, cycle length was increased at wk 7-8, but not at wk 14-15. There was a treatment-related increase in the number of resorptions/female, and 20% fewer live implants. Total number of implantation sites was unchanged by restriction. These data show that commonly measured reproductive parameters of the rat are not uniquely sensitive to decreases in body weight even to 70% CBW, implying that smaller changes seen in studies with test agents should not confound data interpretation.

THE EFFECTS OF DIETARY RESTRICTION ON REPRODUCTIVE ENDPOINTS IN SWISS MICE. D K Gulati, E Hope, R F Chapin. EHRT, Lexington, KY, and 1 NTP/NIEHS, RTP, NC.

Weight loss is one common sequel of toxicant exposure that may adversely affect reproduction. To try to relate weight loss and reproductive changes, groups of 20 male and female Swiss mice were maintained at 90%, 80%, and 70% of control body weight (CBW) for up to 16 weeks. Males were mated with 2 untreated females at wks 8 and 15. Females were vaginally lavaged wks 6-7 and 14-15. During wks 15-16, females were mated with untreated males, and necropsied on gd 14, and uterine contents assessed. At both time points, the 70% CBW group had fewer cycling females, and those that cycled had longer periods. At necropsy, the 70% CBW females had more resorptions and fewer total implants than controls. At their first mating, control males sired more litters than any restricted group; at wk 15, only the 70% CBW males sired fewer litters than controls; each litter had fewer live pups. At necropsy, most organ weights decreased less than body weight; testis weight was most resistant to this decline. There was a "dose"-related decrease in epididymal sperm density (significant at 70% CBW) and increase in abnormal sperm (in the 70 and 80% CBW groups). These data show that mice are sensitive to the effects of diet restriction, at just 10% difference from control.

A ONE GENERATION REPRODUCTION STUDY WITH ELEMENTAL PHOSPHORUS. E C Robinson, R S Nair, Monsanto Co., St. Louis, MO, and J L Schardien, International Research and Development Corp., Mattawan, MI.

Groups of 15 male and 30 female rats were given 0, 0.005, 0.015, or 0.075 mg/kg/day of elemental phosphorus (EP) in corn oil by gavage. Dosing began 80 days before mating and continued during mating, gestation, and lactation for two litters. All parental animals were observed twice daily for signs of toxicity, and detailed clinical observations were made weekly. Parental animals were weighed and food consumption was measured weekly during treatment. Specific observations included fertility, gestation length, behavior abnormalities, litter size and viability, pup survival through weaning, pup weight at birth and during lactation, and general appearance and behavior of pups. The most significant finding in this study was the death of 7 females in the Flb litter and 6 females in the Flb litter on gestation day 21 or 22 in the high dose group. No compound-related mortality was observed in males. A slight decrease in the number of viable fetuses was also observed in the high dose group especially in the Fla litter. The NOEL was considered to be 0.015 mg/kg/day. Additional studies are underway to further investigate this unusual effect.


In the rubber industry, MBT is used as an accelerator and as an intermediate in the manufacture of accelerators. Its effects on reproductive performance and fertility were examined in Sprague-Dawley rats (one litter per generation). MBT was administered to 28 rats/group in diets containing 0, 250, 8750, and 15000 ppm. Diet was provided to F0 and F1 animals for a minimum of 70 days prior to cohabitation, during cohabitation, and until scheduled sacrifice. Survival, clinical signs, reproductive indices, litter size, and pup birth weight and viability were comparable for control and treated animals of all generations. Body weights were significantly reduced for high dose F0 males and females prior to breeding and for F1 pups from the mid and high dose groups and F2 pups from all MBT dose groups beginning day 14 of lactation and continuing until sacrifice. Treatment-related weight and histopathological changes were seen in kidneys (primarily in males) of F0 and in livers and kidneys of F1 parental rats. Under the conditions of this study, minimal toxicity was observed in F0, F1, and F2 rats from all MBT-treated groups; however, no reproductive effects were observed in any generation treated with up to 15000 ppm MBT. (Supported by the CMA Rubber Additives Panel)

The Sprague Dawley rat was used to assess the reproductive and teratologic effects of the highly treated reclaimed water derived from secondary wastewater by the Denver Water Department. A two-generation reproduction study with teratology phase was conducted using test groups receiving a 500x concentration of reclaimed wastewater water or a 500x concentration of wastewater processed by an ultrafiltration process and control groups receiving commercially obtained distilled water or a 500x concentration of Denver's present high quality water. Fifty rats per sex in the first generation (F1) received the specified dosing regimen. From the offspring of this generation, 35 rats per sex/group (F2) received the appropriate drinking water through growth and maturation and during three breeding (F2a, b and c) and gestation periods. The offspring from the third breeding (F2c) will be evaluated for teratologic effects following gestation day 20 sacrifice. The evaluation of data from two generations of growth, breeding, gestation and lactation indicated no adverse reproductive effects from exposure to any of the dose-water regimens. The results of the teratology phase will be reported.

REPRODUCTIVE TOXICITY OF A CHEMICAL MIXTURE (MIX) ADMINISTERED IN THE DRINKING WATER TO SWISS MICE. *JL Heindel, JD George, PA Fail, and TB Grizzle. Research Triangle Institute, *National Toxicology Program, NIEHS, Research Triangle Park, NC.

The reproductive toxicity of a mixture of 25 chemicals (MIX) formulated to simulate groundwater supplies near hazardous waste dumps (Yang et al., FAAT 13, 366, 1989) was evaluated in CD-1 mice by the continuous breeding protocol. Mice received MIX in drinking water at 1%, 5%, and 10% of a technically achievable stock solution. F0 mice were allowed to breed through 18 weeks of exposure. However, litter size was significantly but minimally (10%) decreased at the 10% dose and the ratio of males born alive was slightly depressed at the 5% and 10% doses. F0 water consumption was depressed at 5% and 10% MIX probably due to poor palatability with normal feed consumption and body weights. F1 mice also had normal fertility with decreased adjusted live pup weight and a depressed ratio of males born alive in the presence of decreased water consumption (10% MIX). Body weights at necropsy were unaffected. Relative kidney/adrenal weight was elevated for males and females at 5% and 10% MIX; nephropathy was noted at 10% MIX. There was hepatic inflammation in the 5% and 10% MIX treated females. Sperm concentration was reduced 20%, at 1%, and 10% MIX. At 5% and 10% MIX the estrous cycle was significantly disrupted. Thus, despite the presence of low concentrations of several known reproductive toxicants, MIX caused only minimal reproductive effects in F0 and F1 mice. Supported by NTP, NIEHS, Contract No. NOI-ES-65141.

EFFECTS OF 4-SUBSTITUTED AMPHETAMINES ON INTRAUTERINE DEVELOPMENT AND PREGNANCY OUTCOME IN MICE. HS Butler, BC Foster and J Moffatt. Bureau of Drug Research, Health Protection Branch, Ottawa, Canada.

Amphetamines remain significant drugs of abuse and addiction. There is a lack of information on the comparative developmental toxicity of 4-substituted amphetamines such as 4-hydroxyamphetamine (4-HA), 4-methoxyamphetamine (4-MEA), 4-ethoxyamphetamine (4-ETA), 4-propoxyamphetamine (4-PPA) and 4-benzylxoyamphetamine (4-BEA). In this study, Swiss-Webster mice (9 animals/group) were given single doses (100 mg/kg) of an aqueous solution of different amphetamines by gavage on gestational days 6 through 18 (plug day = 1). The concurrent controls received distilled water alone. Mice were kept under standard animal husbandry conditions and had free access to food and water. As indicated by maternal body weight reduction or death, 4-MEA was most toxic while 4-PPA was least toxic to the dams. In comparison with the controls, the incidence of resorptions was low in the 4-PPA and 4-BEA groups, and high in the 4-ETA and 4-MEA treated mice. Apparently well formed but dead pups were delivered prematurely by 4-MEA treated dams. Delayed parturition occurred in 4-PPA and 4-BEA treated groups. Treatment with 4-BEA not only resulted in high incidence of cannibalism within 24 h after birth but also caused an increase in cumulative pup mortality during the first 3-weeks of age. Body weight gain was significantly lower in 6-wk-old male offspring exposed prenatally to 4-BEA than that of the controls. The results suggest that these amphetamines exhibit a wide variation in causing maternotoxicity and in producing adverse effects on the conceptus, parturition, pup survival and postnatal development.

REPRODUCTIVE TOXICITY OF 4-VINYLCYCLOHEXENE (VCH) IN MICE AS EVALUATED BY THE CONTINUOUS BREEDING PROTOCOL. PA Fail, JD George, TB Grizzle, J C Seely, and JL Heindel. Research Triangle Institute, PATHCD, Inc., and National Toxicology Program/NIEHS, RTP, NC.

VCH has been reported to reduce the number of primordial follicles and to cause ovarian tumors in B6C3F1 mice. We have therefore evaluated the reproductive toxicity of VCH using a continuous breeding protocol. Swiss (CD-1) mice were gavaged daily with VCH in corn oil at 0, 100, 250, or 500 mg/kg. For the F0 generation, VCH exposure for 18 weeks at any dose did not alter reproductive competence (litters/pair, pups/litter, % born alive) or feed or water consumption even in the presence of an 8% decrease in body weight (BW) of F1 pups and their dams at 500 mg/kg VCH. In the second generation (F1), reproductive competence and water consumption also were unaltered. At sacrifice, at 500 mg/kg VCH, F1 males and females had lower BW (7-8%), females had larger livers, and F2 pup BW was decreased by 5%; F1 males had decreased spermatid head count with normal spermatid number and testis and epididymal weight; F1 females had decreased numbers of primordial growing, and antral follicles. Thus, VCH, at a dose that decreased BW and reduced the number of developing gametes, did not alter reproductive competence in either F0 or F1 CD-1 mice in the time frame in this study (NIEHS/NTP Contract No. NOI-ES-65141).

Sensitive and biologically meaningful litter scores for the statistical analysis of rat resorption rate (r/m where r = resorptions, m = implants) were derived in a three-step process. First, since control resorption rate is much higher at low (e.g. 1-5) and high (e.g. 18-20) values of m, a likelihood score was derived for each (r, m) couplet based on the incidence of that couplet in 1379 control Sprague-Dawley rat litters examined between 1978 and 1988. These scores were similar to the raw values r. Second, since treatment-induced increases in resorption rate tended to occur as increased numbers of resorptions within affected litters rather than as increases in the number of affected litters (based on 126 litters from 7 groups with slight increases in resorption rate), the scores for control-like litters (r = 1, 2, 3) were downgraded. Third, the modified score from step 2 was divided by an expected score given m to make the scores essentially independent of litter size. Simulation studies were used to show that statistical analysis using the final "robust" scores provides more power for detecting treatment effects than several competing statistical methods, and is relatively unaffected by differences between treatment groups in average litter size.

POSTNATAL LEAD (Pb) EXPOSURE INCREASES D. AND D. DOPAMINE RECEPTOR NUMBER DURING DEVELOPMENT. D V Widzowski, D A Cory-Slechta, and J N Finkelstein. Environmental Health Sciences Center, Univ. of Rochester School of Med. & Dent., Rochester, NY.

Postnatal Pb exposure has been shown to alter development of dopaminergic (DA) systems, changes which have been suggested as mechanisms of Pb's behavioral effects. Previous studies have usually examined Pb-induced DA alterations at only one time point after postnatal exposure. DA systems in the rat develop during the first 4 weeks postnatally. This study examined the effects of low level Pb exposure on the ontogeny of DA systems to determine the time course of Pb effects. Lactating dams were exposed to lead acetate (0, 350, or 1000 ppm) in drinking water. Saturation binding analysis indicated that Pb exposure beginning on day 0 (birth) dramatically increased both DA D_1 and D_2 receptor number (B_max) in the striatum, midbrain, and frontal cortex. Doubling receptor number as early as 7 days of age. Low Pb level exposures (350 ppm) increased B_max more than high level exposures (1000 ppm), suggesting a U-shaped dose effect function (e.g striatal D_1, B_max day 14: 622.1352, 1046 fmoi/mg protein for 0, 350, 1000 ppm Pb); there were no consistent changes in receptor affinity (K_i). Neither body weights, brain weights or regional protein content were significantly altered by Pb exposure. These results suggest a possible neurochemical correlate for behavioral supersensitivity observed in rats exposed postnatally to Pb as manifested in altered drug discrimination performance.

Substrate-Dependent Calcium-And Lead Effects on Retinal Mitochondrial Respiration and Oxidative Phosphorylation. C J Medrano and D A Fox. College of Optometry, U. of Houston, Houston, TX.

Our previous studies demonstrate that perinatal Pb exposure produces a selective rod photoreceptor degeneration. To test the hypothesis that Pb-induced rod cell death may be due to Ca-mediated alterations in mitochondrial energy metabolism, we examined the effects of Ca^{2+} or Pb^{2+} on State 4 and 3 respiration and oxidative phosphorylation (ADP/O) in isolated rat retinal mitochondria. To examine possible substrate-dependent differences, we used NAD-linked substrates glutamate plus malate (G/M) or FAD-linked substrate succinate with rotenone (S/R). With G/M, State 4 is decreased 7-29% and 9-18% with 70mM-300mM Ca^{2+} or 1.2pM-200uM Pb^{2+}, respectively. With S/R, [Ca^{2+}] = 100uM or [Pb^{2+}] = 2.5pM produce similar decreases in State 4. With G/M, State 3, respiratory control ratio and FCCP-induced respiration are decreased 33-76% and 37-53% with 6-300uM Ca^{2+} or 28pM-200uM Pb^{2+}, respectively. With S/R, State 3 and FCCP-induced respiration decrease 59-46% and 21-27% with 300uM Ca^{2+} or 200uM Pb^{2+}, respectively. ADP/O was unaffected by Ca^{2+} but decreased 21-27% with 200uM Pb^{2+}. Thus, Ca^{2+} or Pb^{2+} preferentially inhibit NAD-linked respiration with Pb^{2+} possessing a 2-3 log unit greater potency. These results suggest that lead's direct effect on mitochondrial energy metabolism may play a role in the Pb-induced selective rod degeneration. Supported by ES 03183.

LEAD (Pb) INDUCES FUNCTIONAL D. AND D. DOPAMINERGIC SUPERSENSITIVITY. D A Cory-Slechta and D V Widzowski. Environmental Health Sciences Center, Univ. of Rochester School of Med. & Dent., Rochester, NY.

Changes in dopaminergic (DA) systems have been suggested as a mechanism of Pb's behavioral effects. To determine whether a role of DA systems in such effects was credible, the hypothesis that Pb produced functional changes in DA sensitivity was examined using drug discrimination (DD) procedures. Rats exposed to 0, 50 or 250 ppm Pb acetate in drinking water from weaning were trained to discriminate either 0.05 mg/kg i.p. quinpirole (D_1 agonist) or 3.0 mg/kg i.p. SKF38393 (D_2 agonist) from saline. Several lines of evidence demonstrated that Pb exposure enhanced DA sensitivity to both D_1 and D_2 agonists: 1) Pb rats learned the DDs in fewer sessions than controls, indicative of functionally higher training doses; 2) Pb sensitivity dose-effect curves were shifted upwards and leftwards of controls; 3) 0.08 mg/kg s.c. haloperidol blocked drug-lever responding of D_2/saline controls, but not of Pb rats. The generality of DA supersensitivity in Pb rats was confirmed by increased drug-lever responding to other DA agents, including cocaine (7.5 mg/kg i.p.), apomorphine (0.08 mg/kg i.p.) and d-amphetamine (1.0 mg/kg i.p.). Pentobarbital (5.0 mg/kg i.p.), a non-DA compound, produced no differential sensitivity, indicating that the DA effects were selective. The context of these experiments and did not reflect non-specific performance effects. Both Pb exposure levels were associated with DA supersensitivity which occurred in the absence of any group differences in response rate.
Previous studies have suggested that chronic Pb exposure results in impaired regulation of CNS dopamine (DA) synthesis in rats. The present study was designed to directly assess TH activity in exposed animals compared to controls, employing a pharmacological model that assesses the functional status of dopaminergic synthesis-modulating autoreceptors. At birth, dams received 0.2% Pb acetate in drinking water while control dams received distilled water. Offspring were weaned and maintained on the same solution until termination at 60 or 120 days. Rats were given saline or a DA agonist (EMD 23448 or CGS 15855A) 45 min before sacrifice followed 15 min later by gamma-butyrolactone (GBL). Regional TH activity was measured by a modification of the tritium release method. DA content was determined by liquid chromatography. The ability of EMD 23448 to prevent the GBL-induced increase in DA content was significantly diminished in caudate-putamen (C-P) of exposed rats compared to controls, similar to previous observations. However, an analogous effect of Pb on TH activity in this drug model was not observed using CGS 15855A in rats either 60 or 120 days of age. These findings suggest that chronic Pb exposure has no effect on autoreceptor-mediated regulation of TH in DA neurons when TH activity is measured ex vivo. (Supported by ES 04359 and the Children's Miracle Network)

Using a single capillary passage technique (Oldendorf, 1970), the transport of Mn across the rat blood-brain barrier (BBB) was characterized. The initial rate (15 sec.) of accumulation of Mn²⁺ (0-1000 µM) in rat brains was dependent on the injected Mn concentration. The hyperbolic rate vs. Mn concentration profiles were diagnostic of a simple diffusion process. Reciprocal plots of the uptake profiles have a zero intercept on the 1/V, axis, further conforming to a simple diffusion process. Using the same technique, the effect of transferrin (TF), the principal Fe-carrying protein of plasma, on the initial rate of brain Mn uptake was studied. Common carotid injection of freshly mixed Mn²⁺ with TF (at a 1:1 to 1:10 molar ratio) did not result in a significant change in brain Mn levels compared to animals injected with Mn²⁺ in the absence of TF. However, when Mn²⁺ was incubated in the presence of TF at a 1:10 ratio (10 and 100 µM, respectively) for 1-5 days, the initial rate of Mn uptake across the BBB was time-dependent. Since Mn binds to TF exclusively in its trivalent oxidation state, and since molecular oxygen is known to oxidize Mn²⁺ to Mn³⁺, increased brain uptake of Mn under these conditions appears to be related to the generation of trivalent Mn species. (Supported by ES 05223).

The cellular composition of primary mixed neural cultures may vary between preparations. Differences of sensitivity to toxic chemicals between cell types cannot be adequately evaluated by such endpoints as lactate dehydrogenase release. Potential histochemical markers, for the morphological and functional integrity of neural cells, were studied in mouse primary cerebrocortical cultures. Neuron specific enolase (NSE), glial fibrillary acidic protein (GFAP), myelin basic protein (MBP), vimentin (Vi) and glutathione-S-transferase (GST) were revealed by immunohistochemistry, and naphthyl butyrate esterase (NBE) and gammaglutamyl transferase (GGT) by enzyme histochemistry. No MBP reactivity was found, suggesting the absence of oligodendrocytes NSE and GFAP identified neurons and astrocytes, respectively. In cultures exposed to 0.1 mM imipramine for 1 hr, NSE and GFAP allowed the identification of the specific neuronotrophic effect of this chemical. Vi was found in both cell types, as it is in the developing nervous system in vivo. GST was revealed in both neurons and glia, which correlated with CNDB-transferase activity assays. GST immunoreactivity was not altered by exposure to methyl iodide (5 mM for 5 min), indicating that the irreversible inhibition of GST enzyme activity, known to be produced by methyl iodide, is not a consequence of proteolysis. NBE activity was only observed in a few astrocytes (2-5%) of control cultures but was induced after methyl iodide exposure and expressed by about 90% of the treated glial cells. It is expected that such markers will provide means for revealing subtle injuries directly relevant to primary neurotoxic and/or glutate effects associated with many neurotoxins.

Circulating MEL and prolactin (PRL) levels are decreased in steers on Acremonium coenophialum (endophyte) infected tall fescue (TEF). Erog peptide alkaloids produced by the endophyte and found in TEF are suggested as the cause of reduced PRL and other toxicities in livestock. Using the rat as a model, PRL was reduced with both BEC and extracts from BEC. Subsequently, a preliminary study was conducted to determine if BEC also reduced pineal MEL and NAC5HT (precursor to MEL) in rats. Male Sprague-Dawley rats (4 wk, n=20) were maintained (6 wk) under a 12 hr light/dark cycle, randomized to weight and dosed (0.25 mg/kg; i.p.) with BEC or vehicle (0.85% saline) 3 hr prior to lights out. Six hr later, rats were killed under red light, pinealas collected, pooled (5/group; n=2 groups/trt) and stored (-80°C) until analyzed (HPLC/BEC) for MEL and NAC5HT. BEC treated rats vs controls had numerically reduced NAC5HT (P<0.001) and MEL (2.494 ± 0.771 vs 2.772 ± 0.110 ng/mid. pineal, P<0.05) respectively, with significantly increased MEL/NAC5HT ratios (0.188 ± 0.004 vs 0.136 ± 0.011, P<0.03). The data suggest properly timed BEC administration may reduce pineal NAC5HT and MEL and thus warrants further investigations.
AN INVESTIGATION INTO THE APPARENT NEUROTOXICITY OF THE PHYTOTOXICINS FOUND IN BRUNFELSIA CALYCNIA VAR. FLORIBUNDA. C B Spinhour, Jr., J C Reagor and W Flory. Texas Veterinary Medical Diagnostic Laboratory, College Station, TX.

Brunfelsia calycula var. floribunda is an ornamental garden shrub belonging to the family Solanaceae and is quite commonly found in the United States. Toxicologic studies were performed on mice and rats with crude aqueous homogenates of shrub material after the suspected death of a canine. These experiments demonstrated that all parts of this woody plant are neurotoxic, but unequally so. All plant preparations produced signs similar to those produced by a central nervous system convulsant, such as any of the diamine compounds found in plants of the Lathyrus spp. There were no pathognomonic gross pathologic or histopathologic findings associated with the intoxications produced in laboratory animals with Brunfelsia preparations. There are, however, distinct species differences between the responses of mice and rats to exposure to these plant extracts. The toxicons from this shrub are water soluble and highly stable. The ability of aqueous extracts stored at 4 °C to reproduce the original clinical syndrome and percent mortality remained unchanged over a period of four months. Possible mechanisms of toxicity and the chemical nature of the toxicons are discussed.

INTERACTIONS OF ETHANOL AND DIAZEPAM ON HIPPOCAMAL LONG TERM POTENTIATION. M J Wayner, J L Polan-Curtain and D L Armstrong. University of Texas at San Antonio, San Antonio, TX.

Within a limited range of doses, ethanol (ETOH) and diazepam (DZ) when co-administered produce an enhanced anxiolytic effect of either drug alone. These combined effects on long term potentiation (LTP) in the hippocampus of rats under urethane anesthesia were studied in an attempt to provide an explanation at a more fundamental neuronal level. Male Sprague-Dawley rats received 0.5, 1.0, 1.5 or 2.0 g/kg 25% ETOH by gavage or 1.0 mg/kg DZ by intraperitoneal injection thirty minutes prior to tetanic stimulation of the perforant path and measuring the resulting LTP in terms of potentiation of the summated excitatory postsynaptic potential. The effects of both drugs alone and in combination on LTP will be presented. In general, although ETOH decreased LTP (maximum decrease of -0.13 ± 4.15% at 2.0 g/kg) and DZ increased LTP (47.09 ± 10.16%) enhanced interactive effects were observed with combined doses. A neuronal mechanism is now available for further studies at the neuronal level.

DIFFERENTIAL BEHAVIORAL RESPONSES OF WISTAR-KYOTO (WKY) AND SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AFTER CONTINUOUS COCAINE INFUSION. Z J Yu, M F Huang, K L Tharp, B Hoskins, R W Rockhold, and L K Ho. Dept. of Pharmacology and Toxicology, Univ. Mississippi Med. Ctr., Jackson, MS.

Studies of behavioral responses to cocaine were conducted in 9-week-old WKY and SHR following continuous i.v. infusions of cocaine (60 mg/kg/day) or saline (120 µl/kg/day) for 3, 7, or 14 days. Twenty-four hours after the infusions were terminated, the rats were challenged with cocaine (40 mg/kg, s.c.). Body weights did not differ between groups during the infusion period. Rectal temperatures increased after 2 days of cocaine infusion and declined to normal over the 14-day infusion period. The SHR had a higher mortality rate (13%, n = 108) than did WKY (3.7%, n = 106) during the cocaine infusion periods although plasma concentrations of cocaine were maintained at a plateau of 167 ± 23 mg/ml in both strains. Similar increases in locomotor activity were observed following cocaine challenge to saline-infused WKY-SHR. The pattern of cocaine-induced stereotypies did differ between strains, i.e., bobbing dominated in WKY while rearing and sudden turning dominated in SHR. No differences were observed in plasma concentrations of cocaine between acute and challenge injections. In contrast, there was a marked reduction in post-challenge locomotor activity as the infusion period lengthened, indicated development of tolerance. However, no tolerance appeared to develop to the cocaine-induced stereotypies. (Supported DA 04264).


Increased alcohol consumption by adolescents and teenagers has heightened awareness of potential endocrine and developmental alterations. The current study was designed to determine whole-organ weight, plasma corticosterone, and adrenal function in the developing rat. One month old male Sprague Dawley rats were administered 6% ethanol in drinking water and controls received 12% sucrose in the drinking water to balance caloric intake. After one month of treatment animals were sacrificed at 0900 by decapitation, and blood, pituitary and adrenal glands collected. Plasma was collected and stored at -20°C until assayed for ACTH and corticosterone (CS) by radioimmunoassay (RIA). Five anterior pituitary glands per group were quartered and challenged with 100 µM corticotropin releasing factor (CRF) for 90 min at 37°C under 95% air/ 5% CO_2. The remaining five anterior pituitary glands in each group were immediately frozen and stored in liquid nitrogen until extraction with 0.1N HCl for ACTH determination. Adrenal glands were quartered and challenged with 100 µM ACTH for 90 min at 37°C, 95% air/ 5% CO_2. Media were analysed for either ACTH (pituitary) or CS (adrenal) by RIA. Plasma ACTH and CS were unaffected by ethanol consumption. Pituitary response to CRF was not altered by ethanol. The lack of difference in ACTH release was not due to differences in pituitary content of ACTH. However, chronic ethanol consumption did decrease adrenal responsiveness to ACTH stimulation. In vitro corticosterone production was 1.21±0.14 µg/adrenal in controls and 0.70±0.06 µg/adrenal in ethanol consuming rats. The results of this study suggest that chronic ethanol exposure in the developing rat decreases the ability of the adrenal gland to respond to ACTH stimulation, suggesting a decreased ability of not adrenal to respond to stress.

Single exposure oral toxicity studies were performed in Sprague-Dawley rats with undiluted VP, an intermediate in coatings manufacturing. Rats were treated with a single peroral (gavage) dose of 2 or 4 ml VP/kg body weight in an acute toxicity study and sacrificed 14 days later. Additional rats were treated with 0, 2, or 3 ml VP/kg body weight in a follow-up neurotoxicity study and sacrificed 3 or 15 days later. Behavioral and histological evaluations (light and EM) were performed. Two of 4 animals at 4 ml/kg and all 6 animals at 3 ml/kg died within two days of treatment. Microscopic lesions of the nervous system were not observed for animals that died. Behavioral findings for the remaining animals included gait alterations (lowered posture, hind limb splay, waddling, and/or exaggerated hind foot placement), decreased hind limb grip strength, increased landing foot splay, and hypoaesthesia. Microscopic lesions observed following treatment with 2 or 4 ml/kg VP included minimal to marked myelin sheath swelling and degeneration that appeared to be most consistent with a primary myeloneuropathy. These changes occurred primarily within the ventral and lateral funiculus of the spinal cord and extended into the central portions of the medulla and pons in some cases. The severity of the lesions increased with increasing dose and tended to be greater with increased time after treatment.

382 LACK OF NEUROTOXIC EFFECTS FROM INHALED STYRENE MONOMER IN MALE RATS. J P Yermakoffs, B M Ryan, N S Hatoum, W D Johnson and R G Farmer. Amoco Corporation and IIT Research Institute, Chicago, IL.

Styrene monomer is used in making polymeric plastic, protective coatings, styrenated polyesters, copolymer resins and as a chemical intermediate. In an effort to assess its neurotoxic potential, Crl-CD® male rats (23/group) were exposed to styrene vapor by inhalation for 6 hrs per day at target concentrations of 50, 100, 200 and 1400 ppm for 28, 14, 7 or 1 consecutive day(s), respectively. Four concurrent control groups of equal size were exposed to filtered air following the same regimen. A functional observational battery (F.O.B.) was performed to evaluate neuromuscular function, reflex response and sensory perception a week prior to exposure (i.e., baseline) and after the first exposure. F.O.B. was also performed at intervals selected for equivalent "cumulative dose" (i.e., concentration x duration) for comparative purposes, as well as at 2 and 4 weeks following the last exposure to assess delayed effects and/or recovery. Three rats/group were sacrificed immediately following each F.O.B. (except baseline) and tissues were fixed in situ. Selected nervous tissues were processed for microscopic examination with special staining of myelin, neuronal bodies and axons. None of the rats died during the study. No treatment-related neurotoxic effects were detected in any F.O.B. parameter at any time during the study. No exposure-related microscopic lesions were observed in the brain, sciatic nerve or spinal cord. Therefore, exposure to 50, 100, 200 or 1400 ppm styrene monomer for various durations did not result in any neurotoxic effects in the male rat.


Determination of radiation dosage, regimen & time needed to produce delayed brain irradiation necrosis (IBIN) was studied using 20 male New Zealand white rabbits. The cerebral cortex of each rabbit received a single dose of radiation using a Theratron 780 Cobalt unit with a radiation source of 60 Co gamma rays. Group 1 (n=6), group 2 (n=6) & group 3 (n=8) received a dose of 2499, 3249 & 3993 rads respectively. After irradiation, a daily functional observational battery and biweekly body weights and neurobehavioral observations were performed. Selected animals were sacrificed at 4, 8, 16 & 28 wks after irradiation. Alopecia at the irradiation site was noted in 6/6 (group 2) and 6/8 (group 3) rabbits. Behavioral changes indicative of brain necrosis occurred towards the latter stage of the study. Pathological evidence of brain necrosis was first seen at 20, 16 & 28 wks for groups 3, 2 & 1 respectively. In all, 2/6 in group 1, 5/6 in group 2 & 2/8 in group 3 showed brain necrosis. It was concluded that a single dose of 2499 to 3993 rads could induce IBIN in some rabbits. A dose of 3250 rads with a 7-month post dose observation period was selected for a future study.


MBT, a major rubber vulcanization accelerator, was tested for neurotoxicity in rats according to EPA TSCA protocols. After an initial range-finding study, MBT was administered by gavage to groups of 12 rats per sex at 0, 500, 1250 or 2750 mg/kg for acute testing, or at 0, 5,000, 15,000 or 25,000 ppm in the diet for 13 weeks. Animals were examined by conducting a functional observational battery (FOB), determination of a motor activity test (MAT) and also by extensive neuropathology evaluations in the subchronic study. In the acute study, both sexes of rats had decreased motor activity at 12 hours post-dosing in the 2750 mg/kg group and females treated with 1250 mg/kg also exhibited decreased motor activity. The FOB showed transitory changes suggestive of a generalized toxicity and no clear indication of neurotoxicity. Assessments for CNS disorders, muscle tone, equilibrium and sensory function did not show any evidence of treatment-related effects in the acute or the subchronic study. No significant effects were observed for the MAT or on gross or microscopic pathology in the subchronic study although there were significant effects on body weight gain and food intake in the 15,000 and 25,000 ppm groups. (Supported by the CMA Rubber Additives Panel)
FOUR-WEEK INHALATION TOXICITY STUDY OF ETHYL tert-BUTYL ETHER IN RATS. B M Ryan, J S Ferguson, N S Hatoun, M J Klan and R O Farmer. JH Research Institute and Amoco Corporation, Chicago, IL.

Ethyl tert-Butyl Ether (ETBE) is a fuel additive with octane boosting capacity. Its addition also increases oxygen content of the fuel resulting in a more complete combustion. Currently, ETBE is being considered for use in reformulated gasoline, therefore, in an effort to assess its subchronic toxicity, ETBE was administered by inhalation as a vapor at target concentrations of 0, 500, 2000 and 4000 ppm to 10 C57/B16 rats/group. Rats were exposed 6 hours, 5 days/week for four weeks. Parameters monitored included body and organ weights, lung volume, hematology, clinical chemistry, gross and microscopic pathology, in addition to a functional observational battery (F.O.B.) which evaluated neuromuscular function, reflex response and sensory perception once/week prior to exposure (i.e., baseline), and after 1, 5 and 20 exposures. None of the rats died during the study. Body weight/body weight gain and lung weight/volume were not affected by exposure. Signs of CNS depression (stasis/latency) were observed in the 4000 ppm group during the daily exposure periods with complete recovery being noted shortly after exposure termination. Changes in body temperature and hindlimb splay pattern were also detected in the 4000 ppm group. No significant alterations were observed in the other F.O.B. parameters evaluated. Significant increases in absolute and relative organ weights were noted in the liver, kidneys and adrenals of the 4000 ppm males as well as an increase in liver weights for females of the 2000 and 4000 ppm groups. White blood cell counts were increased in females of the 2000 and 4000 ppm groups. No histopathologic findings were associated with the organ weight changes or increased WBC counts. Thus, repeated exposure to ETBE produced transient signs of CNS depression at 4000 ppm and associated changes in body temperature and possibly hindlimb splay. Also, exposure at the two highest levels caused select organ weight increases with no underlying histopathology.

ASSESSMENT OF OFFSPRING DEVELOPMENT AND BEHAVIOR FOLLOWING GESTATIONAL EXPOSURE TO INHALED METHANOOL IN THE RAT. NE Stanton, EM Cragon, LE Gray, CM Gordon, EU Bushnell, M Mole and DB Peale, US EPA and NIST Technology Services, RTP, NC.

The prospect of widespread human exposure associated with its use as an alternative fuel has sparked concern about the toxic potential of inhaled methanol (MeOH). Previous studies have revealed congenital malformations following inhalation of MeOH (Nelson et al., JDT, 1985, 5, 727-736) but these studies have not included postnatal behavioral assessment. In the present study Long-Evans females were placed in exposure chambers containing 15,000 ppm MeOH or air for 7 hr/day on Gestational Days (GD) 7-19. Maternal body weights (BW) were recorded daily and blood methanol determinations were performed at the end of exposure on GD7, 10, 13, and 17. Following birth (Postnatal Day 0 [PD0]), a number of tests were performed on the offspring, including: 

- offspring mortality and BW (PD1, 3), motor activity (PD13-21, 30, 60), olfactory learning (PD18), behavioral thermoregulation (PD20-21), T-maze learning (PD23-24), acoustic startle response (PD24, 60), pupertal landmarks (PD31-56), and passive avoidance (PD60).

MeOH levels remained at about 3000 ug/mL through-out exposure. MeOH transiently reduced maternal BW (4-7%) on GD8-10, and offspring BW (5%) on PD1 and 3. The other tests failed to reveal significant effects. Prenatal exposure to high levels of inhaled MeOH appears to have little effect beyond PD0 in this series of tests.

EFFECTS OF EXPOSURE TO 50 OR 400 PPM TOLUENE ONFLASH EVOKED POTENTIALS AND EEG POWER SPECTRUM IN MAN. James D Prah, U S Environmental Protection Agency, Research Triangle Park, NC.

Toluene is commonly found in the environment and is an abused solvent. The goal of this study was to examine the effects of toluene on electrophysiology. Males (n=33) were exposed to 50 or 400 ppm toluene for 2 hr. After 88 min of toluene exposure, an "odd-ball" auditory evoked potential paradigm (AEP) with 40 odd trials was presented. This test was followed by a flash evoked potential (FEP) test in which the subjects received 60 paired flashes; each flash was separated by .2 sec and the pairs were pseudorandomly delivered. Data were collected for 2.0 sec during each paradigm. The last 1.0 sec of each data vector was used for EEG power spectrum analysis. Comparisons of the two groups revealed marginally reduced positive peaks in the AEP and significant reductions in the positive peak amplitude of the FEP in the 400 ppm group. EEG power spectrum post-AEP and post-FEP revealed significant reductions in delta, theta, and alpha band power in the 400 ppm group. Subjects could not determine which level they received, thus were blind to exposure level. These results indicate that toluene at 400 ppm for 2 hr produced significant decrements in FEP and EEG power spectrum and trends toward reduced AEP and FEP responses (BPV). Minimal reduction in CNS responsiveness may explain these results. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.


Male Sprague-Dawley rats received daily intravenous doses of 0, 100, 200, 300 or 400 mg/kg misidazole 5 days/week. Animals were evaluated for behavior, tibial nerve ß-glucuronidase activity, nerve conduction velocity, and neuropathology following termination of treatment and during a 4-week recovery period. During the treatment phase, decreased conduction velocity and paralysis were noted. Slight decreases in olfactory and auditory evoked potentials were observed at 400 mg/kg, and significant body weight gain and food consumption suppression occurred at both 300 and 400 mg/kg. No treatment-related effects were apparent for motor activity, acoustic startle response, forelimb grip strength, tail and toe pinch reflexes, ß-glucuronidase activity, or nerve conduction velocity. However, 400 mg/kg rats exhibited a significant deficit in rotorod performance of increasing magnitude from Study Day 15 to 43, as well as diminished hindlimb grip strength (Day 15). Necrosis of brain tissue, primarily in the central cerebellum, vestibular, olivary, and cerebellar regions, occurred at 400 mg/kg on termination of treatment. Gliosis of brain tissue, indicative of tissue reparative processes, was observed in these regions 4 weeks post-dosing at 300 and 400 mg/kg. Results of this study indicate that short-term intravenous administration of misidazole to rats produces central nervous system toxicity.
The dose-setting goal for subchronic neurofunctional studies is to select exposure concentrations that are high enough to challenge the nervous system, but not so high that they cause complications from organ toxicity or excessive sedation that make neurofunctional data difficult or impossible to interpret. This defines, in a sense, a neurofunctional maximum tolerated dose. Acute solvent induced sedative alterations in nervous system function (CNS challenge) readily can be assessed with electroencephalograms (EEG) and evoked potential (EP) electrophysiology.

Acute EEG and EP data were used in high-concentration selection for a subchronic neurotoxicity evaluation of 1,1,1-trichloroethane (1,1,1-T), a solvent with anesthetic properties. This study was conducted as part of a United States Environmental Protection Agency (USEPA) consent agreement. EEG, flash evoked potentials (FEPs) and somatosensory evoked potentials (SEPs) were recorded before exposure started and during exposure at approximately 6 hours after exposures were initiated. Acute neuropharmacologic EEG and EP changes were observed at 2000 ppm and at 1000 ppm. Other studies demonstrate mild hepatic effects at 2000 ppm. Consequently, USEPA approved a 2000 ppm high-concentration for the 1,1,1-T subchronic neurotoxicity study.

*Study sponsored by the Halogenated Solvents Industry Alliance.

A COMPARISON OF THE EFFECTS OF DICHLOROMETHANE AND 1,2-DICHLOROBENZENE ON PEAK N190 AMPLITUDE OF FLASH EVOKED POTENTIALS IN RATS. DW Herr, MS Bercegenay, and WK Boyes. Neurotoxicology Division, USEPA, Res. Tri. Plk, NC.

Peak N190 amplitude of flash evoked potentials (FEPs) is decreased following exposure to several solvents. Lipid solubility is an important factor which affects the potential of a xenobiotic to gain access to the central nervous system. We are examining the ability of dichlorinated solvents with varying lipid solubilities (log Ps) to alter the amplitude of FEP peak N190 in Long Evans rats. Animals were chronically implanted with epidural electrodes over the visual cortex and FEPs were recorded after dosing (ip, in corn oil) with 0.0635, 0.125, 0.25, or 0.5 of the LD50 of dichloromethane (DCM; log P = 3.38; 53 106, 219, 430 mg/kg). Both DCM and DCB increased peak latencies and decreased colonic temperature. DCM did not significantly alter the amplitude of peak N190. However, the amplitudes of peaks N90 and P90 were decreased by DCM 15 min after dosing. DCB significantly decreased peak N190 amplitude at 0.5, 1, 2, and 4 hr after dosing. Other peak amplitudes were not significantly altered by DCB. Data indicate that the relative ability of compounds to alter FEP peak N190 amplitude may be related to their lipid solubilities. However, alterations in other portions of FEPs indicate that lipid solubility is not the only factor which determines the potential of a substance to produce neurologic effects. This work was supported by a NRCC Research Associateship.
Rats exposed to power-frequency electric fields exhibit an appreciable reduction in the nocturnal rise in melatonin content of the pineal gland. Because nocturnal release of melatonin is a key element in the regulation of circadian rhythms, electric field exposure might disrupt circadian integration, producing diverse effects. We are conducting an experiment to determine if combined 60-Hz electric and magnetic field exposure produces melatonin suppression in a nonhuman primate. Using an exposure apparatus providing 6 kV/m electric fields and 0.5 G magnetic fields, baboons (Papio cynocephalus) are being exposed for 12 hours a day for a period of six weeks. All six animals (three experimental and three-sham exposed) have been fitted with catheters so that blood samples may be collected automatically by a saline infusion and blood withdrawal system. Once a week, sampling is conducted for 24 hours; blood is taken at 2-hour intervals for analysis of melatonin. The subjects have been sampled three times prior to the initiation of field exposure; sampling will continue bi-weekly through exposure and post-exposure periods. This research is sponsored by DOE’s Office of Energy Management.

Vardenafil (V) has been shown to have a number of insulin-like effects and has been demonstrated to be beneficial in the treatment of type 1 diabetic rats when included in the drinking water. However, some signs of toxicity were reported in the V-treated animals. Moreover, V accumulated in all tissues analyzed. In the present study, the effect of repeated intraperitoneal administration of 4,5-dihydroxy-L-phenylalanine (DA), ascorbic acid, and dopamine methyl ester (DMPE) alone or 2-mercaptoethanol (ME) on the distribution and excretion of V was determined in male Sprague-Dawley rats which had previously received sodium metavanadate, sodium orthovanadate, or vardenafil sulfate pentahydrate in the drinking water at concentrations of 0.15 mg/kg (NaVO₃), 0.25 mg/kg (Na₂VO₃), and 0.35 mg/kg (Na₂H₂PO₄) for 3 weeks. Chelating agents were administered for 2 weeks at doses of approximately equal to one-tenth of their respective LD₅₀. Urine was collected on days 1, 3, 7, and 14 of treatment. 24-h after the final chelator injection the rats were killed and the concentrations of V were determined in their tissues. Treatment with DMPE was effective in mobilizing V after Na₂VO₃ or Na₂H₂PO₄ administration, whereas DA was also the most effective chelator in the removal of V from the rats after vardenafil sulfate administration. Surprisingly, ascorbic acid was not effective in increasing the urinary elimination of V on in decreasing tissue V concentrations.


This study was concerned with the impact of pesticides in its formulation plants on certain male hormones (LH, FSH, and testosterone), and certain trace elements (Zn, Cu, and Fe). Our studied populations were exposed to pesticides, organophosphates, carbamates, and pyrethroids for an average exposure of 2 to 35 years and an average age of 20 to 60 years, and all were males.

The immunologically serum level of the studied hormones was significantly higher among exposed subjects than in controls, however, no relation to their duration of exposure was observed.

The serum level of zinc (Zn) was significantly decreased, but copper (Cu) and iron (Fe) were increased among exposed subjects. These changes also do not appear related to either age or duration of exposure to pesticides.

Negative Side Effects of Oral Vardenafil and Vardenafil Treatment in STZ-Diabetic Rats. J. L. Aragón, M. González, J. P. Gabaldón, and C. L. Keen. Laboratory of Toxicology and Biochemistry, School of Medicine, University of Barcelona, 43001 Reus, Spain.

Vardenafil and vardenafil, two forms of vardenafil, have been reported to exert insulin-like effects in vivo and in vitro. In the present study we compared the effectiveness of oral sodium metavanadate (NaVO₃), sodium orthovanadate (Na₂VO₃), and vardenafil sulfate pentahydrate (VDA, H₂O·5H₂O) treatment in alleviating some signs of diabetes in streptozotocin-induced diabetic rats. Vardenafil compounds were administered in aqueous solutions of NaCl (80 mM) at concentrations of 0.20 mg/kg (NaVO₃), 0.30 mg/kg (Na₂VO₃), and 1.1 mg/kg (VDA, H₂O·5H₂O) for two weeks. Control rats, either diabetic or non-diabetic, drank solutions of NaCl (80 mM). Although some signs of diabetes (hyperglycemia, hyperlipidemia, polydipsia) were significantly alleviated by the vardenafil treatment, negative side effects were also observed in all of the vardenafil-treated diabetic rats. These effects included some deaths, decreased weight gain, increased serum concentrations of urea and creatinine, and tissue vardenafil accumulation, which are consistent with the reported toxicity of vardenafil in non-diabetic rats. Vardenafil sulfate was the most effective compound of those tested in normalizing blood glucose levels. However, the results here reported suggest that chronic administration of vardenafil or vardenafil in the drinking water is a viable alternative treatment to insulin in human diabetes. (Supported by CIEN, Spain, Grant SAB_0553).
408 COMPARATIVE NEUROTOXICITY OF SHORT-CHAIN ALKYL-METALLIC COMPOUNDS. L K Chang, Deps. of Pathology, Pharmacology & Toxicology, Univ. of Arkansas for Medical Sciences, Little Rock, AR

Organometallic compounds, especially the short-chain alkyl metals, are, in general, potent neurotoxins to the central nervous system. The most noticeable ones are those of mercury, lead, and tin. Each of these metallic compounds, has fairly specific target loci in the CNS with characteristic pathologic lesions. Methyl and ethyl mercury, being very similar in chemical nature, are very neurotoxic in their parent forms producing neuronal changes in the cerebellum, caudate nucleus, and dorsal root ganglia. The tertiary and triphenyl lead, which are used as gasoline additives, on the other hand, requires metabolic transformation to the trialkyl form before becoming neurotoxic. The trialkyllead produces lesions in the cerebral cortex, the brainstem, and the hippocampus. Trimethyl and triethyl tins, although share very similar chemical structures, displayed extremely different pathologic properties: TMT being neurotoxic producing neuronal necrosis in the limbic system (amygdala, pyriform cortex, hippocampus), and TET being eye- and optic nerve damage, producing edematous changes in the myelin of the white matters. Among these three categories of alkyl metals, only organomercury shows a strong and direct correlation between metal distribution in the brain and the loci of pathology produced. These observations suggest direct cytotoxicity and a more "indirect" toxic action of the organolead and organotin compounds in the central nervous system.

409 METAL SEQUESTERING BY THE CHORIZOID PLEXUS: IN VIVO AND IN VITRO CHARACTERIZATION. W Zheng and H V Apohian, Dep. of Pharmacology & Toxicology, Dep. of Molecular & Cellular Biology, University of Arizona, Tucson, AZ.

The characteristics of the choroid plexus in sequestering Pb in vivo and taking up Cd in vitro have been investigated. Tissue contents of the metals were determined by atomic absorption spectrophotometry or radioactive isotope. When rats were injected i.p. with 10, 20, 30 or 50 mg Pb acetatekg and tissues removed four hr later, the concentration of Pb in the lateral choroid plexus increased proportionally with the increase of dose. Pb concentrations in the brain cortex and cerebrospinal fluid (CSF), however, were not significantly changed. During the 24 hr period after injection of 50 mg Pb acetatekg, i.p., the lateral choroid plexus continued to concentrate this metal ion, while the CSF and brain cortex had much lower levels of Pb. In rats given 175 μCi polonium-210 sc. daily for three days, the radioactivity was five fold greater in the choroid plexus than in the brain cortex. When ouabain was incubated in vitro with the rat lateral choroid plexus in artificial CSF, ouabain caused a 40% inhibition of Na+,K+-ATPase activity. At a concentration of 1.5 mM, ouabain inhibited the uptake of 106Cd by the choroid plexus by 57%. The uptake of Cd from the CSF side of the choroid plexus appears to require energy. Total (GSH + GSSG) and reduced GSH in the brain cortex were significantly higher than those in the choroid plexus. Cystine, however, was four fold greater in the choroid plexus than in the brain cortex. Neither cysteinylglycine nor gamma-glutamylcysteine was detected in the choroid plexus.

410 CAN DMPS BE USED TO DETERMINE THE BODY BURDEN OF MERCURY IN HUMANS? H V Apohian, D C Bruce, M M Apohian and W Allen†. University of Arizona and †Associates in General Dentistry, Tucson, AZ.

Recent experiments, published in peer-reviewed journals by reputable investigators, have pointed out the hazards of dental amalgams. To determine whether there is a correlation between the number of dental amalgam surfaces in the mouth and the urinary excretion of mercury (Hg) after giving the chelating agent 2,3-dimercaptot-1-propane-sulfonate (DMPS), ten normal young men were first assigned an amalgam score, a measure of the number of amalgam surfaces in their mouths. After fasting for 11 hrs, they were given 300mg DMPS po. Urinary excretions of total Hg were determined. Six urinary profiles have been completed as of this time. The mean 4 hr urinary Hg excretion after DMPS administration increased approximately 9-fold as compared with that found for the 4 hr period before DMPS administration. A direct correlation of the amalgam score number with Hg excretion after DMPS administration was found. The use of DMPS as a test to determine the body burden of the mercury for humans appears promising.

411 EFFECTS OF OVARIECTOMY AND CADMIUM (Cd) ON BONE CALCIUM. N Sacco-Gibson, S Chaudhry, J Abrams, D Peterson, N Bhattacharya. Argonne National Laboratory, Argonne, IL.

This study evaluated the effects of Cd exposure on bone calcium following ovariectomy. Fourteen female beagles (7-9 bgold) with 42 prepuberal skeletons (100 g/kg body weight) were divided into four groups: shams (SO; n=3); ovariectomized (OV; n=4); shams exposed to Cd (SO; n=3); ovariectomized exposed to Cd (OV; n=4). Cd was given in capsules for 4 weeks, increasing dosage weekly (1.5, 5, 15, 50 ppm), followed by exposure for 4.5, 2.0, and 15 weeks in drinking water (15 ppm). Repeated measures of bone mineral density (BMD) were made by dual photon absorptiometry. After the last Cd-water exposure, ribs, tibiae, humeri and lumbar vertebrae (L2-4; L5) were taken from each dog (except SO, kept for further study). No consistent differences between treatment and control groups were observed in dry or ash weight, Ca content, ash/dry, Ca/dry, and Ca/ash ratios. However, 56Ca, 44Ca/dry, and 44Ca/ash were significantly higher (18 to 38%) in bones of OV+ and OV- compared to SO-. In contrast, significant decreases in BMD of L5-4 were observed in OV+ dogs (baseline to sacrifice) (OV+: -7.2±1.1%; OV-: -4.0±1.9%; SO-: -1.0±1.7%). Our results suggest: 1) ovariectomy sensitizes bone to cadmium effects and 2) bone mineral loss due to Cd exposure, such as in Itai-Ita disease, may be due to direct effects. (U.S. DOE Contract No. W-31-109-ENG-38; NIH ES04816-01.)
Previously, we studied the ability of cadmium (Cd) to initiate or promote tumors in B6C3F mice and, contrary to expectation, found Cd promoted barbiturate-promoted and diethylnitrosamine (DEN)-initiated liver tumor formation. In this study the time sequence of Cd inhibition of DEN-initiated tumor formation was studied. DEN (90 mg/kg, ip) was given at time 0 followed by Cd (1000 ppm) in drinking water for 2-48, 4-48, 6-48, 16-48, and 32-48 wks. The study ended at 48 wks. DEN-induced elevations in liver tumor incidence (20 tumor bearing mice/25 total) over control (5/25) were prevented by Cd regardless of period of administration (DEN + Cd: 2-52 wks, 2/25; 4-48 wks, 1/25; 8-48 wks, 1/25; 16-48 wks, 2/25; 32-48 wks, 6/24). Cd alone (2-48 wks) eliminated (0/25) spontaneously occurring liver tumors (5/25). DEN-induced lung tumor rate (25/25) and multiplicity (7.28 tumors/lung) were also reduced by Cd (maximal decreases 28% and 80%, respectively). Some evidence of specific toxicity toward initiated cells was seen histologically as Cd-induced necrototoxicity in DEN-induced hepatic lesions. These results indicate Cd prevents or reduces tumor formation in the B6C3F mouse liver and lung regardless of the exposure interval and apparently by cell-specific cytotoxicity. This chemotherapeutic potential deserves further study.

Fractional retention of ingested metals in neonates exceeds that in adults, perhaps because intestinal InP decreases with development, and/or because of changes in luminal composition, intestinal transport rate, or ability to measure a fall in InP with age because morphological changes invalidate comparisons on the basis of uptake/g or/cm. We are proposing as basis for comparison use of the rate of ethanol (Et) uptake. This consists of passive diffusion following first order kinetics, with a half time (t1/2) depending on untrusted layers (UL) and the area of the limiting membrane, but not its chemical composition. Absorption of cy and Cd at low concentration in both young and adult rats is also first order. (Et)cy should be influenced like (Et)Et by UL, but also by InP. In mature rats, (Et)cy/((Et)Et) = 1.5±0.5, compared to 1.6±0.6 in 21-24 day old weaned rats, i.e. InP for cy does not change and is absorbed at 2/3 the rate of Et at both ages. In contrast, (Et)cy/((Et)Et) rose with age from 1.9±0.8 to 3.4±1.2 (p<0.01), a change apparently not due to alterations in Cd binding to mucin. Per unit surface area, therefore, jejunal in young post-weaning rats takes up Cd twice as fast as in adults, i.e. (InP)Cd decreases with post-weaning development. (USPHS grants ES-04840, ES-00159).

Cellular senescence is a genetically programmed cessation of cellular proliferation. This process is dominant over immortality. Senescence has been proposed to be involved in tumor suppression, and evidence suggests that multiple genes regulate these processes. With the use of hamster-human cell hybrids, a putative senescence gene was mapped to human chromosome 1 and another has been localized to the Chinese hamster X chromosome. In this study, the control of cellular senescence by the human X chromosome was examined using the technique of microcell fusion. A normal human X chromosome in a mouse-human hybrid clone was introduced via microcell fusion into a nickel-transformed, immortal male CH cell line (Ni-2/TG) with a deletion of Xq. About twenty percent of resulting hybrids sensed. Karyotypic analysis of the non-senescent hybrid clones revealed that none of these clones retained the complete X chromosome, but retained only a small fragment of the human X. Introduction of another human X chromosome from a tumorigenic human osteosarcoma cell line into Ni-2/TG cells also caused typical signs of cellular senescence in about twenty percent of hybrids. However, transfer of normal human X chromosome into a tumorigenic Chinese hamster ovary (CHO) cell line had no effect on the growth of the cells. These and other findings indicate that nickel carcinogenesis in CH cells involves the loss or inactivation of a senescence gene which is suggested to reside on both CH and human X chromosome. (Supported by grant ES05512).

Cadmium uptake in hepatocytes is characterized as occurring by both simple and facilitated diffusion. Because of the known antagonism between Cd and Ca, we examined whether the calcium channels play a role in the uptake of Cd in hepatocytes. Rat hepatocyte primary cultures were exposed to 3 μM CdCl2 in the presence of up to 250 μM diltiazem or verapamil or 100 μM nifedipine or nitrendipine at 4 and 37°C for up to 30 min. These calcium channel blockers inhibited the Cd uptake in a concentration-dependent manner causing a maximum inhibition of 25%. Both verapamil and diltiazem decreased the Km and Vmax of Cd uptake. At 4°C the hepatocytes accumulated only about one-fifth the amount of Cd taken up at 37°C. This was not further inhibited by the channel blockers. Furthermore, the metabolic inhibitors, NaF, ECD, and ouabain had no effect on verapamil's ability to inhibit Cd uptake at 37°C. 30 μM La, Fe, Cu and Zn inhibited the accumulation of 3 μM Cd by 12, 20, 27 and 70%, respectively, but had no effect on Cd efflux. These data suggest that a part of the Cd enters the hepatocyte through calcium channels, without requiring energy. Furthermore, Zn, La, Cu and Fe reduce Cd accumulation by lowering its uptake rate rather than enhancing its efflux. (Supported by PHS grant ES-03187).
416 HYDROXYLATION OF PHENOL TO HYDROQUINONE CATALYZED BY A HUMAN MYELOPEROXIDASE-SUPEROXIDE COMPLEX.
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Benzene, a known human myelotoxin and leukemogen is metabolized by liver cytochrome P-450 monooxygenase to phenol. Further hydroxylation of phenol by cytochrome P-450 monooxygenase results in the formation of mainly hydroquinone, which accumulates in bone marrow the target organ of benzene. Here we report that phenol hydroxylates to hydroquinone is also catalyzed by human myeloperoxidase in the presence of a superoxide generating system, hypoxanthine-xanthine oxidase. No hydroquinone formation was detected in the absence of myeloperoxidase. Superoxide dismutase and mannitol (a hydroxyl radical scavenger) both enhanced the myeloperoxidase-dependent formation of hydroquinone from phenol. Histidine (a single oxygen scavenger) resulted in >90% inhibition in hydroquinone formation. Based on these results we postulate that hydroxylation of phenol to hydroquinone occurs during the decay of myeloperoxidase-superoxide complex and that heme-bound singlet oxygen may be responsible for phenol hydroxylation. These results also suggest that myeloperoxidase-dependent hydroquinone formation from phenol could play a role in the production and accumulation of hydroquinone in bone marrow, the target organ of benzene-induced myelotoxicity. Supported by NIH grant P42ES04705 and U.C. Toxic Substances Program.

418 EVIDENCE THAT GROWTH HORMONE IS NOT RESPONSIBLE FOR REGULATING SEXUALLY DIMORPHIC P-450 ENZYMES AND STEROID 5a-REDUCTASE IN RAT LIVER.
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Differences in the pattern of growth hormone secretion in male rats (i.e., "continuous" secretion in females were "pulsatile" secretion in males), are thought to be the underlying cause of sex differences in liver microsomal P-450 enzymes and steroid 5a-reductase. A new strain of dwarf rats (NIMP/AS) has recently been shown to have little or no detectable circulating growth hormone due to a selective defect in pituitary growth hormone secretion. We have examined the levels of some activity of P-450a (also P-450h), P-450i (IC13), P-450m (IIA2) and P-450c (IIA2) and steroid 5a-reductase in liver microsomes from male and female dwarf rats, to test the hypothesis that the expression of these sexually dimorphic enzymes is dependent on growth hormone. The levels and activity of P-450a declined post-pubertally in male but not female dwarf rats, whereas the levels and activity of P-450c declined post-pubertally in female but not male dwarf rats. The levels of P-450h and P-450i increased post-pubertally only in male dwarf rats, whereas the activity of P-450i and steroid 5a-reductase increased post-pubertally only in female rats. The magnitude of these sex-dependent developmental changes was essentially indistinguishable from those observed in normal rats. These results were unexpected, and suggest that growth hormone is not the pituitary hormone responsible for regulating the levels of sexually dimorphic enzymes in rat liver. Supported by ES03765 and ES00166.

417 COUMARIN7-HYDROXYLATION BY HUMAN LIVER MICROSOMES: PRONOUNCED INTER-INDIVIDUAL DIFFERENCES REFLECT DIFFERENCES IN P-450 IIA3 LEVELS. R. Pearce and A. Parkinson.
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Rat IIA1 and IIA2 are two closely related liver microsomal P-450 enzymes that catalyze the 7a- and 15a- hydroxylation of testosterone, respectively. Antibody against rat IIA1, which cross reacts strongly with IIA2, recognizes a single protein in human liver microsomes. However, human liver microsomes do not catalyze the 7a- and 15a- hydroxylation of testosterone. The results of the present study suggest that the protein in human liver microsomes recognized by anti-IIA1 is cytochrome P-450 IIA3, and that this enzyme is responsible for catalyzing the 7-hydroxylation of coumarin. The rate of coumarin 7-hydroxylation varied ~12 fold among liver microsomes from 22 human liver samples (200-2500 pmoles/mg protein/min). This variation coincided with inter-individual differences in the levels of IIA3, as determined by immunoblotting. Anti-IIA1 completely inhibited (>95%) the 7-hydroxylation of coumarin by human liver microsomes. Propportionately more antibody was required to inhibit those microsomal samples with high coumarin 7-hydroxylase activity than those with low activity. A single high affinity enzyme (Km = 0.5 nM) catalyzed the 7-hydroxylation of coumarin, regardless of the rate of coumarin 7-hydroxylation. Consequently, inter-individual differences in coumarin 7-hydroxylase activity were observed over a wide range of substrate concentrations (0.1 to 1000 µM). Interestingly, just as human liver microsomes do not appear to contain IIA1 or IIA2, it does not appear that rat liver microsomes contain IIA3, inasmuch as rat liver microsomes do not catalyze the 7-hydroxylation of coumarin (<20 pmoles/mg protein/min). These results suggest that coumarin 7-hydroxylase activity is a reliable marker for IIA3 levels in human liver microsomes, and that the level of this P-450 enzyme shows considerable inter-individual variation. Supported by NIH grants ES-03765, ES-00166 and ES-07079.

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Tissue specific changes in P-450 and its associated NO activity were observed in the guinea pig following a single subcutaneous injection (75 µmol/kg) of sodium arsenite. Arsenite, a known inducer of stress proteins, caused a 2- to 5-fold increase in pulmonary microsomal P-450 concentrations 24-48 hr after administration. On the other hand, total hepatic microsomal P-450 content did not change significantly whereas there was a marked decrease (3- to 5-fold) in that of renal microsomes. NO activities were assayed with several isozyme selective substrates: 7-ethoxyresorufin (ERF), 7-pentoxyresorufin (PRF), 4-aminohiphenyl (AB) and erythromycin. Both tissue selective and isozyme selective differences were noted. For example, AB N-hydroxylation was increased by arsenite administration in lung but not liver. ERF O-deethylation was inhibited in all three tissues but to a lesser extent in lung. With PRF as substrate an increase of hepatic, but not pulmonary activity was noted 6, 12 and 24 hr after treatment. The mechanisms responsible for these selective changes is currently under investigation. Supported by NRC of Canada grants NT-9722 (to JRF) and NT-9528 (to MCG).

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METABOLISM AND DISTRIBUTION OF 14C LABELED ACROLEIN IN SPRAGUE-DAWLEY RATS.

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Acrolein labeled with 14C in the 2,3 position was administered to 4 groups of Sprague Dawley rats (59). The group 1 was treated (iv) with a single dose (2 mg/kg), while all other groups were treated by intubation. Group two was given a single dose (2 mg/kg), group three 14 doses of unlabeled acrolein followed by one of 14C labeled material and group four, one dose of labeled acrolein at 15 mg/kg. The usual excreta were counted, tissues were sampled and 14C distribution assessed. Few differences were noted between group 3 (single dose) and group 4 (multiple dose) indicating that metabolic pathways probably do not change after multiple doses. The iv. dosing did result in a pattern consistent with acrolein binding to blood elements and the high dose (15 mg/kg) did demonstrate different excretion patterns relative to other doses. With the exception of the iv. treated group, all others showed the largest 14C concentration in the liver. Most 14C was excreted from all groups within 24 hours.

METABOLISM OF 3,3'-DICHLOROBENZIDINE BY HAMSTER HEPATIC S9. A Ghosal, R T Rosen and M M Ibs. Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ.

Studies were carried out to (i) characterize the isolable metabolites of the animal carcinogen 3,3'-dichlorobenzidine (DCB) and (ii) assess the relative contribution of oxidative and reductive metabolism to binding of the metabolites to DNA. NADPH-supplemented hamster hepatic S9 metabolized 14C-DCB to isolable products, three of which were identified by HPLC-mass spectrometry as (i) benzidine [Bzd (M/z = 184)], (ii) 3-chlorobenzidine [ClBz (M/z = 218)], and (iii) an oxygenated derivative [OxyClB (M/z = 268)]. A dehalogenated, monoarylamide compound [X (M/z = 121)] was also isolated. The Km was 100 mM and 500 mM for the formation of OxyClB and ClBz, respectively. The formation of X or Bzd was inhibited at a DCB concentration of 35 mM or higher. Below 35 mM DCB, the contribution of OxyCDB, X, ClBz, and Bzd to total isolable metabolites was 25.9%, 53%, 6.1%, and 15%, respectively. Hepatic S9 also catalyzed the activation of 14C-DCB to derivatives that bound covalently to added DNA. A 100% argon atmosphere inhibited the latter reaction and OxyCDB formation by 37% and 92%, respectively, but did not affect the formation of X, Bzd, or ClBz. The data suggest that (i) DCB is dehalogenated, perhaps by reductive mechanisms, to ClBz as well as Bzd (ii) reductive metabolism contributes to DNA adduct formation by DCB in vitro. Supported by EHHS, Busch and EPA grants.

METABOLISM OF 3-METHYLINDOLE TO A MERCAPTURATE AND SEVERAL OXIDATIVE METABOLITES. G L Skidmore, M L Appelros, D J Smith, J R Carlson and G S Yost. 1Department of Pharmacology and Toxicology, 112 Skaggs Hall, University of Utah, Salt Lake City, UT, and 2Department of Animal Sciences, Washington State University, Pullman, WA.

The systemic pneumotoxic 3-methylindole (3MI) requires bioactivation to elic toxic effects. Previous studies using microsomal incubations have indicated that 3MI is probably activated to a methylene imine by a two-electron oxidation. Formation of the methylene imine was inferred by the formation of glutathione (GSH) adduct from microsomal incubations that were supplemented with GSH. For the present studies mice, rats and goats were dosed with radiolabeled or unlabeled 3MI and the urine from each species was analyzed for the excretion of the mercapturate corresponding to the previously identified GSH adduct. Rats and goats excrete a 3MI metabolite in the urine that was characterized as 3-[N-acetylcyctamine-S-y]-methylindole by HPLC, UV spectrophotometry, high-field NMR, and mass spectrometry. The identification of the mercapturate of 3MI demonstrates that bioactivation to a methylene imine occurs in vivo. Mice did not produce detectable amounts of the mercapturate even though previous in vitro experiments showed that mouse-lung microsomal incubations supplemented with GSH can produce the GSH adduct. Other products from hydrolysis of a mouse 3MI-GSH adduct may be formed, such as the cystine and glycylcysteine adducts, but have not been identified. Mice metabolized 3MI to 3-hydroxy-3-methylindole, indole-3-carboxylic acid, and several ring-hydroxylated and glucuronic-conjugated metabolites. Supported by Grant HL36495 from the U.S. Public Health Service, National Institutes of Health. G.S.Y. is a U.S. Public Health Service Postdoctoral Awardee (HL02191).


Carcinogenic arylamines are known to be activated by N-O-acetyltransfer of their N-arylated-hydrazonic acid metabolites. The dog, which lacks a cytosolic N-acetyltransferase, has previously been shown to possess 3 different N-deacetylases in the hepatic microsomes. In the present study, we demonstrate that one of these enzymes also possesses N-acetyltransferase, N-O-acetyltransferase and carboxylsterase activities. Dog liver microsomes were solubilized with Triton X-100 and resolved on an ion-exchange column into 3 different N-deacetylases. The fraction that possessed N-acetyltransferase activity was further resolved on a Sephadex S-200 column. The N-acetyltransferase fraction which was >200 kDa also had N-O-acetyltransferase and N-deacetylase activities. The Km for acetyl CoA and 2-amino-fluorene for the acetylation of 2-aminofluorene were 85 and 750 , respectively. The antibody, which was raised against the dog 60 kDa microsomal enzyme that has N-deacetylase, N-O-acetyltransferase and carboxylsterase activities, did not react with this enzyme. These results suggest that these microsomal enzymes may be involved in the carcinogenesis of arylamines in the dog. Supported by USPHS grant CA23800
RAPID, SENSITIVE, AND VERSATILE TECHNIQUES FOR THE ANALYSIS OF ACIDIC URINARY METABOLITES OF XENOBIOTICS. C K Winter, T M Dinoff, A D Jones* and J Q Sipes**. Department of Entomology, Univ. of California, Riverside, CA, *Facility for Advanced Instrumentation, Univ. of California, Davis, CA, and **Department of Pharmacology and Toxicology, Univ. of Arizona, Tucson, AZ.

Techniques for the sample preparation and analysis of acidic urinary xenobiotic metabolites have been developed that allow for the rapid and accurate detection of metabolites at concentrations in the low-to-sub-picogram range. Sample preparation techniques involve solid phase extraction and/or phase transfer catalysis. Metabolites are derivatized to pentafluorobenzyl esters and are analyzed by gas chromatography / negative electron capture mass spectrometry. Accurate and sensitive quantitation for some metabolites has been achieved through selected ion monitoring and the use of stable isotopically labelled internal standards. The techniques are applicable to a wide variety of acidic metabolites; examples include the analysis of a model compound, S-benzyl mercapturic acid, mercapturic acids of the lipid peroxidation product trans-4-hydroxy-nonenal, metabolites of the insecticide malathion, and metabolites of 4-vinylcyclohexene, a byproduct of synthetic rubber production and a known ovarian carcinogen in female mice.

STOCHASTIC SIMULATION OF ALTERED HEPATIC FOCI (AHF): USE IN BIOLOGICALLY-BASED RISK ASSESSMENT. PB Conolly, DS Marsman, JA Popp and TL Goldworthy. CIIT, Research Triangle Park, NC.

Stochastic simulation was used to estimate parameter values for the 2-stage cancer model of Moolgavkar and co-workers (MVK model; JNCI 66:1037, 1981). In this model, mutation of a normal cell produces an intermediate cell (IC) which may die or expand clonally. IC mutation generates a malignant cell. The stochastic model used for this study describes the rate of normal cell mutation to IC and the growth or extinction of IC clones. The hepatic correlate of IC clones is the putatively pre-neoplastic AHF. Stochastic simulation offers the advantage over analytical forms of readily incorporating time-dependent changes in parameter values associated with dosing schedules and animal aging. AHF data from male F-344 rats initiated with diethylnitrosamine (DEN) and promoted with phenobarbital or Wy-14,643 were accurately simulated by using measured AHF cell turnover rates and estimating the number of hepatocytes initiated by DEN. Thus, when AHF size and number are known, stochastic simulation can be used to infer the carcinogen-stimulated mutation rate of normal cells (initiation) and birth and death rates of cells in AHF (promotion). These estimates, in turn, can be used for biologically-based carcinogen risk assessment based on the MVK model.

CARCINOGENIC RISK ASSESSMENT: FACT OR FANTASY? A K Thakur and A Parthasarathi: Hazleton Washington, Vienna, VA.
Sponsor: D J Brusick.

Regulatory decisions about many chemicals are generally made with empirical and so-called mechanistic models of carcinogenicity. Often investigators today do not pay much attention to the statistical and biological inadequacies behind these models. Not all chemicals act the same way on their respective target sites. As a consequence, none of these models can be taken as a general purpose model. Physiological, biochemical, pharmacological, and pharmacokinetic differences exist among different species. These models are mostly inadequate to account for them. Further, in many cases, bioassay performed under regulatory toxicology only provide inadequate and inappropriate designs for risk assessment using low-dose extrapolation. Fallacies in low-dose extrapolation using these models will be demonstrated with several "potent carcinogenic" chemicals.

INDUCTION OF HEPATOCYTE DNA SYNTHESIS IN MALE B6C3F1 MICE FOLLOWING SUBCHRONIC EXPOSURE TO 1,1,2-TRICHLOROETHYLENE (TCE). R E Rush, D E Rodwell, J C Siglin, J J McKenzie, M D Mercieca, and J E Elsung. Springborn Lab. Inc. Spenservile, OH and Medical College of Ohio, Toledo, OH.

Recently much interest has been directed to understanding the role that the induction of cell proliferation plays in the chemical carcinogenesis process. The present investigation examined the effect of treatment of mice with TCE (a non-DNA reactive carcinogen) on hepatic DNA synthesis. TCE was administered by oral gavage at doses of 0, 10, 100, 500, and 1000 mg/kg/day to male B6C3F1 mice for 1, 2, 3, 6, or 13 wks. At each time period mice were killed and necropsied. Three days prior to sampling, each mouse received a surgically implanted osmotic minipump containing BRDU. DNA synthesis was determined by immunohistochemical detection of BRDU. TCE induced a duration and dose dependent increase in DNA synthesis in the murine hepatocytes at doses of 100 mg/kg and above after 2 wks of treatment that returned to normal levels by 4 wks. This increase was accompanied by increased liver wt/body wt but not increases in serum AST and ALT levels.
HEPATOCELLULAR PROLIFERATION IN RODENTS DURING 90-DAY 1,4-DICHLOROBENZENE ADMINISTRATION. S R Eldridge, T L Goldsworthy, J A Popp and B E Butterworth. CITI Research Triangle Park, NC.

1,4-Dichlorobenzene (DCB), a non-DBA reactive compound, induced hepatocellular carcinomas in mice, but not in rats, in an NTP bioassay. A cell proliferation study was undertaken to determine whether increased cell proliferation was correlated with DCB-induced hepatocarcinogenicity. DCB was administered in corn oil by gavage at the bioassay doses to male and female B6C3F1 mice (600 and 300 mg/kg/day) and to female BALB/c rats at the highest bioassay dose (600 mg/kg/day) 5 days/week for up to 13 weeks. Cell proliferation was detected by labeling hepatocytes in 3-phase with 5-bromo-2'-deoxyuridine (BrdU) delivered for 3 days by subcutaneously implanted osmotic pumps during weeks 1, 3, 6 and 13 of treatment. An increase in liver weight as a percentage of body weight compared to controls was observed in high dose male and female mice and female rats at all time points, and in low dose male and female mice at weeks 6 and 13. No significant elevations in liver-associated plasma enzymes were found in either treated species, indicating a lack of overt hepatotoxicity. Histopathological evaluation revealed no evidence of hepatotoxicity in all groups. However, centrilobular hypereosinophilia was observed in high dose male and female mice at week 13. The percentage of hepatocytes in S-phase (labeling index) was significantly increased compared to controls in high dose male mice at weeks 1 (7.3±1.0 vs. 0.1±0.05) and 3 (1.3±0.55 vs. 0.08±0.04), high dose female mice at week 1 (7.5±1.35 vs. 2.9±1.8) and female rats at weeks 1 (35±2.1 vs. 1.2±0.44) and 6 (3.9±1.3 vs. 0.79±0.21). Mice exhibited a centrilobular pattern of labeled hepatocytes, whereas rat hepatocytes were labeled throughout the lobules. These data indicate mitogenic stimulation of cell proliferation in the liver of DCB-treated mice suggesting involvement in the carcinogenic activity of DCB. The observation of cell proliferation also in the rat indicates that additional factors are involved in the species-specific effects of DCB.


To identify differences in colonic cell proliferation a comparison was made between two sulfated polysaccharides: carrageenan (CARRA) a regulated food additive of high molecular weight, and poligeenan (POLL) an analogous product of low molecular weight shown to cause intestinal lesions in certain animal species. Both were fed at the 5% level in NIH 07. Thymidine kinase (TK) activity was used as a marker of cellular proliferation. TK activity in both groups increased an average of 3 fold the first 28 days. Histological analysis revealed diffusely flattened or cuboidal epithelial cells of the mucosa in the POLI group. Some reduction of mucus content was observed in both groups. A second set of animals was fed the test materials for 28 days followed by a 28 day recovery on NIH 07. TK activity in the CARRA group returned to control levels while the POLI group remained elevated. This difference in recovery time may prove useful in differentiating "adaptive" from potentially harmful alterations in colonic proliferation.

HEPATOCELLULAR PROLIFERATION IN MICE EXPOSED TO UNLEADED GASOLINE (UG) VAPOR. LF Tilbury, O R Moss, B E Butterworth and T L Goldsworthy. Chemical Industry Institute of Toxicology, RTP, NC.

Chronic exposure to UG vapor (2056 ppm) was reported to induce an increase in liver tumors in female but not male B6C3F1 mice. An inhalation experiment exposing mice to UG under the conditions of the bioassay was conducted to better understand the role of hepatocellular proliferation with respect to the dose- and sex-specific tumor response. Male and female B6C3F1 mice were housed in one cubic meter single pass flow-through inhalation chambers and exposed to 67, 202 or 2056 ppm UG vapors 8 hr/day, 5 day/wk, for up to 13 weeks. Liver to body weight ratios were significantly higher than controls in male and female mice exposed to 2056 ppm UG at weeks 1, 3, 6 and 13. An elevation of liver-specific serum enzymes was noted in treated animals, nor were there any significant histopathological changes in the liver, indicating a lack of overt hepatotoxicity. Hepatocyte proliferation as expressed in labeling index (LI), was measured immunohistochemically after 5-bromo-2'-deoxyuridine administration via an osmotic mini-pump implanted three days before the animals were killed. A six to tenfold increase in LI compared to controls was observed in male and female mice exposed to 2056 ppm UG for one week, with a return to control levels at weeks 3, 6 and 13. Mice exposed to 67 and 252 ppm UG did not show any increase in LI. A previous experiment using a recirculating exposure system demonstrated concentrations of 2000 ppm UG exhibited increased hepatocyte proliferation in female mice when compared to male mice (Toxicologist 9, 207 [1989]). Data presented here demonstrate a high exposure UG effect on cell proliferation rates in both sexes only at week 1 and suggest that additional factors may play a role in the sex-specific hepatocarcinogenicity of this complex mixture.

LIVER CELL REPLICAATION IN RESPONSE TO LYS171883 (LY) AND OTHER PEROXISOMAL PROLIFERATORS (PP) P J Eche and T L Lamier. Toxicology Division, Lilly Research Laboratories, Eli Lilly & Co., Greenfield, IN.

Two of the proposed mechanisms by which PP induce hepatocellular carcinomas (HC) in rodents involve a) oxidative stress and DNA damage related to the induction of peroxisomal 8-oxidation (8-Ox), and b) sustained mitogenic properties that may account for tumor promotion. We have begun to address these hypotheses with respect to LY, which induces HC in female B6C3F1 mice. LY increased 8-Ox 2 to 3-fold over two yr at 0.075% in diet but not at 0.0225%. Both doses caused an equal incidence of HC after 2 yr. Thus, induction of 8-Ox does not correlate with HC in mice given LY. Hepatocyte replication was measured using bromodeoxyuridine given by minipump for 7 days. LY (0.0225, 0.075 and 0.25%) caused dose-related increases in the labeling index (LI; % labeled) in the first 7 days of treatment in mice (1.6, 3 and 5-fold), and there were corresponding increases in liver weight. There was no increase in LI at any dose in the last 7 days of a 90 day study, although liver weights remained elevated. Clofibrate and nafenopin increased LI initially in mice and rats, but not after longer treatment periods. In contrast, WY-14643 increased LI even after longer term treatment. Thus, a sustained mitogenic effect on hepatocytes is not characteristic of all of the PP. If such an effect is involved in the induction of HC by LY and other PP, the effect may be on a specific subpopulation of liver cells, eg., spontaneously initiated cells (Kraupp-Gradl et al., Cancer Res. 50: 3701-3706, 1990).

A wide variety of chemicals have been shown to produce hepatic peroxisome proliferation (PP) in the mouse and certain of these compounds are also hepatocarcinogens. In this study we have investigated the relationship between PP and cell replication in rat liver. Male Sprague-Dawley rats were fed control diet or diet containing either 0.0125% and 0.05% nafenopin (NAP), 0.05% methyl clofenapate (MC), 0.025% WF-14,643 (WF) or 0.05% clofibrate (CA) for 1 and 15 wk. All four compounds produced marked liver enlargement and a sustained induction of peroxisomal (palmitoyl-CoA oxidation) and microsomal (auric acid 12-hydroxylation) fatty acid oxidizing enzyme activities. Enzyme induction was less marked with 0.0125% NAP than with 0.05% NAP which was similar to that produced by the other three compounds. Replicative DNA synthesis was studied by implanting 7 day Alzet osmotic pumps containing [3H]thymidine during wk 0-1 and 14-15. After 1 wk replicative DNA synthesis (assessed as radioactivity incorporated into homogenate DNA by scintillation counting) was increased in all treatment groups to 170-225% of control levels. Hepatocyte Labelling Index (determined by autoradiography of liver sections) was increased in all treated groups. After 15 wk hepatic DNA radioactivity levels were 150 and 200% of control in MO and WF treated rats, respectively, whereas in NAP and CA had no effect. These results demonstrate that the relationship between the magnitude of PP and induction of cell replication depends on the compound being studied and that some peroxisome proliferators produce sustained stimulation of replicative DNA synthesis in the rat. (Supported by U.K. Ministry of Agriculture, Fisheries and Food).

HYPOMETHYLATION OF THE Ha-ras ANDraf PROTO-ONCOGENES IN MOUSE LIVER FOLLOWING PROLIFERATION OF HEPATO CYTES. J S Ray, S L Bell and J I Goodman. Department of Pharmacology and Toxicology, Center for Environmental Toxicology, Michigan State University, East Lansing, MI.

A relatively low content of DNA-5-methylcytosine (MeC) is one of the requirements for gene expression. We have hypothesized that a decrease in MeC (i.e., hypomethylation) may facilitate the aberrant expression of proto-oncogenes involved in tumorigenesis. This can occur if DNA maintenance methylation is not faithful during cell proliferation. Adult male B6C3F1 mice were subjected to a 2/3 partial hepatectomy (PH) and liver samples were obtained at the time of PH and 7 days later. The methylation status of Ha-ras andraf was assessed by restriction of DNA with the methylation sensitive isoscilozymeMspI and HpaII, Southern blotting, hybridization with radiolabelled probes for Ha-ras orraf, and autoradiography. Hypomethylation was evidenced by the loss of a methylated site in each proto-oncogene at 7 days post- PH. Phenobarbital (PB) stimulates proliferation of hepatocytes. B6C3F1 mice were treated with 0.05% (w/v) PB in their drinking water for 14 days; this also resulted in hypomethylation of Ha-ras andraf and a stimulation of cell proliferation in the liver of the adult B6C3F1 mouse may result in hypomethylation of proto-oncogenes, a condition which could facilitate their expression and contribute to hepatoma development. Supp. by NIH/ES05299.

Dose-Related Hepatocellular Proliferation (PF) in Clofibrate(Cf)-Treated Rats: Morphometric Analysis and Relationship to Historical Tumor Incidence. Tanaka, K., Smith, P.F., Keenan, K.P., Eydeloth, R.S., and Stromberg, P.A., Ohio State Univ., Columbus, OH and Merck Sharp & Dohme Research Labs, West Point, PA.

Cf, a peroxisome proliferator (PxP) increases hepatocellular carcinoma in a dose-dependent fashion in rats. The relationship between peroxisome PF and rodent liver tumorogenesis, recent evidence also suggests an association between the tumorigenicity ofPxP's and sustained cell PF. To investigate the predictive potential of this endpoint in a 3-mo study, rats were fed Cf doses equivalent to those used in the chronic bioassay, and cell PF was determined after 1-wk and 3-mo using a continuous bromodeoxyuridine (BrdU) labeling technique. Adult Sprague Dawley rats were fed Cf at 1,500, 4,500 or 9,000 ppm. Six animals/group were killed after 1 or 15 wk; Seven days prior to termination, rats were implanted with osmotic mini-pumps containing BrdU to determine the 7-day hepatocyte labeling index immunocytochemically. A dose-related increase in liver weights and hepatocyte labeling index was seen after 1 wk of treatment. Determination of the labeling index after 3-mo of Cf administration is underway. The hepatomegaly suggests that the earlier trend will continue. These results suggest hepatocyte PF appears to contribute to the hepatomegaly, and that persistent cell replication may play a role in tumorigenesis with long-term Cf treatment.


PB and DEHP are nongenotoxic hepatocellular carcinogens when administered chronically to mice. The mechanism(s) by which these enhance tumorigenesis remains unclear. One potential mechanism may involve the induction of cell proliferation within the liver, which may preferentially enhance the replication of preneoplastic cells. The present study evaluated the hyperplastic response of individual hepatic foci and adenomas induced in neonatal C3H/He mice to short-term PB and DEHP administration. Sixty male mice received a single ip injection of diethylnitosamine at 13 days of age. Mice were weaned at 4 wks of age and remained untreated until 12 wks of age. Mice were then divided into 3 groups of 20 each and received either no treatment; PB (500 ppm) in the drinking water; or DEHP (12000 ppm) in the diet. Four mice from each group were killed after 2, 4, 7, 14, and 21 days of promoter treatment. Mice received an osmotic mini-pump containing bromodeoxyuridine (BrdU) 48 hrs prior to sacrifice. Immunohistochemistry was performed to identify BrdU-laden cells and a labeling index (LI) was determined for each focus and adenoma. PB significantly increased BrdU labeling in hepatic focci at 2 and 4 days compared to controls. In contrast, DEHP significantly reduced LI in hepatic foci and adenomas at all time points. These data suggest a differential mechanism of enhancement of tumorigenesis, at least at the onset of promoter administration, by two known hepatic tumor promoters.
A single subtoxic dose of CCl₄ (100 µl/kg) to rats is known to induce hepatocellular regeneration and tissue repair within 2 to 6 hr, resulting in prompt recovery from liver injury within 24 hr. Colchicine (CLC, 1 mg/kg, i.p. in saline) injection 2 hr prior to CCl₄ (100 µl/kg, i.p. in corn oil) results in antimitosis at early time points and the CCl₄ toxicity is prolonged up to 72 hr. The present studies were designed to evaluate whether hepatocellular antimitosis at early (6hr) and later time points (48 and 72hr) by CLC would result in further enhancement of CCl₄ toxicity in male S-D rats. Hepatocellular regeneration was measured by thymidine (3H-T) incorporation in hepatic nuclear DNA and by mitotic index. Hepatotoxicity was evaluated by serum ALT, AST, liver histopathology and histomorphometry. The role of hepatocellular regeneration and tissue repair in CCl₄ autoprotection was further investigated by challenging CLC pretreated animals with low dose of CCl₄ (100 µl/kg, ip) and 24 hr later with high dose of CCl₄ (2.5 ml/kg, ip). There was marked elevation in serum enzyme levels, as well as increased hepatocellular damage, low level of mitotic index and failure in autoprotection in CLC pretreated rats. These findings provide substantial evidence for a critical role played by hepatocellular regeneration and tissue repair in CCl₄ toxicity. (Supported by AFOSR-88-0008).

A common effect of many hepatocarcinogens in the male B6C3F1 mouse is their induction of hepatocyte DNA synthesis and cell proliferation. The present study was undertaken to examine the time course and dose dependency of this cell proliferation following continuous treatment with the hepatic carcinogen PB. Male B6C3F1 mice received either 0, 20, 100, or 500 mg/kg PB in their drinking water. After 1, 2, 4, 6, and 13 weeks, 3 mice from each treatment group were killed and DNA synthesis was evaluated by autoradiography. Three days prior to sampling each mouse received an osmic minipump containing 3H-thymidine. Only the 500 and 100 mg/kg PB treatments induced DNA synthesis. DNA synthesis peaked after 2 weeks of PB treatment with a 15 fold increase over control with 500 mg/kg and a 10fold increase with 100 mg/kg. At 6 and 13 weeks of treatment, none of the PB doses increased DNA synthesis over control values. Thus, PB induced a time and concentration dependent increase in DNA synthesis that peaked at 2 weeks and returned to control levels by 6 weeks.

Ozone is known to induce lipid peroxidation of lung tissue, although no direct evidence of free radical formation has been reported. We have used the electron paramagnetic resonance (EPR) spin-trapping technique to search for free radicals produced in vivo during ozone exposure of rats to ozone. C H Kennedy, G H Hatch, R Stade, and R P Mason, Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

The EPR spectrum of a 4-POBN radical adduct (a = 15.02 G, a' = 3.27 G) was detected with EPR spectroscopy in lung extracts from lungs of rats treated with 4-POBN and then exposed to ozone. Only a very weak signal was detected in the corresponding solution from rats exposed to 0 ppm ozone (air with CO₂ only). The concentration of the radical adduct increased as a function of ozone concentration. After administration of 4-POBN, rats were exposed for either 0.5, 1.0, 2.0 or 4.0 hrs to either 0 or 20 ppm ozone (with CO₂). The radical adduct concentration was significantly different for the ozone-exposed groups at exposure times of 2.0 and 4.0 hrs. A plot of EPR signal intensity vs lung weight/ body weight revealed a linear correlation (r² = 0.8). These results demonstrate that ozone induces the production of free radicals in rat lungs during inhalation exposure and suggest that free radicals may be involved in the toxicity of ozone.

Humans exposed to O₃ have a PMN influx into the lungs. We have shown that O₃-exposed AA induced an increase in human peripheral blood PMN polarization (shape change) in vitro (Am Rev Resp Dis, 141:249, 1990) suggesting that O₃-degraded AA possess chemokinetic and/ or chemotactic properties. We examined if GSH could inhibit the PMN polarization induced by O₃-degraded AA. AA (30 µM in PBS) was exposed in vitro to 0.1 ppm O₃ (30 min), and then incubated with GSH (37°C, 1 hr). PMN were then treated (10 min, 37°C) with each test solution, fixed in formaldehyde, and polarization determined by light microscopy. PMN treated with O₃-exposed AA incubated with 0 or 10 mM GSH had similar polarization values (44 ± 9 and 42 ± 6% polarized, respectively; p = NS). PMN treated with O₃-degraded AA treated with 100 mM GSH had a 90% decrease in polarization (4 ± 1%, p < 0.05). HPLC analysis showed that a decrease in the amount of 3 carbonyl compounds was associated with a decrease in polarization after 100 mM GSH incubation. These data suggest that GSH reactivity with aldehydes/ketones may mediate the biological activity of O₃-degraded AA. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy. Supported by CR812738 and ES04951.
Various exposure scenarios can induce O3 in rats. To ascertain the persistence of O3 and the potential role of lung lining fluid antioxidants in this phenomenon, bronchoalveolar lavage (BAL) from rats, exposed to 0.25 ppm O3 (12h/d for 6 or 14wk), was assayed for ascorbic acid (AA) and protein (lung damage index). O3 was expressed as a delay (min) in pulmonary function response to O3 challenge i.e., awake rats were exposed to 1 ppm O3 until they exhibited a 50% increase in breathing rate. Upon response, they were immediately anesthetized, intubated, and lavaged. Half of the rats were lavaged only. A pressure controlled system was used to deflate lungs to residual volume (-23cm H2O) and inflate them with warm saline (+23cm H2O) and evacuate the BAL (-23cm H2O). O3 was seen at 6 and 14wk and lasted 7 days post exposure (1d -14 min, 3d -34 min, and 7d -20 min). All O3 exposures, including the challenge, affected AA but none altered protein. AA was increased after 6wk (66%) and 14wk (128%) and decreased by 7d post exposure to 17% and 36%, respectively. O3 challenge reduced AA levels in BAL significantly (-40%). Data indicate that O3 in rats, induced by daily low-level O3 exposure, persists for at least a week post exposure and suggest that AA in lung lining fluid may be, in part, responsible. (This abstract does not necessarily reflect EPA policy).

**Comparison of intermittent with continual Ozone exposure for 14 weeks in rats.** K M Crissman, J Norwood, R Slade, T R Goleichter*, J A Last*, and G E Hatch. ETD, HERL, U.S.E.P.A., RTP, NC. *Univ. of Cal., Davis, CA.

In order to assess the effects of intermittent versus continual O3 exposure to O3, male BALB/c mice were exposed 12 hr/d to 0.25 ppm O3 for 3d/wk or 7d/wk and sacrificed at 1, 3, 6, and 14 weeks. Lung tissue and bronchoalveolar lavage fluid (BALF) supernatant and cells were assayed for protein, lipid phosphorous and the major antioxidants - ascorbic acid (AH2), uric acid, glutathione and α-tocopherol. Lung tissue was assayed for total collagen (hydroxyproline) and three types of collagen-specific crosslinks. No changes were seen in lung collagen content but there was a suggestion of a dose-related alteration in the trifunctional crosslink, hydroxyproline. BALF protein was increased at 3 and 6 weeks in the 7d/wk rats and 23% in both 3d/wk and 7d/wk rats at 14 weeks. BALF AH2 was increased in both the 3d/wk and 7d/wk rats at all time points with the 7d/wk rats having about twice the response of the 3d/wk rats. Thus, although collagen changes were not readily detectable at these exposures, BALF protein and AH2 showed large changes, and the continually exposed rats had larger responses than the intermittently exposed rats. (This abstract does not necessarily reflect EPA policy.)
INTERACTION OF [18]OZONE WITH RAT LUNG TISSUE AND DNA AND PROTECTION BY ANTIOXIDANTS. S P Mukherjee1, and G E Hatch2.1Duke Univ. Dept. of Medicine, Durham; 2US-EPA, HERL, MO-82, Research Triangle Park, NC.

We examined the possibility that inhaled ozone (O₃) may interact with lung tissue as an oxidant and cause DNA injury. O₃ reactions leading to incorporation of 18O into lung parenchyma and DNA in vivo were estimated following exposure of male rats (F344, 60d old) to 18O₃ (0.4 ppm to 2 ppm, 2 to 4 hrs). Lyophilized tissues were reductively pyrrolized to CO₂ and analyzed by isotope ratio mass spectrometry to determine [18O]/[16O] ratios. A significant increase in [18O] binding was observed in both the lung tissue and the extracted lung DNA in the [18O] exposed group compared to air exposed. Incorporation of 18O-oxygen (as opposed to O₃) at these levels was negligible. In vitro exposure of a 1% solution of calf thymus DNA to 18O₃ also showed [18O] incorporation into DNA. Albumin and mucin, both having extracellular anti-oxidant property, also bound 18O in vitro. Physiological concentrations of ascorbate and glutathione generally lowered [18O] incorporation into DNA as well as proteins. The small amounts of 18O detected in vivo and in vitro exposed tissues and DNA suggests that O₃ reaction with DNA does occur. (This abstract does not necessarily reflect EPA policy.)

ASSESSMENT OF ANTIBACTERIAL DEFENSES FOLLOWING OZONE EXPOSURE. M A Salguero, P Park, and M Gilmore. Health Effects Research Laboratory, U.S. EPA, Research Triangle Park, NC, and Center for Environmental Medicine, UNC Chapel Hill, NC.

Exposure to O₃ has been shown to increase susceptibility of mice to bacterial infection; however, the underlying mechanism has not been well elucidated. This study investigated the effect of O₃ exposure on the ability of mice to combat an infectious challenge of Streptococcus pneumoniae. Following a 3hr exposure to either air, 0.4, or 0.8ppm O₃, 5 and 9 week old mice received an aerosol infection of bacteria. Intrapulmonary killing of the bacteria was impaired in the O₃ exposed mice. The effect was most severe at the higher dose in the younger mice, and showed good correlation to subsequent mortality assessed over a 20 day period. Alveolar macrophages (AM) from O₃ exposed mice had an impaired ability to phagocytose the bacteria. Additionally, levels of prostaglandin E2 (PGE2) known to depress AM function, were increased in the bronchoalveolar lavage fluid of O₃ exposed mice. The data show that O₃ inhalation can dramatically reduce the defensive capability of the lung and this is associated with a reduction in AM phagocytosis. The defect is more marked in younger mice suggesting that they may be more susceptible to oxidant exposure. Further studies are required to distinguish between direct toxicity of the O₃ on the AM, and indirect suppression due to pharmacologic mediators. This abstract does not necessarily reflect EPA policy.

A Mechanism for Ozone (O₃)-Induced Bradycardia and Hypothermia in Rats. J S Trappe, A A Alleru, W P Watkinson,1 D F Doerfler and D L Costa*, NCI-Environmental Sciences, RTP, NC and *US EPA, RTP, NC.

Exposure of unrestrained rats to O₃ results in a progressive bradycardia and hypothermia at concentrations as low as 0.37 ppm. To investigate the mechanism by which O₃ produces these responses, heart rate (HR) and core temperature (Tcor) were measured in awake unrestrained F-344 rats one day after a telemetry transmitter was surgically implanted. Classical physiological probes were used in an attempt to block the O₃-induced effects by pretreating the rats with either atropine, spantide or vehicle and exposing them to 2 hr of 1.0 ppm O₃. Potentiation of the O₃ responses was attempted by pretreating the rats with propranolol, phosphoramidon or vehicle and then exposing them to 2 hr of 0.37 ppm. Atropine, the muscarinic blocker, significantly (38%) blocked the HR response, whereas spantide, the substance P (SP) receptor blocker, partially (25%) antagonized this response. Conversely, pretreatment with phosphoramidon, which inhibits SP catabolism, caused a potentiation of both the O₃-induced HR (61%) and Tcor (225%) responses. Propranolol, the β receptor blocker did not affect either HR or Tcor. We hypothesize that the release of SP from afferent sensory nerves during O₃ exposure may trigger efferent acetylcholine release that produces O₃-induced bradycardia and hypothermia in rats. (This abstract does not reflect EPA policy.)

EFFECTS OF OZONE ON ADULT AND AGED LUNG OXYGEN CONSUMPTION, GLUCOSE METABOLISM AND GPDPH ACTIVITY. P RASKA-EMERY, J U BALIS, and M R MONTGOMERY. Colleges of Public Health and Medicine, Univ of South Florida and VA Hospital, Tampa, FL.

Fischer-344 male adult (4-6 mo) and aged (24-26 mo) rats were exposed to 0-3.0 ppm O₃ for 8h, sacrificed immediately, and O₂ consumption, c14-glucose metabolism and GPDPH activity were determined. For O₂ consumption, the exp to 0.5 ppm O₃ produced a stimulation in both age gprs. Decrements in O₂ consumption were only evident in aged rats after 1.563.0 ppm. Glucose metabolism showed a marked difference between adult rats. Control values in aged rats were 40% of adults. Exp to 0.5 ppm was stimulatory in adults and aged, while 1.563.0 ppm decreased glucose metabolism in both gprs. No age-related difference in GPDPH activity between control and exposed was seen. However, in both age gprs, 0.5 ppm O₃ resulted in a significant increase in activity (33-41%); 1.563.0 ppm were without effect. The combined results show a biphasic response of adult and aged lung to severe, acute O₃ exp. One-half ppm O₃ for 8h is stimulatory for all three parameters examined in both age gprs. Three ppm O₃ inhibits O₂ consumption and glucose metabolism in both age gprs but is ineffective on GPDPH activity. Supp. by NIH AG07801 and HL34793 and VA HRS.
PULMONARY MECHANICS IN ADULT FISCHER 344 RATS DURING OZONE-INDUCED ACUTE ALVEOLAR INJURY.

J F Paterson, M R Montgomery, M D Hammond, J T Sharp and J U Balis. J A Haley VA Hospital and University of South Florida, Tampa, FL.

As part of a study on the effects of acute ozone stress on pulmonary energetics and the surfactant system, we investigated the sequential changes in pulmonary mechanics in male rats exposed to 3 ppm ozone for 1, 2, 4 and 8 h. Control and ozone exposed rats were anesthetized and lung mechanics assessed immediately post-exposure using the “Feds Lab” pulmonary evaluation and diagnostic system adapted for small animals. After 4 and 8 h of exposure, there was significant decrease in total compliance (ml/cmH20/kg) from control value of 0.84 to 0.72 and 0.57, respectively. The inspiratory compliance was decreased significantly (18% & 30%) at 4 and 8 h, whereas the expiratory compliance showed a significant reduction (37%) only at 8 h. Inspiratory resistance was reduced significantly at 2, 4 and 8 h, but both expiratory and total resistance (cmH20/l/sec) were unchanged by the 8-h period of exposure. Arterial blood gases at 8 h revealed a significant decrease in PaO2 = 55 mmHg (Control = 92 mmHg). These findings indicate that ozone-induced alveolar injury results in pulmonary dysfunction that mimics the early phase of adult respiratory distress syndrome. (Supported by NIH HL34793 and AG07801, and HSR of VA.)

MOLECULAR DOSIMETRY AND TIME-COURSE OF FORMATION OF N-(2-HYDROXYETHYL)VALINE IN RATS FOLLOWING MULTIPLE EXPOSURES TO ETHYLENE OXIDE (EO). VE Walker, TR Fennell, JP MacNeela, MJ Turner, Jr and JA Swensenn. CINT, Research Triangle Park, NC and UNC, Dept of Pathology, Chapel Hill, NC.

Investigations centered around EO have provided the basis for the concept of using adducts in hemoglobin (Hb) as a surrogate measurement for DNA adducts in target tissues. The relationship between chronic exposure to EO and the molecular dose of N-(2-hydroxyethyl)valine (HEVal) in Hb was investigated in male rats exposed to 0, 5, 10, 30, 100 or 300 ppm EO 5 hr/day by inhalation for 4 weeks, or exposed to 300 ppm for 1, 3, 5, 10 or 20 days (5 days/wk). HEVal was determined by Edman degradation. The dose-response curve for HEVal was linear between 3 and 100 ppm EO, with concentrations ranging from 5.2 ± 0.3 to 145 ± 29 (SEM) nmol/g globin (N = 5). Above 100 ppm EO, the slope of the dose-response curve increased. Chronic administration of 300 ppm EO led to linear formation of HEVal for five days, followed by a decrease in the slope of the formation curve with further exposures. The concentration of HEVal after 4 wk was 14 times greater than that found after 1 day of exposure (42 ± 3 nmol/g globin). Comparison of HEVal in Hb to 7-(2-hydroxyethyl)guanine (7-HEG) in DNA of the same rats showed that the formation of each adduct was nearly equal after a single dose of EO, but HEVal accumulated to 2 to 4 times the concentration of 7-HEG (depending upon the tissue) after 4 wk of exposure. The occurrence of a nonconstant relationship between HEVal and 7-HEG, during chronic exposures to EO, indicates that the use of Hb adducts as a surrogate for DNA adducts requires an understanding of both DNA and Hb adduct formation and persistence under conditions relevant to human exposure.

FORMATION & PERSISTENCE OF 1,3,5-TRINITROBENZENE ADDUCTS WITH BLOOD PROTEINS AND TISSUE DNA.


Blood protein adducts of chemicals have been used as biological markers of exposure. We investigated the ability of 1,3,5-trinitrobenzene (TNB) to form adducts with blood proteins and tissue DNA. Forty male rats were gavaged once with 14C-TNB (1.06 mmol/kg, 9.47 mcCi/mmol) and divided equally into eight groups and were sacrificed after day 1, 2, 3, 4, 7, 14, 21 and 28, respectively. Adducted TNB with blood proteins, albumin (ALB), globulin (GLBU) and globin (GLB) was determined. ALB and GLBU adducts reached maximum (218 pmol/mg, 100%) and (61 pmol/mg, 100%) by day 1 and by day 7 about 90-95% of the adducts were lost. In contrast, GLB adducts reached maximum by day 2 (200 pmol/mg, 100%) and after 28 days 20% of the GLB adducts remained. TNB also formed stable adducts with DNA by day 1 in the spleen (16 pmol/mg, 100%) and by day 3 in both the stomach (112 pmol/mg, 100%) and the liver (75 pmol/mg, 100%). After 28 days the residual adduct level in the liver and stomach was 25% and of spleen was 100%. Hence, protein and DNA adducts of TNB may be useful as markers for exposure assessment. (Abstract does not necessarily reflect EPA policy) Supported by US Army I.A. Project Order No. 90FP0012.

LOW-DOSE DNA ADDUCT DOSIMETRY BY ACCELERATOR MASS SPECTROMETRY (AMS).


DNA adduction was measured following exposure to low doses of [2-14C]-2-amino-3,8-dimethylimidazo[4,5-d]quinoxaline, (MeOx) [2-14C]-2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and [14C]-2,3,7,8-tetrachlorodibenzo-p-dioxin by AMS, a technique used in the earth sciences but not previously in toxicological research. The ability to measure low concentrations of rare isotopes suggested that biomedical research was a potentially powerful application for this technology. Sensitivity of the method was found to be one adduct per 1011 nucleotides. No DNA adduct formation could be detected in TCDR treated rodents. DNA adducts in cynomolgus monkey lymphocytes following exposure to 500 µg/kg IQ peaked between 6 and 18 hrs following exposure. Sensitivity was limited mainly by the abundance of 14C in contemporary carbon. Hosts depleted in radiocarbon are being developed, potentially increasing sensitivity another 3 orders of magnitude. These results demonstrate the high sensitivity of AMS for tracing molecules following administration of low levels of isotopically-labeled xenobiotics. In addition to 14C measurement, AMS offers potential to conduct studies with other isotopes, particularly 3H and 41Ca. (Performed under the auspices of the US DOE by LLNL under contract #W-7405-ENG-48 and partially supported by NCI grant CA0811 and by NIEHS. IAG #222Y01-ES-70136).
Exposure to chemical carcinogens can often be identified by detection of DNA adduct lesions. Primary cultures of isolated rat and human hepatocytes were exposed for 24 hours to 10 μM of 2-acetylaminofluorene (AAF), 4-aminobiphenyl (ABP), or benzo[a]pyrene (BaP). The isolated DNA from the exposed cells was analyzed using the 32P-postlabeling assay. Total ABP-DNA adducts were 1.7 fold greater in human cells (3.8 fmole) than in rat cells. Total AAF-DNA adducts were 2.4 to 3.5 fold greater in human hepatocytes (5.3-7.5 fmole), while total BaP-DNA adducts were 3.4 to 11.7 greater in hepatocytes from humans (6-21 fmole) than from rats. The predominant DNA adducts for each carcinogen were the same between rat and human cells. The dG-C4 AF was the major AAF-DNA adduct. The dG-C4-ABP was the major ABP-DNA adduct. In the rat dG-N2-BaP and two unidentified adducts were nearly equal in amount, while the major BaP-DNA adducts in the humans was the dG-N2 BaP. These results are comparable to the predominant adducts found with rats exposed in vivo. Similarly, DNA adducts from samples of human peripheral blood of individuals exposed to environmental BaP pollutants are comparable to hepatocyte cultures. Human hepatocytes demonstrated 2 to 12 fold greater DNA binding than rat hepatocytes. Thus, human hepatocyte culture system can provide useful information for assessing human carcinogenic hazards.

Determination of Hemoglobin Adducts of Acrylamide and its Epoxide Metabolite Glycidamide in Rats. E. Benmark, G. J. Callaman and L. G. Costa,Dept. of Environmental Health, University of Washington, Seattle, WA.

Glycidamide (GA), a reactive epoxide metabolite, is formed in vivo in rats treated with acrylamide (AA) (Callaman, Bergmark and Costa, Chem. Res. Toxicol., vol 3 (5) 1990). AAs and GA covalently bind to hemoglobin (Hb) and the levels of the adducts formed can be used to determine in vivo doses for monitoring exposure to acrylamide and for risk estimation of toxic effects. We have investigated the formation of AA and GA hemoglobin adducts in rats given various doses of AA or GA (0.5 - 100 mg/kg) by a newly-developed GC-MS method which simultaneously measures the cysteine adduct levels of both compounds in hydrolyzed hemoglobin samples. After AA injection, the AA adduct levels increased approximately linearly with the given dose, whereas levels of the GA adducts suggested that the enzyme system responsible for its formation becomes saturated at higher concentrations of AA. After injection of AA, adduct levels increased linearly with the given dose, although, on a mole/kg basis, the levels were lower than the AA adduct. In vitro experiments indicated that this was partly due to GA's lower reactivity towards cysteine-SH and possibly its slightly higher elimination rate in blood. The percentage of AA metabolized to GA ranged from 25% at low doses to 8% at higher doses. The cumulative Hb adduct levels of AA and GA in animals administered AA in acute versus chronic regimens is currently under investigation. (Supported in part by grant ES-04696 from NIEHS and CR-816768 from EPA).


Even though there are a number of sources for human exposure to acrylamide, reliable biomarkers of exposure are not available. In an effort to develop such a biomarker, we are characterizing peptides derived from trypsin digests of acrylamide-adducted hemoglobin. For this, radiolabeled acrylamide was incubated with purified human hemoglobin (Ao) and decomposition products removed by dialysis. When the adducted hemoglobin was separated by reverse-phase HPLC, radioactivity eluted with the α and 8 subunits, suggesting covalent binding. Digestion of individual subunits with trypsin followed by reverse phase HPLC indicated that most of the radioactivity associated with the α subunit coeluted with a single peptide. Similar results were observed for the 8 subunit except that significant amounts of radioactivity eluted with the solvent front, suggesting that radioactivity was released to the aqueous phase during digestion. Currently, these preparations are under further characterization by electrospay ionization mass spectrometry. This approach will aid in the identification of the adducted peptide and subsequent preparation of an acrylamide-specific antibody. Supported by US EPA Contract 14478 under a Related Services Agreement with the US DOE under Contract DE-AC05-76RLO 1830.

α-Toluidine Blood Protein Adducts: HPLC Analysis with Fluorescence Detection after a Single Dose in the Adult Male Rat. K L Cheever, G D DeNard, & T F Swarengen. NIH, DBBS, ETB, ETS, Cincinnati, OH

Hemoglobin (Hb) and albumin (Alb) adducts of the suspect human carcinogen α-toluidine (OT) were quantified in blood samples collected from rats after a single blood injection. α-Toluidine hydrolysis of Hb-adducted [14C]OT followed by extraction with ethylacetate resulted in recovery of 66% of the bound radioactivity. HPLC analysis revealed a single radiolabeled peak which was identified as OT by GC-MS. In subsequent experiments the Hb and Alb adduct levels were determined by HPLC analysis of this split product using fluorescence detection. 4-Ethylamidine was used as internal standard. The detection limit for OT was approximately 450 pg/injection or 3 pmol/μg Hb. Mean adduct levels for Hb increased rapidly over the first 4 hr with the highest (ng/μg Hb ± SD) 3.7 ± 0.5 detected 24 hr after OT (50 mg/kg body wt). In contrast, adduct levels for pooled Alb samples increased from 0.7 ng/μg Alb at 2 hr to 2.5 ng/μg Alb at 4 hr, but were not detectable 24 hr after dosing. Hb adducts showed a linear relationship for OT doses of 10, 20, 40, 50, and 100 mg/kg body wt. The Hb adduct level (6.3 days) was determined after a single 100 mg/kg OT dose. Hb adduct levels were quantifiable (0.1 ± 0.2 ng/μg Hb) by HPLC/fluorescence 28 days after 100 mg/kg OT. Although Hb and Alb adducts differ in stability, a ratio of such OT-adducts may be useful in long-term industrial biomonitoring for evaluation of OT exposure.
In vivo metabolic activation of BaP produces the ultimate carcinogen BaP diol epoxide (BaPDE) which can bind covalently with blood proteins. These adducts, whose level reflect the absorbed dose of BaP, can be quantified in a multi-step analytical procedure: 1-mild acid hydrolysis which yields BaP tetraol; 2-Cleanup on C-18 cartridges; 3-Ethyl acetate/water partitioning and 4-HPLC/fluorescence analysis. When the hydrolysate (step 1) was spiked with standard BaP tetraol, we recovered about 70% of the added amount. When extensive enzymatic proteolysis of hemoglobin (Hb) or albumin of BaPDE-treated rats was performed, no tetraol was seen in the HPLC analysis indicating that the BaP tetraol appearing after acid hydrolysis truly originates from an adduct. For an adduct level of 117 pmol/g Hb or 898 pmol/g albumin the intra-assay coefficient of analytical variation obtained for 4 determinations was 7.8% and 4.2% respectively. In rats treated i.v. with a single dose of 2.75 μmol BaPDE, adducts levels were 74.9 pmol/g Hb and 211 pmol/g albumin 24h after the injection. Five and 20 days later the Hb adducts were reduced to 53 and 20% of this level whereas the disappearance of the albumin adducts was faster with corresponding figures of 29 and 0% (below detection). HPLC/fluorescence analysis of adduct-derived BaP-tetraol is sensitive and reliable and the concomitant measurement of Hb and albumin adducts yield useful information about exposure to the parent BaP. (Supported by IRSST-Québec)

The usefulness of small wild mammals as indicators of the degree of contamination of certain ecosystems has been suggested by some authors. We have examined the possibility to use the measurement of benzo(a)pyrene (BaP)-hemoglobin and BaP-albumin adducts in wild woodchucks to assess environmental contamination by this carcinogenic PAH. The study was conducted in the Province of Québec. Blood from 8 animals was obtained in the control area which is located on the south shore of the St-Lawrence river, 350 km downstream from Montréal. Blood samples from 9 animals were also collected 10 km downwind from a major aluminum plant located 450 km NNE from Montréal. After isolation of hemoglobin and albumin, the samples were subjected to mild acid hydrolysis to release the adduct-derived BaP tetraol which was analyzed by HPLC-fluorescence. The level of both hemoglobin and albumin adducts (geometric mean [range]) was significantly higher for the woodchucks living in the contaminated area (1.18 [0.02-12.84] and 59.6 [10.0-1130.5] pmol/g protein) compared to the animals captured in the control area (0.40 [0.01-2.72] and 7.6 [0.8-29.3] pmol/g protein). The level of protein adducts in the horned mammals living in open fields seems to be a useful biocriterion of the level of contamination derived from industrial or other sources. (Supported by CAFIR Université de Montréal)
DERIVATION OF $K_m$ AND $V_{\text{max}}$ FOR THE METABOLIC CONVERSION OF ACRYLAMIDE TO GLYCIDAMIDE FROM HB ADDUCT LEVELS IN Vivo IN RATS

Carl Johan Calleman¹, Louis G. Stern², Krista Maschoff², Emma Bergmark¹, and Lucio G. Costa. Depa. of Environmental Health¹ and Applied Mathematics², University of Washington, Seattle, WA.

Many hazardous compounds require metabolic activation to reactive electrophilic agents for the induction of toxic effects. In such cases, the rate of metabolic activation of the parent compound, A, to an electrophile, RX, may be assumed to be a saturable function of the concentration of A and the rates of change of the in vivo concentrations of the parent compound (A) and the metabolite (RX) can be described by the following differential equations, where $k_1$ and $k_2$ are the rates of elimination of A and RX, respectively:

$$\begin{align*}
\frac{d[A]}{dt} &= -k_1[A] + k_2[RX]
\frac{d[RX]}{dt} &= \frac{V_{\text{max}}[A]}{K_m + [A]} - \frac{k_2[RX]}{K_m + [A]}
\end{align*}$$

Using the relationship between the in vivo dose, D, and the adduct level, $(D/V)/Y$, formed in the reaction of RX with a nucleophile Y with the rate constant $k_Y$: $D = \frac{[RX]}{k_Y}$, as derived by Ehrenberg et al. (Mutat. Res., 24 (1974) 83), it was possible to deduce an exact relationship between the adduct level and the initial concentration of A, by integrating the above equations twice over time.

By means of this relationship: $D(V)/Y = V_{\text{max}}(1 + [A]/K_m + V_{\text{max}}/k_1)/k_2$, $K_m$ and $V_{\text{max}}$ for the metabolic conversion of acrylamide to glycidamide were estimated to be $10 \mu M$ and $14.25 \mu mol/kg bw/hr$, respectively, from the adduct levels formed in vivo by glycidamide with HB in rats. (Supported in part by NIHES grant ES-04696 and CR-819768 from the EPA).

NICOTINE AND COTININE: DETECTION BY ELISA IN BLOOD, SALIVA AND URINE. S D. Stanley, M Huffman, R. Sadove, C G Gairola, J N Diana, R T Dowell, H-H Tai, D Watt and T Tobin. Graduate Center for Toxicology and Dept. of Veterinary Science, University of Kentucky, Lexington, KY.

We have raised antibodies against the tobacco alkaloids (S)-( )-nicotine and its major metabolite (S)-( )-cotinine. These antibodies were then developed as a one step Enzyme-Linked Immuno-Sorbert Assay (ELISA). Upon evaluation we found these antibodies to be highly specific for parent drug and showed minimal cross-reactivity with nicotine metabolites and other structurally related compounds. The nicotine ELISA has an I-50 for nicotine of approximately $100 ng/mL$, while the cotinine ELISA shows an I-50 for cotinine of about $1.0 ng/mL$. Both ELISA’s detected their respective analytes in plasma samples from rats, urine samples from mice and humans, and in serum samples and saliva samples from human subjects. Experiments with NIST standards for cotinine in human urine showed that this ELISA is relatively accurate ($\pm 18\%$) indicator of the presence of cotinine. These ELISA’s will enable reliable quantitative measurement of nicotine or cotinine as an indicator of "mainstream" and "sidestream" exposure to tobacco smoke. (Supported by The Tobacco and Health Research Institute, University of Kentucky.)

STRESS PROTEIN SYNTHESIS AND ACCUMULATION AS AN INDICATOR OF TOXICANT EXPOSURE AND EFFECTS. S D Dyer, K L Dickson, and E G Zimmerman, Institute of Appl. Sci., Univ. of North Texas, Denton, TX.

Sponsor: J R Coates.

The stress protein response is a ubiquitous protective mechanism that enables cells to tolerate environmental perturbations. This response results in the decreased transcription of "normal" genes and increased transcription of stress protein genes, the products of which are involved in protein folding, multimeric assembly, and receptor interaction, to name a few. Gill, brain, and striated muscle tissues of fathead minnows exposed to toxicants such as arsenite, chromate, copper, lindane, and diazinon elicited the stress response. Suites of stress proteins induced were toxicant specific. Synthetic rates and accumulation were significantly correlated with concentration and mortality. These results indicate the potential usefulness of the stress response as a biomarker of exposure and effects.

DEVELOPMENT PATTERNS OF CONTAMINANT-SENSITIVE ENZYMES IN NESTLING STARLINGS (STURNUS VULGARIS). J L Hofius and M J Hooper, The Institute of Wildlife and Environmental Toxicology, Clemson Univ., Clemson, SC. Sponsor: R W Mixstrom

Artificial nestboxes on hazardous waste sites or agricultural areas encourage adult European starlings to nest in contaminated habitats. Development patterns of contaminant-sensitive enzymes from their nestlings (days 0-21 post-hatch) are dynamic indicators of exposure to site specific contaminants. Cholinesterase in brain and butryrylcholinesterase (BChE) in plasma increase dramatically through development while plasma acetylcholinesterase decreases then stabilizes at low activities. Field assessments using BChE patterns identified organophosphate exposure scenarios dependent on application method and compound. Ethoxyresorufin-O-deethylase (EROD) and glutathione-S-transferase (GST) activity showed differential development patterns, with striking increases over time in kidney GST activity. Liver activity was more stable through nestling development. Exposure assessments of sites contaminated by past fire school and pesticide rinseate activities will be demonstrated.
Organ specific toxicity such as nephrotoxicity is often investigated by in vivo animal models or in vitro. It would be beneficial if these findings could be verified in a human in vitro system. In collaboration with the Institute for the Advancement of Medicine, we were able to establish a human kidney slice system which makes this possible. Control cortical kidney slices approximately 250 μm were evaluated using intracellular K⁺ content, protein synthesis and organ injury transport for 3, 6, 12, 18 and 24 h. Control slices were viable for this time period using all viability parameters. Cisplatin (10, 50 and 100 μM) and mercuric chloride (100, 250 and 500 μM) were used as nephrotoxins in this human kidney slice system. Cisplatin seemed to be more nephrotoxic in this human in vitro system than in previously investigated in vitro animal systems whereas mercuric chloride was similar in both systems. These studies suggest that suspected nephrotoxins should be analyzed in human tissue to verify the potential of toxicity in the human. (NTH GM 38290)

The agricultural fungicide NDPS produces renal proximal tubular damage when administered to rats. Although metabolism is believed to play a role in this process, the ultimate toxic species has yet to be identified. In this study, we investigated the in vivo nephrotoxicity of two potential p-hydroxylated metabolites of NDPS, N-(3,5-dichloro-4-hydroxyphenyl)succinimide (NDPS-p-OH), and its succinimide ring hydrolysis product, N-(3,5-dichloro-4-hydroxyphenyl)succinic acid (NDPSA-p-OH). NDPS-p-OH, NDPSA-p-OH (0.2 or 0.4 mM/kg) or vehicle were administered i.p. to male Fischer 344 rats (4 rats/group). Nephrotoxicity was assessed at 24 and 48 h by monitoring changes in urine volume, blood urea nitrogen levels, accumulation of organic ions (lactate, ammonium and p-aminohippurate) by renal cortical slices, kidney weights, and morphology under light microscopy. Neither analog produced significant changes in any of these parameters at the two doses studied when compared to controls, and therefore lack the toxicity of the parent compound. In other studies, we have obtained tentative evidence that NDPSA-p-OH is a minor metabolite of NDPS. However, these observations suggest that NDPS-p-OH and NDPSA-p-OH may not be involved in NDPS-induced renal damage. Supported by P.H.S. grants ES05189 (P.J.H.) and DK31210 (G.O.R.).
Chlorpromazine (CPZ) has been shown to attenuate the nephrotoxicity induced by the fungicide N-(3,5-dichlorophenyl)-2-hydroxy succinimide (NDHS) in mice. The purpose of this study was to examine the effects of CPZ on the renal toxicity mediated by NDHS metabolites, NDHS. Male Fischer 344 rats (4/group) were administered CPZ (1.0 or 5.0 mg/kg, ip) or vehicle 1 hr before NDHS (0.1 mmol/kg, ip) or vehicle. Renal function was monitored at 24 hr intervals for 48 hr post-NDHS or NDHS-vehicle. CPZ (5.0 mg/kg) pretreatment had no effect on NDHS-induced diuresis, increases in blood levels, proteinuria, and glucosuria, but did not attenuate NDHS-induced proteinuria and glucosuria, and did not decrease renal cortical slice accumulation of organic ions or proximal tubular necrosis. CPZ (5.0 mg/kg) slightly attenuated NDHS-induced proteinuria and glucosuria, but did not reduce the accumulation of organic ions or proximal tubular necrosis. These results indicate that CPZ only weakly attenuates NDHS nephrotoxicity and suggests that CPZ might attenuate NDHS nephrotoxicity by altering NDHS biotransformation. (Supported by NIH grant DK 31210).

In vitro interactions of S-(2-chloromethyl)-L-cysteine (CCE) with isolated renal lysosomes and cytosol from rats. M. Kasa, S. Chakraborti, and C. DeMott. Département de médecine du travail et d'hygiène du Milieu, Université de Montréal, Montréal, Québec, Canada.

CCE, a putative metabolite of 3,2-dichloroethane, forms through glutathione conjugation with nephrotoxic in rats, producing proximal tubular necrosis (J. Pharmacol. Exp. Ther. 242: 741-748, 1987). In vitro toxicity of CCE towards renal lysosomes and cytosol has been examined in the present study. Isolated renal lysosomes and cytosol from adult male Sprague-Dawley rats were incubated at 37°C with 0, 1, 2.5 and 5 mM CCE for 30 min. Lysosomal membrane fragility was assessed by the activities of N-acetyl-B-D-glucosaminidase (NAG) and acid phosphatase (AP) and toxicity in the cytosolic fraction by lactic dehydrogenase (LDH) activity. With increasing concentration of CCE, the free activity of either NAG or AP in the lysosomes was increased; that is, the portion of the enzyme activity that is demonstrable in the absence of detergent. In the cytosol, a parallel decrease of the AP activity with increasing concentration of CCE was observed. Both these effects due to CCE were found to be both dose- and time-dependent. These results indicate that both lysosomes and cytosol among others could also be the potential targets for the nephrotoxic action of CCE. (Supported by the Medical Research Council of Canada, MA-9705).

Effect of partial hepatectomy (HP) on the metabolism, distribution and nephrotoxicity of p-methylthiobenzamide (PMTB) in the rat. G. Trager, V. Davidson, C. Stogell, and D. Cox. Dept. Pharma. and Tox., Univ. of Kansas, Lawrence, KS.

PMTB produces injury to the liver and kidney. Toxicity is mediated via its biotransformation into a reactive S,S-dioxide metabolite. The objective of this study was to examine the role of hepatic metabolism in the production of PMTB-induced renal toxicity. Renal injury was assessed in partially hepatectomized and sham-operated rats and the effect of this procedure on the distribution of metabolism of PMTB was examined. Plasma urea levels and renal cortical slice uptake of organic ion were used to monitor renal function. HP enhanced the renal injury resulting from a 24-hr treatment with PMTB (1.2 mmol/kg). An HPLC method was used for the measurement of PMTB and its metabolites p-methylthiobenzamide-S-oxide (PMTBSO) and p-methylbenzamide in plasma and kidney. HP delayed the removal of this dose of PMTB from plasma and allowed greater concentrations to occur in plasma and kidney at 6 and 15 hr. Levels of the two metabolites were similar in both groups at 6 hr, but were increased in plasma and kidney of the HP group at 15 hr. The results indicate that the increase in nephrotoxicity induced by HP is associated with an increased renal accumulation of PMTB and PMTBSO which are both precursors to the reactive metabolite. (Supported by grant RR-6605).


Intraperitoneal administration of AT-125 at 10 mg/kg in rats inhibited renal gamma-glutamyl transpeptidase (GGT) by 95% (Davis, Mary E., Toxicol. Appl. Pharmacol. 92: 44-52, 1988). Prior to studying the effects of GGT inhibition by AT-125 on the glutathione mediated toxicity of another compound, the nephrotoxicity and hepatotoxicity of AT-125 alone was evaluated in this study. AT-125 was administered by ip injection at 10 mg/kg to two groups of male Fischer-344 rats at 12 hour intervals. One group was terminated at 24 hours and the other group was terminated at 48 hours. Two control groups were dosed with the vehicle and terminated at the same intervals. All rats were subjected to a gross necropsy. Blood samples were collected for clinical chemistry evaluation. Brain, liver and kidney weights were recorded. The kidneys and livers were examined microscopically. All animals survived to termination and no clinical findings were observed. Clinical chemistry values and absolute and relative organ weights were comparable among the groups. No treatment-related microscopic alterations were observed in the kidneys and livers. AT-125, when administered by ip injection for one or two days at 12 hour intervals, did not produce kidney or liver damage in Fischer-344 male rats.

Cephaloradine (CPH) causes a variety of biochemical and functional changes in tubule cells resulting in acute proximal tubular necrosis when given in large single doses. Recently we reported that CPH also decreased alkaline phosphatase (ALP) activity in rat renal proximal tubule (RPT) preparations. CPH at 1.0 mM depressed rat (male, F-344) RPT (0.5 mg protein/mL hormone- and 2% BSA-supplemented culture medium) ALP activity (significantly at one hr, increasing to up to 60% with 10 mM CPH by 8 hr); -glutamyltransferase (GGT) activity was unchanged. Under these conditions, lipid peroxidation (TBA reaction products) was minimal and CPH-induced LDH release at 8 hr was insensitive to promethazine. At 5.0 and 10.0 mM CPH reduced basal and nystatin-stimulated respiration (12% and 44%, respectively) prior to LDH release. Male F-344 rats given CPH at 800 mg/kg/d for 3 d showed marked necrosis of renal tubules, compared with vehicle-only controls, and a 45% reduction in ALP; GGT was depressed 16%. The extent of CPH-induced ALP depression in RPT in vitro at 8 hr could be repressed if tissue incubation was replaced with CPH-free medium. ALP depression occurs with other cephalosporins and may provide a marker for clinical detection of renal injury by these antibiotics. (Supported in part by NIH Contract No. ES-651455).

BIOCHEMICAL DIFFERENCES BETWEEN α2u-GLLOBULIN (α2u) AND MOUSE URINARY PROTEIN (MUP): EXPLANATION OF MURINE RESISTANCE TO DEVELOP HYALINE DROPLET NEPHROPATHY (HDN). D Caudill, T N Asquith and D Lehman-McKeeman. Proctor & Gamble Co., Cincinnati, OH.

It is well-established that α2u is pivotaly involved in HDN. However, whereas MUP is structurally-homologous to α2u, mice do not develop HDN. Experiments were conducted to compare the capacity of α2u and MUP to bind d-limonene oxide (dLO) and to evaluate the renal handling of the two proteins. Equilibrium saturation binding studies showed that α2u bound dLO, but there was no interaction between dLO and MUP. The renal reabsorption of α2u and MUP was evaluated by comparing urinary excretion in untreated and Na maleate-treated (to inhibit tubular reabsorption) animals. In controls, excretion of α2u and MUP, quantified by HPLC, was 16.3 ± 1.6 and 14.6 ± 1.1 mg/day, respectively. The total filtered load of the proteins (Na-maleate-treated), was 40.9 ± 2.1 and 15.2 ± 1.2 mg/day for α2u and MUP, respectively, thereby indicating that 60% of filtered α2u is reabsorbed by the kidney and that MUP is not reabsorbed. These results were confirmed by 2-D gel electrophoresis which showed that MUP was absent among mouse kidney proteins. Thus, MUP is sufficiently different from α2u that it does not bind dLO. More importantly, MUP will not cause HDN because it is not reabsorbed into the kidney.


The renal papilla is rich in GAGs and histochemical studies have shown that changes in GAG content or structure occur early in the development of renal papillary necrosis (RPN). The aim of this study was to evaluate urinary GAG excretion as a potential early, non-invasive marker of RPN, using two known papillary toxicants, 2-bromoeschamine (BEA) and indomethacin. Following a single dose of BEA (150 mg/kg) to female SD rats, mean urinary GAG excretion was increased 2.5 and 1.5-fold during the periods 0-8 and 8-24 hr post-dose respectively, returning to control levels thereafter. In subsequent studies rats were given a single dose of BEA (150 mg/kg) or indomethacin (75 mg/kg) and urine collected 0-24 hr pre- and post-dose. In each case, 8/10 rats had histologically-detectable papillary lesions and for those animals mean GAG excretion was increased 2-fold over the pre-dose values (p<0.001). Pre- and post-dose GAG excretion did not differ in control rats. These results suggest that increased urinary GAG excretion may be a marker of xenobiotic-induced RPN.

EXPLORATION OF THE TUMORIGENIC POTENTIAL OF A UNIQUE MALE RAT PROTEIN - ALPHAB2GLOBULIN. C L Alden, M E Chestnut, R A LeBeouf, J L Burns, T E Burrell, J A Polley and R Raineri. The Proctor & Gamble Co., Cincinnati, OH, the University of Illinois (Burrell), and Hazleton Laboratories, Vienna, VA (Polley and Raineri).

Several societally-important chemicals have been demonstrated to induce alphab2globulin nephropathy such as d-limonene and unleaded gasoline. Substantial progress has been made in characterizing the effect of these chemicals and further defining prerequisite events in the pathogenesis of this syndrome. An alphab2globulin increase is hypothesized to be the proximate event in the toxicologic and tumorigenic sequelae associated with administration of these xenobiotics over the male rat's lifetime. In this scenario the xenobiotic simply serves as a mediator of the alphab2globulin overload. In support of this hypothesis, alphab2globulin has recently been demonstrated to cause morphologic transformation in the Syrian hamster embryo (SHE) cell transformation assay. Initial results indicate that transformation was stable based on transient exposure to alphab2globulin with persistence of the morphologic (phenotypic) alteration. Other proteins such as albumen and lysozyme did not induce transformation suggesting a protein-specific phenomenon. Results suggest the SHE assay as a useful model for mechanistic studies of this syndrome.
Chemically-induced α2u-globulin (α2u) accumulation in the kidneys of male rats leads to degeneration/regeneration of the proximal tubule cells (PTC). Chemical binding to α2u alters the hydrolysis rate of the protein which is believed to be responsible for its accumulation by the PTC. Using 2,4,4-trimethyl-2-pentanol (TMP-2-OH) as a representative chemical, a quantitative biologically-based description of the relationship between TMP-2-OH exposure, pharmacokinetics, interaction with α2u, and accumulation of α2u (bound and free) in renal PTC and resultant PTC death, was developed for the male F344 rat. Physiological parameters such as tissue volumes, blood flows and rates of kidney filtration, α2u synthesis, reabsorption and degradation were obtained from the literature. TMP-2-OH was determined to bind to α2u in vitro with an apparent binding affinity of 0.2 μM. This value was used in the model along with TMP-2-OH tissue solubility data measured by vial equilibration. The model accurately simulated the increase in the concentration of α2u present in the kidneys of male rats 24 hours after treatment with TMP-2-OH (80 mg/kg, p.o.) when the degradation rate of TMP-2-OH-α2u complex was 4X less than the free protein. Simulated studies indicated that peak concentrations of α2u present in the PTC were sensitive to the degradation rate of TMP-2-OH bound α2u, while the residence time of α2u in the PTC was largely determined by the binding affinity. With appropriate laboratory validation the model should provide insight into the mechanism of α2u accumulation and the subsequent PTC death.

Decalin is an acyclic hydrocarbon which has been shown to cause a male rat specific α2u-globulin nephropathy (Karerva et al., 1987). An acute manifestation is the accumulation of intracytoplasmic protein droplets in the proximal tubular epithelial (PTE) cells. Experiments were conducted to assess lysosomal enlargement and the location of α2u-globulin (α2u) within the PTE of male rats dosed orally with decalin (500mg/kg/day for 3 days). The lysosomes of the PTE cells of male rats treated with decalin were approximately 3 times the size of untreated control rats. Concurrent immunocytochemical localization of α2u showed α2u accumulation mainly on the luminal surface of the PTE cells, with little α2u localized within the enlarged lysosomes. The location of α2u corresponds to the normal position of endosomally engulfed materials. The results of this study suggest that decalin-induced lysosomal enlargement may not be due to α2u accumulation within the affected lysosomes.

Male rat nephropathy is characterized by increased hyaline droplet formation in renal tubular cells, and has been associated with exposure to several hydrocarbon compounds and an increased cellular accumulation of α2u globulin (α2U). The appearance of hyaline droplets in renal tubular cells is primarily due to proteins contained within the endosomal and lysosomal compartments. Histochimical staining procedures for horseradish peroxidase (HRP) and acid phosphatase (AP) were used to detect decalin-associated alterations in the endosomal and lysosomal compartments of renal tubular cells. Decalin exposure: (1) increased the size and produced the AP stain intensity of individual lysosomes, and (2) altered the endosomal distribution of HRP when compared to control animals. These findings indicate that decalin exposure affects the morphology as well as the function of the endosomal-lysosomal pathway in renal tubular cells.

DMSA has been shown to be an effective chelator for lead (Pb). Two groups of rats were given 0.5% Pb acetate for 6 mo. (ED6) & sacrificed at 12 mo. An ED6 group was treated with 3 x 5 day courses of 0.5% DMSA (DMSA) every 2 mo. & sacrificed at 12 mo. Normal controls (CD6) were pair-fed. Blood Pb (BL; µg%) GFR (ml/min/100g) & renal cortex Pb (µg/g) & silicon (Si; µg/g) were assessed. BL in DMSA rats (19.1±2.2) was significantly higher than ED6 (3.0±0.6) but lower than ED6 (30.4±6.3). GFR was significantly increased in DMSA (1.7±0.17) as compared to CD6 & ED6, & was significantly higher in CD6 than in ED6 (0.9±0.06 vs 0.8±0.17). Kidney Pb was higher in DMSA than in ED6 (132±128 vs 54±25) while kidney S1 was significantly lower in DMSA (5±8 vs 12±4). Tubulointerstitial changes were equal in DMSA & DMSA. Data indicate: 1) high dose of Pb results in lower GFR even after its discontinuation; 2) DMSA causes persistent increase in GFR, which is independent of pathological changes in kidney Pb. The relationship of the increased GFR to reduction in S1 is conjectural.
BETA-2-MICROGLOBULIN: A MARKER TO ASSESS RENAL TUBULOPATHY IN YOUNG AND OLD RATS. D. Chevalier, R. Owem, Y. Bailly, P. Delort and P. Duprat. MSD-Chibret, route de Marsas, Riom, France.

Sodium chromate (3 mg/kg/day, SC) was used as a model of renal tubulotoxicity assessed in young (9-10 weeks old) and old (89-91 weeks old) Syrian hamsters as a method using periodical Beta-2-microglobulin (B2M) ELISA techniques in combination with routine urinalysis methods (same time intervals) and histopathological evaluation of kidneys at termination. Control animals were used for comparison with Na chromate treated rats. Results from old untreated control animals clearly demonstrated a good correlation between albumin, B2M and chronic progressive nephrosis (CPN). Increase in albuminuria directly paralleled the CPN severity. Result of B2M indicated that the tubular function was unchanged in rats having a slight CPN and albumin excretion below 15 mg/16 hrs. Conversely, when albuminuria was above 15 mg/16 hrs and CPN more severe, tubular function was gradually more affected. In Na chromate treated young rats, routine urinalysis failed to detect tubular effects and only some debris of tubular origin were present in urine of old treated rats. A marked increase of B2M was observed in young and old treated rats independently of the age-related kidney disease and B2M increase paralleled the tubulopathy. In conclusion, B2M determination is a reliable marker of renal tubulopathy in rats of different ages.

ACTIVATION OF THE POLYOL PATHWAY MAY CONTRIBUTE TO INCREASED RISK OF RADIOCONTRAST AGENT NEPHROTOXICITY IN DIABETES. D. L. Kaplan, P. L. Mann, P. G. Zager and M. A. Smith, Dep of Tox., Univ. of New Mexico, Albuquerque, NM. Spons: G. B. Carson.

The incidence of radiocontrast (RC) agent nephrotoxicity is higher in diabetic than non-diabetic patients. RC nephropathy involves both glomerular and tubular cells. We postulate that activation of the polyol pathway contributes to the increased susceptibility of diabetics to RC nephrotoxicity. Mesangial cells modulate GFR by altering the capillary surface area available for filtration. Toxic insult to mesangial cells can impair glomerular function. The present study was performed to determine if mesangial cells isolated from galactose-fed rats, the classic model for studying the effects of polyol accumulation, demonstrate increased susceptibility to RC toxicity. A cellular model was developed to study the effects of RC agents on mesangial cells isolated from rats maintained on diets of 50% galactose (MCG) and 50% dextrin (MCD). MCG's and MCD's were plated in 96 well trays at a density of 6.25 x 10^4 cells/ml. Cells were exposed to Hypaque 90 at concentrations of 10 mM to 1 uM for 1, 2, 4 & 24 hrs. Cell viability was determined by fluorescein isothiocyanate-propidium iodide staining. 3H-thymidine incorporation was used to determine cell proliferation rates. Hypaque 90 produced no detectable cytotoxicity at any time points or concentrations tested. An increase in cell proliferation was observed 1 hr after exposure to 1 uM Hypaque. Higher concentrations blunted cell proliferation. No significant effects on 3H-thymidine incorporation were observed at later time points. The effects of 3H-thymidine incorporation were pronounced in MCG's than MCD's. Activation of the polyol pathway amplifies the proliferative response of RC to RC agents. This activation may contribute to the risk of RC nephropathy in diabetes.

INFLUENCE OF DIETARY PROTEIN CONCENTRATION ON SEVERITY OF NEPHROPATHY IN FISCHER 344 RATS. G. N. Rao, J. Edmondson and M. R. Elwell. National Toxicology Program, NIEHS, Research Triangle Park, NC.

Nephropathy is an age-related spontaneous disease of most rat strains and protein content of diets may affect the severity. Spontaneous nephropathy may complicate chemical toxicity. The purpose of this study was to determine the effect of 15% protein nonpurified diet on body weight and severity of nephropathy in comparison with 23% protein NIH-07 diet. Groups of 25M and 25F F344 rats of 6 weeks of age were fed the 23 or 15% protein diet ad libitum for 2 years. Rats were weighed at 1-4 week intervals and the maximum mean body weight attained by each group was determined. Urine was collected during the 24th month and analyzed. Kidneys of rats were fixed during the 24th month and at the end of 2 years were graded for severity of nephropathy (minimal, 1, mild-2, moderate-3 and marked-4) by light microscopic evaluation of H&E stained sections. The results for male rats were 516 and 500g body weights, 3.3 (moderate to marked) and 1.4 (minimal to mild) nephropathy grades, 20.9 and 14.0 mg/dl urine volumes and 1430 and 455 mg/dl of total urine protein for 23 and 15% protein diets respectively. The values for females were 361 and 356g body weights, 1.6 (minimal to mild) and 1.1 (minimal) nephropathy grades, 16.5 and 10.8 ml 24 hr urine volumes and 349 and 173 mg/dl total urine protein for 23 and 15% protein diets respectively. Decrease of dietary protein concentration from 23 to 15% markedly decreased the severity of nephropathy especially in male F344 rats without a substantial decrease in maximum body weight.


Dosage-related increases in urinary protein values determined by Multistix® (Ames Co., Elkhart, IN) were observed for male and female rats treated with ipazilide at dosages of 20, 80 and 160 mg base/kg for 3, 3, and 6 months. No other biologically important clinical, pathological, or bacteriologic alterations were observed. The lack of correlation between proteinuria by other parameters suggested that a false positive reaction due to ipazilide or its metabolites may have been the cause of the apparent proteinuria. To test this hypothesis, ipazilide or its major metabolites were added to pooled rat or dog urine or distilled water (control) to obtain final concentrations of 0.0, 0.25, 0.5, 1.0, 1.5, 2.0, or 4.0 mg base/ml. Protein content was measured for each sample by Multistix® and the Coomassie blue method. The presence of ipazilide or its major metabolites in rat or dog urine or distilled water caused a concentration-related false positive reaction for urinary protein determined by Multistix®. Protein determination by the Coomassie blue method was not affected by the presence of ipazilide or its major metabolites.
Active transport of a toxicant into its target cell has proven to be crucial in producing toxicity in renal cortical slices. Past studies have emphasized organic anionic toxicants; organic cationic toxicants are now addressed. Precision-cut rabbit renal cortical slices were incubated with mepiperphenidol (MP), a known cationic transport inhibitor, for 1-12 hr at 10⁻⁴-10⁻⁶ M. Slices were evaluated for K⁺ content as a general measure of toxicity and for organic cationic (TEA) transport. 10⁻⁴ M MP produced a 50% loss in K⁺ content and TEA transport by 2 hr. 10⁻³ M MP did not affect K⁺ content, but reduced TEA transport 25% by 4 hr. A series of newly synthesized pyridinium or quinolinium cationic structures were then evaluated. A direct correlation was observed between their known ability to inhibit TEA transport and their ability to decrease the K⁺ content of the slices. In fact, the most potent TEA transport inhibitors (20 X > MP) ruptured the slices at 10⁻⁴ M. 1-propylpyridinium (4 X < MP) had little effect on K⁺ content but 1.5X decreased TEA transport in a time and concentration dependent manner (2-12 hr, 10⁻³-10⁻⁵ M). The addition of 1 mM TEA to block MP (10⁻⁴) transport and toxicity had minimal protective effects, which may indicate that the cation transport-related toxicity is occurring outside of the transporter location. Continuing evaluation of MP and this unique series of cation transport inhibitors will establish the role of cation transport in their toxicity to renal cortical slices. (NIH DK41006, T32 ES07091).

**TOXICITY OF ORGANIC CATION COMPOUNDS IN PRECISION CUT RABBIT RENAL CORtical SLICES, HV Sheever, TP Wutz, SH Wright, K Krendel, AJ Gandolfi, Arizona Health Sciences Center, University of Arizona, Tucson, AZ.**

**PROTECTION FROM CHEMICAL HYPOXIA IN ISOLATED RENAL CELLS. L H Lash, Dept. Pharmacology, Wayne State Univ. Sch. Med., Detroit, MI.**

Differences in energy metabolism and redox status in proximal tubular (PT) and distal tubular (DT) cells from rat kidney and susceptibility to chemical-induced injury were investigated by treating cells with iodacetamide (IDA) and KCN ("chemical hypoxia"). Treated PT cells released 40% and 57% of total lactate dehydrogenase (LDH) after 1 and 2 hr, respectively, as compared with 24% and 33% released from control cells. DT cells were markedly more sensitive to injury, releasing 70% and 83% of total LDH after 1 and 2 hr, respectively, compared with 23% and 31% released from control cells. Extracellular acidosis (pH 6.2 vs. 7.4) significantly delayed cytotoxicity in both PT and DT cells. Preincubation of PT or DT cells with 5 mM GSH or 10 mM ATP provided complete protection whereas 10 mM adenosine provided moderate protection and 20 μM α-tocopherol failed to provide any protection. Chemical hypoxia produced marked depletion (75-90% after 30 min) of cellular ATP concentrations but did not deplete cellular GSH.

Maintenance of cellular adenine nucleotides, although important, is not critical, because it was dissociated from cytotoxicity; whereas acidosis and GSH provided complete protection, they had no effect on adenine nucleotides. Conversely, adenosine markedly increased cellular ATP concentrations but was only moderately protective.

(Supported by NIH Grant DK40725.)

**DIFFERENTIAL EFFECT OF AMINOXYACETIC ACID (AOAA) IN TETRAFLUOROHYDROXYCYSTEINE (TFEC) AND DICHLOROVINYL-CYSTEINE (DCVC) TOXICITY TO RENAL PROXIMAL TUBULES (RPT). CE Groves, PJ Hayden, JL Stevens, EA Lock, and RG Schnellmann, Univ. Georgia, Athens, GA, MA Jones Cell Sci Ctr, Lake Placid, NY and "ICI, Cheshire, UK.**

DCVC and TFEC are metabolized by β-lactase to a reactive species which binds covalently to protein and in turn may initiate toxicity. AOAA, an inhibitor of β-lactase, has been shown to prevent cysteine conjugate toxicity in rat renal and LLC-PK1 cells, but has minimal effects in rabbit renal slices. To examine these model differences, the effect of AOAA on TFEC (25 μM) and DCVC (25 μM) toxicity in rabbit RPT suspensions was determined. AOAA (2 mM) reduced TFEC induced LDH release from 71% to 7% and decreased TFEC uptake and cofiant binding by 99%. AOAA (1 mM) decreased TFEC (100 μM) cofiant binding to RPT homogenates by 99%. When RPT were pretreated with AOAA (2 mM) and subsequently resuspended without AOAA, TFEC produced toxicity equivalent to TFEC alone. These findings suggest that AOAA prevented TFEC induced toxicity in RPT by inhibiting TFEC uptake. In contrast, AOAA (2 mM) had no effect on DCVC induced LDH release (75% vs 75%) and decreased DCVC uptake and cofiant binding by 50%. AOAA (1 mM) decreased DCVC (100 μM) cofiant binding to RPT homogenates by 60%. This lack of protection against DCVC toxicity by AOAA may result from limited uptake of AOAA by rabbit RPT. (Supported by NIH ES-04410 and PMA Fdn.)

**THE EFFECT OF EXTRACELLULAR ACIDOSIS ON CHEMICAL TOXICITY IN RABBIT RENAL PROXIMAL TUBULES (RPT) IS DEPENDENT ON THE INTRACELLULAR TARGET. D P Rodheaver and R G Schnellmann, Alcon Labs., Ft. Worth, TX and "Univ. Georgia, Athens, GA.**

Extracellular acidosis has varying effects on chemically-induced toxicity. The hypothesis was examined that the modifying effect of acidic extracellular pH (pH₅) is dependent on the target site of cellular damage. Cell death (LDH release) was determined in RPT suspensions incubated at pH₅ 6.4 or 7.4 after 5 hr exposures to the toxicants. Acidic pH₅ alleviated cell death produced by the mitochondrial inhibitors 0.1 mM antimycin A (84 vs 20%), 0.1 mM rotenone (29 vs 13%), and the protonophores 1 mM FCCP (78 vs 13%), 20 mM monensin (57 vs 19%), 1 mM nigericin (76 vs 10%), and 0.2 mM valinomycin (86 vs 12%). In contrast, acidic pH₅ potentiated 1 mM H₂O₂ (11 vs 50%), 0.5 mM ochratoxin A (4 vs 57%), and 0.5 mM tert-butylhydroperoxide (55 vs 72%) induced lipid peroxidation and cell death in RPT. Acidic pH₅ had no effect on 200 μg/ml nystatin (87 vs 73%) and potentiated 0.2 mM ouabain (34 vs 78%) and 5 μM A23187 (35 vs 58%). We suggest that acidic pH₅ ameliorates cell death when the primary target of a toxicant is the mitochondrial. In contrast, acidic pH₅ potentiates cell death when a toxicant preferentially damages and does not ameliorate cell death when the target is the plasma membrane. Our results with the oxidants and plasma membrane agents are opposite of those seen using hepatocytes (Nieminen et al., BBRC, 1990). (Am Heart Assoc, GA Affil.; NIH ES-04410.)

**DIFFERENTIAL EFFECT OF AMINOXYACETIC ACID (AOAA) IN TETRAFLUOROHYDROXYCYSTEINE (TFEC) AND DICHLOROVINYL-CYSTEINE (DCVC) TOXICITY TO RENAL PROXIMAL TUBULES (RPT). CE Groves, PJ Hayden, JL Stevens, EA Lock, and RG Schnellmann, Univ. Georgia, Athens, GA, MA Jones Cell Sci Ctr, Lake Placid, NY and "ICI, Cheshire, UK.**

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CTN causes nephrotoxicity by an unknown mechanism of action. This study determined if mitochondrial dysfunction played a role in CTN-induced RPT cell death. CTN decreased RPT viability in a concentration-dependent manner, increasing lactate dehydrogenase release from 7% to 43% and 22% 4 hr after exposure to 1 and 0.5 mM CTN, respectively. CTN (1 mM) decreased nystatin-stimulated oxygen consumption (QO2), an indirect measure of mitochondrial state 3 respiration, from 42 to 28, 18 and 10 nmol O2/mg protein/min 0.5, 1 and 2 hr after exposure, respectively. Ouabain-insensitive QO2 rose from 15 to 21 nmol O2/mg protein/min before declining to 13 and 8 nmol O2/mg protein/min 0.5, 1 and 2 hr after exposure, respectively. Similar changes in QO2 occurred after 0.5 mM CTN exposure. CTN (1 mM) decreased intracellular ATP from 7.2 to 4.4, 4 and 2.4 nmol/mg protein 0.5, 1 and 2 hr after exposure and increased ADP and AMP corresponding to the decrease in QO2. CTN (0.5 mM) did not affect adenine nucleotide content during the first 2 hr of exposure. Glycine (2 mM) and glutathione (1-2.5 mM), agents which protect RPT from specific inhibitors of mitochondrial function, partially reduced RPT cell death. Thus, mitochondrial dysfunction may be an important event leading to CTN-induced cell death. (Supp. by ES-05455).

CEPHALOSPORIN TOXICITY IN RABBIT KIDNEY PROXIMAL TUBULE CELLS GROWN ON MILLICELL-HA FILTERS. M J Palomo, B Masters, and O P Flint Bristol-Myers Squibb, Dept. of Invest. Toxicol., Syracuse, NY.

Cephalosporin toxicity in confluent monolayers of proximal tubule cells grown on filters was measured by mitochondrial dehydrogenase activity,ulinum movement, protein synthesis and apical uptake of α-methylglucopyranoside (α-MG). Drugs were added to the basolateral side of the cultures for 48 hours. The most sensitive toxic indicators were protein synthesis and α-MG.

\[ IC_{50} \text{ (50% Inhibition), mg/ml (n=6) } \]

<table>
<thead>
<tr>
<th>Drug</th>
<th>Protein Synthesis</th>
<th>α-MG uptake</th>
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<tbody>
<tr>
<td>Cephalothin (CET)</td>
<td>0.56</td>
<td>0.35</td>
</tr>
<tr>
<td>Cephaloridine (CEL)</td>
<td>0.66</td>
<td>0.71</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>&gt;2.0</td>
<td>&gt;2.0</td>
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<tr>
<td>Ceftazidime</td>
<td>&gt;2.0</td>
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This ranking reflects in vivo toxicities except for CET. Preincubation of CET (1 mg/ml) with rat hepatocyte suspensions for 2 hrs completely inhibited its toxicity, while CEL toxicity was not affected, indicating that CET may be metabolized in vivo to a non-toxic metabolite. Since CEL accumulates to toxic levels in proximal tubule cells in vivo while CET does not, the cells were exposed to the drugs for 5 hr (2 mg/ml) then removed and fresh medium added. Protein synthesis was decreased by CEL to 68% of controls while CET had no effect. Probenecid completely inhibited CEL toxicity at 1 mg/ml. Thus this system is a good model to study nephrotoxicity.

CEPHALOSPORIN CYTOTOXICITY IN SUSPENSIONS OF RABBIT PROXIMAL TUBULES: STRUCTURE ACTIVITY RELATIONSHIPS. G P Rush and G D Ponsler. Lilly Research Laboratories, Lilly Corporate Center, Indianapolis, IN.

Many cephalosporin antibiotics, such as cephaloridine (CLD) and cephaloglycine (COL) have the potential to cause nephrotoxicity. The purpose of this study was to examine the mechanisms of cytotoxicity of a variety of cephalosporin molecules in isolated rabbit proximal tubules. CLD, LY15595 and Cefazidime all contain a pyridinium ring, the redox cycling of this structure has been proposed to result in the formation of reactive oxygen radicals leading to cell damage. Of these molecules, only CLD was nephrotoxic in vivo causing marked renal necrosis and renal dysfunction. In rabbit proximal tubules, CLD was cytotoxic whereas Cefazidime was not. COL, LY1712 in and LY142095 on the other hand, do not contain a pyridinium ring and are all nephrotoxic in vivo and in vitro. Probenecid, which blocks basolateral anion transport in the proximal tubule, blocked COL and LY142095 cytotoxicity in vitro. N-N'-diphenyl-p-phenylene diamine (DPPD), an antioxidant, was effective in attenuating COL and CLD cytotoxicity but had no effect on LY142095 toxicity. Glutamate, glycine and cystine, in combination, were effective in reducing the cytotoxicity of CLD, COL and LY142095. These data demonstrate that the in vitro cytotoxicity of the nephrotoxic cephalosporins tested was dependent on renal transport and presumably intracellular accumulation. Lipid peroxidation did not appear to be a common mechanism among these molecules as DPPD was ineffective against LY142095 cytotoxicity. In conclusion, there were no common structural entities, other than the cephalosporin base structure, that could explain the mechanism of the nephrotoxicity of these compounds.

Gentamicin (GEN) induced nephrotoxicity was previously shown to be inhibited by simultaneous treatment with pyridoxal-5'-phosphate (PLP) in male rats. The observed protective effective of PLP was associated with a decreased renal GEN accumulation. Since female rats are less susceptible to GEN-induced nephrotoxicity and accumulated less tissue aminoglycoside, the objective of this study was to determine whether PLP was also effective in prevention of antibiotic-induced renal damage in females. GEN (70 mg/kg/day) administered (ip) daily for 10 days increased urinary trypsin inhibitor activity (TIA) and renal phospholipid (PL), produced proteinuria and decreased GFR. In simultaneous PLP and GEN administered females, there was no significant difference from GEN alone. However, in PLP and GEN treated females TIA inhibition, proteinuria and decreased GFR was more pronounced when drug treatment was stopped. Data show that during treatment, PLP prevents GEN nephrotoxicity but that cessation of PLP administration results in a rebound enhanced toxicity.

NEPHROTOXICITY OF AMINOGlycosides IN RATS IS ASSOCIATED WITH STIMULATION OF THE RENAL ENDOPLASMIC RETICULUM CALCIUM PUMP. L M De Witt and L. Moore. FDA, CDER, Rockville, MD and Dept. of Pharmacology, USUHS, Bethesda, MD.

It has been postulated that cellular toxicity and death may result from disruption of intracellular calcium homeostasis. The endoplasmic reticulum (ER) calcium pump is an important factor in regulation of cytosolic calcium. This lab has shown that administration of nephrotoxic doses of antimicrobial platinum compounds to rats was associated with an increase in the renal ER calcium pump activity (De Witt et al., TAP, 92: 157-169, 1988). Increased pump activity preceded an elevation in BUN or serum creatinine levels. The activity of the pump was determined by measuring ATP-dependent, microsomal pumping of 45Ca. Intramuscular injections of 100, 200, or 400 mg/kg of neomycin sulfate for two days to rats resulted in a dose-related increase in the activity of the renal ER calcium pump in treated rats 24 hours after the second injection. BUN and serum creatinine levels were significantly elevated only in rats treated with 400 mg/kg. Renal ER calcium pump activity was also increased in rats treated with high doses of gentamicin sulfate for two days. This indicates that an increase in renal ER calcium pump activity may be related to the mechanism of nephrotoxicity of aminoglycoside antibiotics. (Supported by NIHES grant ES03437).

CO-ADMINISTRATION OF CAFFEINE (C) EXACERBATES IBUPROFEN (I) -INDUCED RENAL PAPILLARY NECROSIS (RPN) IN RATS. J A Skarea, V E Dresslera, D E Johnsonb, and L D Lehman-McKeemanb. Richardson-Vicks, Shelton, CT; IRIDC, Mattawan, MI; and Procter & Gamble Company, Cincinnati, OH.

I and other non-steroidal anti-inflammatory drugs (NSAIDs) have been reported to cause RPN in rats. C, which has been reported to enhance the analgesic activity of I in clinical trials, does not cause RPN. In this study, the effect of C on I-induced RPN was evaluated in male and female SD rats dosed orally with I, C or I+C for 6 months. The incidence of RPN in I-treated rats (50, 100, or 200 mg/kg/day) was 7%, 76%, and 85% in males and 14%, 53%, and 55% in females. C alone (25, 50, or 100 mg/kg/day) had no effect on kidney morphology. Co-administration of C with I (25, 50, or 100 mg/kg/day) in I:C ratios of 3:1, 2:1 and 1:1 produced a dose-dependent increase in the incidence of RPN at 3 and 6 months in both sexes. For example, the incidence of RPN for both sexes at 6 months was approximately 65, 70 and 95%, respectively, in the mid-dose I:C group (50:17, 50:25 and 50:50 mg/kg/day) as compared to an incidence of approximately 10% in the 50 mg/kg/day I-only group. Thus, in rats, a species more sensitive to NSAID-induced RPN than humans, co-administration of C exacerbates the I-induced incidence of this renal lesion.

CEPHALOSPORIN INHIBITION OF RENAL GLUCONEGENESIS: STRUCTURE-ACTIVITY RELATIONSHIPS. S S Goldstein and J Nakada. SmithKline Beecham Pharmaceuticals. Department of Investigative Toxicology, King of Prussia, PA.

The cephalosporin antibiotic, cephaloridine (CPH), is known to inhibit renal cortical gluconeogenesis, an effect which has been attributed, in part, to competitive inhibition of micromolar glucose-6-phosphatase (G6Pase) activity. These studies were designed to determine the structure-activity relationships of cephalosporin inhibition of renal gluconeogenesis. Renal cortical slices from male F344 male rats were incubated with [3H]GDP in a bicarbonate buffer supplemented with 10 mM pyruvate. The cephalosporins (5 mM) evaluated in vitro were: CPH, cephalothin (CPL), cephaloglycin (CPG), cephalxin (CPX), cepafarin (CPR) and cefazolin (CPZ). Pyruvate-supported gluconeogenesis was depressed in a time-dependent fashion by all cephalosporins tested. Following 120 min of drug treatment, gluconeogenesis was inhibited by approximately 80% by CPG, 75% by CPH, 56% by CPR or CPL, 31% by CPZ and 12.5% by CPX. Thus, the rank order of inhibition was: CPG > CPH > CPR = CPL > CPZ > CPX. The rank order of inhibition of G6Pase activity in isolated renal cortical microsomes was similar to that observed for renal slice gluconeogenesis. The more marked inhibition of G6Pase by CPG, compared to that of CPH and CPL, suggests that neither the pyridinium nor the thienophene ring are essential for inhibitory activity. Further, a non-cephalosporin beta lactam antibiotic, penicillin-G (P-G) inhibited renal cortical slice gluconeogenesis and microsomal G6Pase activity to a similar extent as CPX. These data suggest that the beta-lactam ring of cephalosporins may play a role in the inhibition of G6Pase, and hence, in renal gluconeogenesis, but other structural/conformational features appear to be important.

EVALUATION OF THE DISPOSITION OF IBUPROFEN (I) ADMINISTERED ALONE OR IN COMBINATION WITH CAFFEINE (C). J A Young, N S Miller, D Caudill and L D Lehman-McKeeman. Miami Valley Laboratories, Procter & Gamble Company, Cincinnati, OH.

Co-administration of C exacerbates I-induced renal papillary necrosis (RPN) in rats. To determine whether this exacerbation results from a dispositional effect, the distribution and elimination of [14C]-I (3 or 100 mg/kg) was compared to [14C]-I+C (2 × 1.2 or 100 + 50 mg/kg) given as a single oral dosage or following a 14-day dosing regimen with unlabeled drug. Peak plasma levels of parent I were not altered by C. I+C (100 + 50 mg/kg) increased the plasma t1/2 of I 2.4-fold when given once, but this kinetic change was not seen in the multiple dose regimen. At peak levels, less than 1% of the I dose distributed to kidney. [14C]-I equivalents concentrated preferentially in the target site, the papilla, relative to cortex or outer medulla, and C did not alter this gradient. In 24 hrs, 70% of I (3 or 100 mg/kg; single or multiple dose) was eliminated in urine. However, I+C (100 + 50 mg/kg; single or multiple dose) reduced urinary elimination of I to 45%. These results demonstrate that C: (1) has no consistent effect on plasma pharmacokinetics of I; (2) does not affect the renal distribution of I and (3) may reduce urinary elimination of I at high dosages. Thus, co-administration of C produced no major changes in the disposition of I that may explain the exacerbation of I-induced RPN.
496 RENAL FUNCTIONAL CHANGES ASSOCIATED WITH D-LIMONENE-INDUCED HYALINE DROPLET NEPHRONEPHROPATHY (HDN). L D Lehman-McKeeman, C P Daston, D Caudill and J A Young. Procter & Gamble Company, Cincinnati, OH.

In this study, renal functional changes associated with d-limonene treatment were assessed. Male and female rats were dosed orally with d-limonene (150 mg/kg) for 4 weeks (5 days/week). Urine concentrating ability, assessed weekly during the first three weeks of dosing, was decreased by 20% in male rats after 5 days of dosing and decreased progressively to 60% of control values at 3 weeks. At 4 weeks, urine protein excretion was increased 2-fold (29.8 ± 2.7 to 59.5 ± 3.6 mg/day) and excretion of o2u-globulin increased from 11.1 ± 1.3 to 19.2 ± 0.7 mg/day in control and d-limonene-treated rats, respectively. Assessment of renal cortical transport function with in vitro slice assays indicated that, in male rats, d-limonene significantly reduced the uptake of α-aminoisobutyric acid (AIB), but had no effect on the transport of PAH, TCA or α-methylglucopyranoside. d-Limonene treatment had no effect on renal function in female rats. These results indicate that d-limonene-induced HDN is associated with perturbations in renal function including a rapid, progressive decrement in urine concentrating ability and specific alterations in protein handling as demonstrated by proteinuria, α2u-globulinuria and decreased in vitro transport of AIB.


Poisoning by ingestion of mushrooms of the genus Amanita is very common in North America and Europe. Among the Amanita chemical constituents, the amanitins seem to be responsible for the lethal toxic effects. In the late stages of the intoxication, which begin approximately 72 hours after ingestion, severe hepatic lesions occur that lead to coma and death. Female Swiss Webster mice were poisoned with α-amanitin 30 minutes before administering the naturally occurring polyacetylenic 1,2-dithiane, Thiarubrine-A, isolated from Ambrosia chamissonis, as a potential antidote. The liver pathology of sacrificed mice, after 72 hrs of treatment, showed a marked protective effect of Thiarubrine-A, at a concentration of 15 mg/kg, against the liver toxin. α-Lipoic Acid showed a lesser protective effect. A plausible mode of action for Thiarubrine-A as a mushroom poisoning antidote will be presented.

498 IN-VIVO TREATMENT OF RICIN INTOXICATION. R H Wannemacher, Jr., W L Thompson, R E Dinterman, and F G Grillo. USDA/NIDR, Fort Detrick, Frederick, MD. WRAIR, Washington, D.C.

Ricin is a highly toxic plant lectin. In Vero cells, monensin A increased and brefeldin A decreased the cytotoxic effects of ricin. A mouse model was used to determine if these two drugs had similar effects against ricin intoxication. Pretreatment with 25 mg/kg of monensin 1 hr before i.p. challenge with ricin resulted in a reduction of the medium lethal dose from 22 to 9 μg/kg. At all challenge doses of ricin, monensin pretreatment resulted in an earlier mean time to death of the mice. In contrast, pretreatment with 10 or 20 mg/kg of brefeldin A had no effect on the survival of mice challenged i.p. or i.v. with a lethal dose of ricin. Similarly, treatment with brefeldin A (10 mg/kg) 1 hr before and 0, 6, 12, and 24 hr after i.v. ricin (15 or 5 μg/kg) challenge had no effect on the survival of mice. These data suggest that monensin has a synergistic effect on ricin toxicity in both in-vivo and in-vitro models. While brefeldin A was effective in-vitro, it appears to have no beneficial effects against ricin intoxication in a lethal mouse model.

499 FUNOMISIN B3: A NOVEL PULMONARY AND HEPATOTOXIC MYCOXOTOXIN OF SWINE. D K Hess, G Notelov, R W. Seoerder, D P Bane, W F Hall, K S Harlin, V R Hasley and W M Haschek, College of Veterinary Medicine, University of Illinois, Urbana and USDA Peoria, IL

Funomisin B3 (FB3), a recently identified mycotoxin encountered in Pusaria moniliforme infected corn, has been detected at 40-265 ppm in the diets of swine affected by pulmonary edema. Female crossbred swine weighing 6 to 13 kg were used to characterize the toxicity of FB3. FB3 (approx. 70% pure) was given daily iv to pig 1 for 9 d for a total of 72 mg (7.9 mg/kg), and to pig 2 for 4 d for a total of 77 mg (4.5 mg/kg). Pig 3 (control) received saline. Clinical signs and gross lesions were not observed, but hepatic lobules were disorganized with scattered hepatocyte necrosis and mitosis. Corn screenings contaminated with FB3 (222 ppm) were fed to pigs 4, 5, and 6, while ground corn was fed to pigs 7 and 8 (controls). Pigs fed screenings developed respiratory distress within 3 to 5 d. Pigs 4 and 7 were killed on d 4; pig 5 was found dead on d 6. Clinical signs in pig 6 regressed; it and pig 8 were killed on d 15. Severe pulmonary interstitial edema, pleural effusion, and individual pancreatic acinar necrosis were observed in pigs 4 and 5. Pigs fed FB3 contaminated feed had liver lesions identical to IV dosed pigs; liver enzymes (ALT, SGOT) were elevated. In conclusion, FB3 whether given iv or orally was hepatotoxic. Corn screenings containing FB3 caused severe pulmonary edema and death.
Crystalline bovine liver rhodanese and sodium thiosulfate were co-encapsulated in murine erythrocytes by hypotonic dialysis. After rescaling and annealing, the resultant carrier erythrocytes were resuspended in saline and administered intravenously to mice. The mice were fasted overnight, and subsequently received cyanide and the cyanide antagonists sodium thiosulfate and/ or sodium nitrite in various combinations.

These carrier murine red blood cells containing rhodanese and sodium thiosulfate provided a striking reduction in blood cyanide concentration, whereas carrier erythrocytes without the enzyme and sulfur donor had no effect on cyanide concentrations. Furthermore, these thiosulfate and rhodanese-containing erythrocytes provided striking protection against the lethal effect of cyanide either alone or in various combinations with sodium nitrite and/or sodium thiosulfate. The use of the erythrocyte as a carrier provides a new approach in the prophylaxis and therapeutic antagonism of chemical toxicants. (Supported by NIH fellowship F32 - ES 05456 and NIEHS grant 03951.)

MDMS, an intermediate in the coatings industry, was subjected to a series of acute toxicity and irritation tests. Perorally, MDMS was slightly toxic to rats (LD50 = 5.68 ml/kg in both males and females) and moderately toxic to rabbits (LD50s = 1.78 and 2.14 ml/kg for males and females, respectively). Percutaneous toxicity (by 24-hr occluded contact) in the rat was very low (LD50 > 16.0 ml/kg), but was much higher in the rabbit (LD50s = 1.47 and 0.73 ml/kg for males and females, respectively). Notably, rabbits died after only 5 to 25 min from MDMS application. To further investigate the effect of route of exposure on toxicity, a few animals were administered a subcutaneous (SC) or intravenous (IV) dose. Two rats per sex survived a SC dose of 1.8 ml/kg. By the IV route (using only one male and one female animal per dose), a male rat died at 0.5 ml/kg and a female survived at 0.25 ml/kg. A male and a female survived respective doses of 0.25 and 0.125 ml/kg. One rabbit/saw died from an IV dose of 0.125 ml/kg but survived 0.05 ml/kg. MDMS did not irritate rabbit skin in a 4-hr occluded procedure (0.5 ml dose) but was slightly to moderately irritating to rabbit eyes (0.01 or 0.1 ml doses). These studies indicate a moderate degree of toxicity and irritancy (lower than that of structurally-similar trimethoxysilane), except for the marked percutaneous toxicity in the rabbit.
COMPARATIVE HISTOMORPHOLOGIC EFFECTS OF A CHEMOTHERAPEUTIC CHLORACYPYRILLUM DYE GIVEN INTRAVENOUSLY TO DOGS AND RATS. A E Roberts, R E Everett and T A Barbolt. Sterling Research Group, Rensselaer, NY.

A chloracypyrillium dye (CPD) intended for use in photodynamic chemotherapy was given to rats and dogs intravenously (IV). Sprague-Dawley rats (12/sex/dosage) and beagle dogs (2/sex/dosage) were given a single IV injection of CPD at dosages of 0, 0.5, 1.0 or 2.0 and 0, 0.5, 1.0 or 2.5 mg base/kg, respectively. Eight rats and 3 dogs died less than 2 h after receiving the highest dosage. For dogs, compound-related histomorphologic lesions were confined to lungs of males and females in the 1.0 and 2.5 mg base/kg groups. For rats, compound-related histomorphologic lesions for rats were observed for lung, choroid plexus, mesenteric lymph nodes and thyroid gland and occurred for males and females in the 1.0 and 2.0 mg base/kg groups. The incidence and severity of vascular irritation at the injection site were increased for all groups treated with CPD. Except for increased irritation at the injection site for rats, 0.5 mg base/kg was a no-observed-effect dosage for dogs and rats under the conditions of these studies. Future studies will be performed to assess recovery from CPD toxicity.


The nitroaromatics 1,3-dinitrobenzene (DNB), 1,3,5-trinitrobenzene (TNB) and N-methyl-N,2,4,6-tetranitroaniline (TNDA) have been detected as environmental contaminants of water and soil near production waste sites and at military test grounds. Acute toxicity evaluations were carried out with these compounds to develop environmental and health effects criteria. Dermal and eye irritation tests and acute dermal toxicity tests in rabbits, acute oral toxicity tests in rats and dermal sensitization (Bushier) tests in guinea pigs were conducted according to EPA standard protocols. The sensitization tests showed that DNB and tetryl are not skin sensitizers while TNB caused a mild allergic reaction. None of these compounds produced skin irritation but positive (DNE) to severe (TNB, tetryl) eye irritation potentials were observed. TNB and tetryl were not toxic at 2g/kg when applied to rabbit skin for 24 hours. However, the dermal LD50 of DNB was 1.99 g/kg. The oral LD50's of DNB, TNB and tetryl were 59 mg/kg, 275 mg/kg and greater than 5g/kg respectively in rats of both sexes. These results show that DNB is more toxic than TNB and tetryl when exposed orally and dermally while TNB and tetryl were more severe eye irritants than DNB. Supported by the US Army Medical Research & Development Command contract No. DAMD17-89-C-9221.


Experimental data indicate that 7.5% NaS in the diet of male rats leads to the formation of urinary silica-containing precipitate and crystals. TES is used in a rat model for silica urolithiasis and associated uterine hyperplasia. Although gross lesions have been reported in the kidney following TES administration, previous studies have not included histology. We treated 5 wk old F344 rats with TES in daily doses of 0, 0.111, 0.223, or 0.334 g/kg/day by gavage. Five rats per sex in each group were to be sacrificed after 1, 2 or 4 days of treatment for histopathological evaluations. Several rats in the mid and high dose groups died early. Rats in these groups also lost weight during treatment and had compromised kidney function, indicated by a dose-related increase in BUN and decreased urinary creatinine and urea. Silicates accumulated in the stomach and kidneys. Histopathological findings in the kidneys of TES-treated rats included acute tubular necrosis, associated with inflammation of the papilla, and renal papillary necrosis. Morphological changes in the bladder after TES were similar to those produced by NaS, with erosion of mucosa and/or submucosal edema and simple hyperplasia. Silica-containing crystals were observed in the urine of TES-treated rats. Supported by a grant from the International Life Sciences Institute.

ACUTE INHALATION STUDIES OF GLYCOL AEROSOLS IN RATS - DIETHYLENE GLYCOL, TRIETHYLENE GLYCOL, AND 1, 3 - PROPANEDEO. T Cascieri, W E Rinehart and G M Hoffman. Hoechst Celanese Corporation, Somerville, NJ; Mendham, NJ; Bio/dynamics Inc., East Millstone, NJ.

The low vapor pressure and generally low acute toxicity of glycols obviates significant vapor exposures. Where data exists, effects have been negligible. However, some industrial uses may allow formation of higher concentrations of mists or fogs and the hazard of these has been questioned. Acute, 4-hour, aerosol inhalation studies have been performed on rats for the three subject materials at maximum attainable concentrations of 4.4 - 4.6 mg/L. The MMAD of particles ranged from 2.6 - 3.1 microns and greater than 96% were below 10 microns. There were no deaths over a 14-day observation period. Observed signs consisted of decreased activity during exposure with rapid recovery on removal, a transient body weight loss with recovery in 3 - 5 days, and nasal discharge or lacrimation suggestive of minor irritation which persisted for several days. Post mortem examinations were unremarkable. A significant hazard from acute exposure to aerosols of these glycols seems unlikely.
Monocarboxylic acids do not produce significant acute systemic toxicity; LD50s usually indicate only moderate or slight toxicity. Irritation of the eye, skin or mucous membranes varies from strong for short chain acids (C1-3) to negligible or nonirritating for longer chain acids (C14-16). Acute inhalation data for vapors is limited to the more volatile compounds. The potential for irritation or toxicity from inhalation of medium chain acids (C3-9) as either vapor or aerosol has been investigated in single, 4-hour studies with rats. For vapor exposures to propionic or butyric acid at 5.1-5.5 mg/L, the only effects noted were signs of slight eye and/or nasal irritation during exposure and for several hours after removal from the chamber. Aerosol exposures to heptanoic and pelargonic acids produced more definite signs of pulmonary irritation, substantial weight loss and fractional mortality (40% and 80%) at 4.6 and 3.8 mg/L, respectively. Exposure to an aerosol of pelargonic acid at 0.46 mg/L showed signs of irritation during exposure and for several days post exposure; however weight loss was more transient and there were no deaths at this level.

Exposures to an aerosol of 2-propanol and acetone produced more minor effects on the eyes and upper respiratory passages, and there were no deaths. No deaths occurred at 1/10th the concentration of the LD50, indicating the lower limit of lethal concentration to be at least 10 mg/L.

Fischer 344 rats were cutaneously treated with undiluted MDEA at doses of 0, 260, 1040, and 2080 mg/kg body weight/day for a total of 9 applications (6 hours/day) over an 11-day period. MDEA produced dose-related moderate to severe skin irritation in both males and females with greater irritation being observed in females. Decreases in food consumption (males only) and body weights were observed. Increased neutrophils, decreased total hemoglobin, hematocrit and mean corpuscular hemoglobin, decreased urine volume, increased urine specific gravity, and increased adrenal weights observed in only females were considered to be secondary to irritation at the site of treatment (and the resulting alterations in fluid balance) or inflammatory stress. Females had increased serum chloride, sodium, urea nitrogen and kidney weights with decreased urine pH. Except for a slight increase in serum chloride, similar changes were not observed in males. Based on these parameters and the changes in food consumption and body weights, cutaneous absorption of MDEA may cause systemic toxicity. The greater degree of skin irritation and treatment-related effects observed in female rats suggested that this sex may be more susceptible to cutaneous treatment with MDEA. Cutaneous toxicity studies with MDEA, diluted in water, at doses of 100, 500 and 750 mg/kg body weight/day were performed. Preliminary findings from this study generally support the above conclusions.

Although acute toxicity data are required by regulatory agencies, most no longer require the classical LD-50 test which yields a statistically precise value. A testing procedure, which used less than 30 animals per study (most cases) and provided sufficient data to meet regulatory requirements, was investigated. Results obtained by this procedure were compared to historical LD-50 data generated with two to four times more animals. The testing scheme was a three phase procedure requiring approximately two weeks to complete. In the first phase groups of three female rats each were dosed at 100, 500, or 1000 mg/kg. After three to four days additional groups of either three or six females were added at dosages intended to further define the maximum tolerated dose (MTD) and the minimum lethal dose (MLD). This provided sufficient data to estimate the LD-50 for female rats. To test for potential sex difference in response, six male rats were dosed with the estimated LD-50 for females. The data obtained by this abbreviated procedure, provided an estimated LD-50 value, and were in close agreement with the original data for MTD, MLD, overt signs, and macroscopic tissue observations. The procedure provided adequate data to determine appropriate dosage levels for repeat-dose ranging.

Earlier studies conducted in this laboratory showed that methyl methacrylate produces a loss of gastric tone in the rodent and human stomach. The purpose of these studies was to determine whether acrylic (A) acid and methacrylic (MA) acid and the A/MA esters, methyl (M), ethyl (E), and butyl (B) could be inferred to be associated with changes in gastric secretory performance. A total of 58 adult Sprague-Dawley male rats were used in these studies. Rats were selected for treatment according to a true random process and separated into 9 dose groups, one of which was used for vehicle control. Gastric secretion was measured in 2 hr pylorus-ligated (Shay) rats under basal conditions and following i.p. administration of saline (S) or 400 mg/kg of treatment compound. Gastric secretion was affected as follows:

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Vol. Output (mL)</th>
<th>Acid Output (µeq/2 hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>2.77 ± 0.95</td>
<td>287.43 ± 125.00</td>
</tr>
<tr>
<td>AA</td>
<td>0.20 ± 0.05</td>
<td>98.83 ± 2.09</td>
</tr>
<tr>
<td>MA</td>
<td>0.39 ± 0.17</td>
<td>17.42 ± 11.29</td>
</tr>
<tr>
<td>EA</td>
<td>0.36 ± 0.22</td>
<td>13.46 ± 3.04</td>
</tr>
<tr>
<td>BA</td>
<td>0.50 ± 0.20</td>
<td>17.85 ± 4.67</td>
</tr>
<tr>
<td>MAA</td>
<td>0.29 ± 0.11</td>
<td>13.25 ± 6.58</td>
</tr>
<tr>
<td>MHA</td>
<td>0.30 ± 0.12</td>
<td>11.51 ± 7.17</td>
</tr>
<tr>
<td>MHA</td>
<td>0.28 ± 0.10</td>
<td>13.60 ± 6.10</td>
</tr>
<tr>
<td>BMA</td>
<td>0.20 ± 0.10</td>
<td>7.64 ± 2.55</td>
</tr>
</tbody>
</table>

In sum, all compounds tested reduced significantly (p<0.001) basal gastric acid secretion.

513 SUBCHRONIC DERMAL TOXICITY STUDY OF AN ANTOMICROBIAL ORGANOSILICON QUATERNARY AMMONIUM CHLORIDE IN RATS. M P Dunn and W H Siddiqui. Dow Corning Corporation, Midland, MI.

A 90-day dermal study was conducted in rats to assess the subchronic toxicity potential of an antimicrobial organosilicon quaternary ammonium chloride (Dow Corning® 5700 hydrolysate). One control and three treatment groups of ten male and ten female Sprague-Dawley rats were dermally treated either with the vehicle (1000 mg/kg/day) or test material at dose levels of 100, 300, and 1,000 mg/kg/day for five consecutive days a week during the 90-day study period. One sham control group of 10 male and 10 female rats was also included in this study. Mortality, behavioral reactions, growth and food consumption were observed and measured along with hematology, blood chemistry, absolute and relative organ weights and pathology parameters. No deaths and no overt signs of toxicity or changes in behavior were noted in any of the groups. No statistically significant differences were noted in either mean body weight or food consumption data between the test and control groups. Clinical pathology and organ weight data did not indicate any treatment-related effects. No significant histopathologic changes of biological or toxicological significance were seen in tissues from terminal sacrifice rats. Based on these results, dermal application of Dow Corning® 5700 hydrolysate for a period of 90 days does not produce any discernible biologic or toxicologic effects on rats at the doses tested.


SC-45647, a guanidine-based high-potency sweetener, is 10,000 times more potent than sucrose. Thirteen week dietary admixture studies were conducted in CD® rats and CD®-j mice. Daily doses of 0, 100, 300, 1000 or 2000mg SC-45647/kg body weight were given to 25 rats/sex/group. After 13 weeks of treatment, 10 rats/sex/group were given control diet for 4 weeks to evaluate reversal of any treatment-related effects. Daily doses of 0, 100, 300, 1000 or 2000mg SC-45647/kg body weight were given to 10 mice/sex/group. There were no treatment-related deaths or antemortem observations, and no differences in food consumption, body weights, or body weight gains in either species. A few sporadic statistically significant decreases in clinical chemistry parameters were noted in male high dose animals. These decreases are not toxicologically meaningful because they were slight, within normal historical ranges, and not accompanied by any histopathological findings. Plasma SC-45647 concentration increased with dose and did not accumulate over time. In conclusion, no adverse effects were observed when SC-45647 was fed to rats and mice for 13 weeks at dosages up to 2000mg/kg/day.

515 TOXICOLOGICAL AND HEMATOLOGICAL EFFECTS OF SICKLEPOD (Cassia obusfolia) SEEDS IN SPRAGUE-DAWLEY RATS: A 13-WEEK FEEDING STUDY. K A Voss and L H Brennecke*. Toxicology and Mycotoxin Research Unit, ARS/USDA, Athens, GA and *Pathology Associates, Inc., Frederick, MD.

Agricultural commodities may be contaminated by potentially toxic seeds from common nuisance plants such as Sicklepod. Sicklepod seeds (SSd) contain glycosides and anthraquinone derivatives and short-term (≤ 2 weeks) studies suggest that the hematopoietic system may be a target organ of SSd toxicity. To investigate the effects of longer-term exposure, 10 rats/sex were fed diets containing 0, 0.15, 0.50, 1.5 or 5.0 % (w/w) SSd for 13 weeks. Mild diarrhea was intermittently found in high-dose animals and body weights of high-dose males were decreased through week 10. Otherwise, toxicologically significant differences in appearance, behavior, body weight, food and water consumption, serum chemical variables or organ weights were not found. Hematological effects including anemia, thrombocytosis, granulocytosis, monocytes and/or myeloid hyperplasia (renal bone marrow) were found in those groups fed diets containing ≥0.50 % SSd after 6 and 13 weeks. Lymphadenopathy, characterized by lymphoid hyperplasia and/or histiocytosis without necrosis or inflammation, was present in the mesenteric lymph nodes of most SSd-fed rats of all sex/groups and was considered a local effect related to absorption of SSd constituents. No other SSd-related histopathologic effects were found. Identification of hematologically active SSd constituent(s) is the subject of ongoing studies.
Olestra, a nonabsorbable fat substitute consisting of a mixture of the octa-, hepta-, and hexa-fatty acid esters of sucrose, was fed to Swiss CD-1 mice (20/sex/group) at 0, 2.5, 5, or 10% (w/w) for 13 weeks. The diet (Purina 5002 Certified Rodent Chow) was supplemented with vitamins A, D, and E at either 5X or 7.5X NRC recommended levels for mice to compensate for any potential decrease in absorption of the vitamins by the high levels of olestra. Survival and growth of the animals were unaffected by olestra. Absolute and relative organ weights of olestra-fed animals were not significantly different from those of control animals. Olestra-fed animals consumed more feed to compensate for the caloric dilution of the diet by olestra. There were no olestra-related effects on ophthalmoscopy, hematology, or clinical chemistry. Olestra produced no gross or histopathological changes. Serum 25-hydroxyvitamin D and hepatic vitamin A concentrations were comparable to controls in olestra groups supplemented with 5X NRC. Hepatic vitamin E concentrations decreased with increasing olestra levels in the diet. The results indicated that olestra produced no observed toxic effects when fed to mice at up to 10% of the diet for 13 weeks.

The incidence and description of ocular lesions and abnormalities observed during ophthalmologic exams of control CD rats on chronic studies. J Clinton and P L Lang, Charles River Laboratories, Inc., Wilmington, MA. Sponsor: R W Fogleman

In order to provide background information about ophthalmologic lesions commonly found in CD rats, data were obtained from pretest and control animals in chronic and subchronic studies performed in industrial or contract laboratories. Data from pretest and control group examinations were summarized and population incidence was established by sex for each lesion at various time intervals. Linear focal retinopathy, persistent hyaloid remnant, vitreous hemorrhage, posterior synechiae, retinal degeneration, and ventral entropion were common findings in pretest animals. Animal groups completing chronic study intervals exhibited significant incidences of keratitis, corneal vascularization, cataract, pale ocular fundi, corneal opacity, and retinal degeneration.

The toxicity of Ocu-18 (Telios Pharmaceuticals, Inc., San Diego, CA) was assessed in two different 28-day studies, by intravenous infusion in the rat or ocular administration in the rabbit. Ocu-18 is an ophthalmic preparation designed to augment healing of wounded corneas. To evaluate the systemic safety, Ocu-18 was infused intravenously daily into 10 rats/sex/group. The groups received 0, 1X, 10X, or 100X the expected human dose. For the assessment of ocular safety, eight rabbits/sex/group received three daily doses of Ocu-18 in the lower cul-de-sac of the right eye. The groups received 0, 1X or 17.5X the expected human dose. Animal survival was not affected by this treatment, although three rabbits died from unrelated events. Animals from both studies were evaluated for changes in body weight, food intake, hematology, clinical chemistry, urinalysis, gross pathology, organ weights, histomorphology, slit-lamp, and macular examinations. Only sporadic and incidental clinical signs were observed for any of these parameters throughout the study. These studies indicate that Ocu-18 has no treatment-related effects by intravenous or ocular administration in these investigations.

The CD rat and CD-1 mouse as described by historical control data provided to Charles River by industry. P L Lang and W H White, Charles River Laboratory, Inc., Wilmington, MA. Sponsor: R W Fogleman

Data from control groups on subchronic and chronic studies are presented in order to provide background information about CD rats and CD-1 mice. Examples of parameters included are distribution of group survival, growth parameters (mean body weight and food consumption), incidence of neoplastic and select nonneoplastic lesions, developmental indices, malformation and variations seen at C-section, ophthalmologic observations, and relative organ weights at various study intervals. Control group means of appropriate data are averaged and the distribution of means at selected time intervals are presented. Group survival data are adjusted by the Kaplan-Meier procedure prior to inclusion. This information can be used by industry and the regulatory bodies to aid in the evaluation of test data obtained on subchronic and chronic toxicology studies using these two models.
THYROXINE (T4) FORMATION FOLLOWING IODINE (I2) ADMINISTRATION IN THE SPRAGUE-DAWLEY RAT. J.K. Thall and R.J. Bull. Pharm./Tox. Graduate Program, College of Pharmacy, Washington State University, Pullman, WA

Iodine has been proposed as a water disinfectant on the manned space station. Previous work in our laboratory has shown that subchronic T2 administration to Sprague-Dawley rats in drinking water significantly increases plasma T4/T3 levels. This was not observed with T3 treatment. We have carried out studies to determine how this increase originates. At 2 hrs following oral dosing we found a 30% increase in plasma T2 and a 30% decrease in plasma T3 levels in animals treated with T4 compared to controls. In an in vitro experiment using wash collected from the rat duodenum, T2 was found to increase the concentration of a chemical that bound to a T4 specific antibody. A similar increase in a radioactive product was observed in blood following oral administration of 125I2, but not 125I-. These data suggest that T2 reacts with deiodinated T4 metabolites in the gastrointestinal tract to resynthesize T4 and elevate its levels in blood. (Supported by NASA Grant No. NAG 9-226).

ACUTE AND SUBCHRONIC TOXICITY OF LEVO-ALPHA-ACETYL-METHADOL (LAAM) AND LEVO-ALPHA-ACETYL-NORMETHADOL (NORLAAM) IN MALE AND FEMALE CD RATS. J.L. Egle, Jr., J.F. Borzelleca and L.S. Harris, Dept. of Pharmacol. & Toxicol., Medical College of Virginia, Richmond, VA

The primary objective of these studies is to provide the toxicological basis for potentially effective therapeutic agents for the treatment of addictive drug disorders. Male and female Charles River CD rats were dosed orally with both compounds acutely and for 30 consecutive days. Respective acute LD50 values (mg/kg) for males and females were 29 and 56 for LAAM and 46 and 88 for NORLAAM. The major sign of acute toxicity was CNS depression. Daily doses of LAAM were 3, 10 and 17.8 mg/kg for males and 5.6, 18.3 and 33.5 for females. For NORLAAM, doses were 4.6, 14.7 and 25.9 for males and 4.4, 13.2 and 21.4 for females. The following were evaluated: morbidity and mortality, behavioral changes, gross and histopathology, clinical chemistry and hematology. Reversibility of effects was assessed 14 days post dosing. Consistent, statistically significant, dose dependent adverse effects observed included a decrease in body weight gain, increased brain/body weight ratio, and a decrease in heart/brain ratio in both sexes and a decrease in total leucocytes in females. There was a dose dependent, but not statistically significant decrease in serum cholesterol and triglycerides in both sexes and a decrease in lymphocytes in females only. Partial recovery was observed 2 weeks after the final dose. Supported by NIDA Contract 271-89-8156.


Desoxycorticonosterone pivalate (Percorten®) was administered to beagles at 0, 5.6, 19.8 or 33 mg/kg per month for six months. The treated males had statistically significant decreases in body weight gain, 18% decrease in body weight in the 33 mg/kg dose group. Polyuria, polydipsia, decreases in serum potassium levels and decreases in blood urea nitrogen levels were observed while the dogs were being treated. The absolute weights of the adrenals, epididymides and testes were decreased. The weights for the 6.6, 19.8 and 33 mg/kg dose groups were respectively: 80%, 70% and 66% of the controls (epididymides); 79%, 75% and 67% of the controls (testes); and 86%, 79% and 69% of the controls (adrenals). When expressed relative to body weight, these decreases in organ weights of the males were still dose-dependent but the differences were less remarkable. In contrast, the relative weights (to body weight) of the kidneys and thyroids/parathyroids were increased dose-dependently. Other effects that were observed included a transient increase in serum sodium levels in some treated males and an elevation of endogenous creatinine clearance in the treated females.


ST 1435, a synthetic progestin, is a potent suppressor of ovulation when administered parenterally and therefore has a potential to be used as a contraceptive agent. In a 90-day toxicity study, ST 1435 was administered via subdermal Silastic implants to a group of female rabbits. The implants releasing approximately 0, 25, 125 or 550 µg/day of ST 1435 were inserted subdermally and left in place until necropsy on day 91. ST 1435 had no effect on body weight. On the other hand ST 1435 depressed uterine weights and increased liver weights at all doses. Also weights of adrenal and thyroid glands in the high dose group were lower than the controls. At the high dose ST 1435 caused adrenocortical atrophy and consistently increased glycanogen content of hepatocytes. The uterus of 4/12 high dose rabbits had mildly dilated uterine glands and endometrial vessels were less congested. None of these histological changes were observable in the mid and low dose groups. At termination of treatment serum albumin, cholesterol and triglycerides were slightly elevated in treated animals whereas serum cortisol levels were lower in the high dose group as compared to controls. We conclude that the major effects observed in this study are related to the postgestational activity of ST 1435 and that the changes observed in the adrenals and liver of high dose group rabbits are indicative of the presence of a weak glucocorticoid-like activity in ST 1435. Other than the glycanogen effects ST 1435 had no systemic toxicological effects in female rabbits.
SUBCHRONIC (13-WEEK) TOXICITY STUDIES OF ORAL PHENOLPHTHALEIN, IN FISCHER 344 RATS AND B6C3F1 MICE. D. D. Dize, M. R. Elwell; R. E. Chapman; N. D. Shelby; M. D. Thompson; J. R. Filler; AND M. A. Steghern. NIEHS/NTP, Research Triangle Park, NC; Liggett & Myers, Durham, NC; AND Microbiological Associates Inc, Bethesda, MD.

Phenolphthalein (ppht) is widely used in over-the-counter laxatives. 13-week toxicity studies of ppht were performed using F344/N rats and B6C3F1 mice. Rats and mice were fed ad libitum with NIH 07 diet containing 0; 3,000; 6,000; 12,000; 25,000; or 50,000 ppm ppht. Papillary produced marginal evidence of toxicity in rats. There was slightly lower weight gain among the 25,000 and 50,000 ppm groups. Kidney (males only) and liver weights were mildly elevated by 12,000-50,000 ppm ppht. Serum bile acids were depressed early (d 5 and 6) by ppht treatment and later (d 92 and 93) exhibited a U-shaped dose response. Treatment-related toxic effects, however, were definitive in mice. The primary treatment-related effects during the mouse studies involved the reproductive and hematopoietic systems. Reproductive changes including depressed testis and right epididymal weights and sperm density, an elevated production of abnormal sperm, and morphologic alterations in seminiferous tubules occurred at all levels of ppht exposure. Hematopoietic changes included bone marrow hypoplasia (12,000-50,000 ppm), increased splenic hematopoiesis (males only; 25,000 and 50,000 ppm), and an elevated incidence of micronucleated erythrocytes (6,000-50,000 ppm).


A 4-week comparative toxicity study of CL 306,293, DUF-785, methotrexate (MTX), and azathioprine (AZA) was conducted. Groups of 10 rats/sex received weekly oral doses of vehicle or compound (5-200 mg/kg). Death occurred at the highest dose of CL 306,293 (200 mg/kg), DUF-785 (100 mg/kg) and AZA (200 mg/kg). Decreased body weight and food consumption were seen with all four agents, but only at the highest doses of MTX (80 mg/kg) and AZA (200 mg/kg). Hematologic findings included decreases in red blood cell parameters with all four compounds, and decreases in platelets with CL 306,293, DUF-785 and MTX. Serum chemistry showed decreases in total protein, albumin and triglycerides with CL 306,293, DUF-785 and MTX. In rats that died, postmortem findings were hypocellularity of bone marrow, lymphoid depletion in lymph node and spleen, and thymus atrophy. In surviving rats there were increased lymph node and spleen weights, decreased thymus weight, hematopoiesis in spleen and liver, and hypercellularity of lymph node.


The oral toxicity of halofantrine HCl was evaluated in a 13-week study in rats and in a 4-week study in dogs. Groups of 10 Sprague-Dawley rats/sex received dose levels of 0, 5, 15, or 30 mg/kg/day by oral gavage. In treated rats, mild dose-related decreases were observed in erythrocyte parameters and marked dose-related increases were observed in serum total cholesterol levels. Serum creatinine levels were decreased in all groups of treated males. Liver/body weight ratios were elevated in high-dose males and females and mid-dose females. No corresponding hepatic microscopic findings were observed. Groups of 2 beagle dogs/sex received dose levels of 0, 5, 25, 50, or 75 mg/kg/day orally by capsule. The two highest dose groups associated with 50% mortality and a number of clinical signs including anorexia, emesis, changes in fecal consistency, tremors, and ataxia. Soft feces was the only clinical sign observed in the 25 mg/kg-dosed group. Total serum cholesterol levels were increased in most treated animals and serum alanine ami-notransferase activity was elevated in several treated animals. On the basis of these studies, dose levels of 0, 1, 5, 12 and 20 mg/kg/day were selected for a 104-week rat study and dose levels of 0, 2, 5, and 20 mg/kg/day were selected for a 52-week dog study.


Beagle dogs were administered CI-981 for 2 weeks (2x/sec/day) or 13 weeks (3x/sec/day) with doses of 0, 20, 80 and 150 or 0, 10, 40 and 80 mg/kg/day respectively. At the conclusion of both studies, dogs were examined for clinical evidence of opacities, sacrificed and the lenses of the left eyes removed, weighed and prepared for biochemical analysis of protein, glucose, potassium, reduced and oxidized glutathione, adenosine nucleotides, phosphofructokinase and glucose-6-phosphate dehydrogenase (G6PD). The right lens was prepared for histologic evaluation. Cataracts were not evident clinically or microscopically in either study. Statistically significant dose related decreases in serum cholesterol were noted in both studies. Significant biochemical changes were not noted in lenses from the 2 week study. In the 13 week study a dose-related trend towards decreased lenticular protein, glucose and potassium was evident. No lenticular effects were apparent at 10 mg/kg. A statistically significant correlation was noted between lenticular protein and Week 13 serum cholesterol. It is not known whether these biochemical changes noted in the lens of dogs precede lenticular opacification.
529 SUBCHRONIC EVALUATION OF A LIVER SELECTIVE HMG CoA REDUCTASE INHIBITOR IN RODENTS AND DOGS. E McGuire, D Bailey, M Dominick, C Morse, R Sigler, K Walsh and R Susick. PATH. & EXP. TOX. PARKE-DAVIS PHARM. RES. DIV. WARNER-LAMBERT CO., ANN ARBOR, MI.

In rats and mice, MLDS of > 5000 mg/kg were observed with oral administration of [R-(R*,R*)]-2-(4-fluorophenyl)-8,9-dihydropyridine-5-(1-methylethyl)-3-phenyl-4-(phénylamino)carbonyl]-1H-pyrole-1-heptanoic acid, calcium salt (CI-981). In a 13-week rat study at doses of 10 to 200 mg/kg, emaciation, inanition, rough pelage, and mortality were observed at 200 mg/kg. Aspartate aminotransferase and alkaline phosphatase in males, and alanine aminotransferase in females, were elevated at 100 and 200 mg/kg. Hyperkeratosis of nonglandular stomach was seen at 100 and 200 mg/kg. Severity of hepatocellular atypia was dose-dependent and still present 4 weeks posttreatment at 30, 100, and 200 mg/kg. In a 13 week dog study, emesis, bloody stools, and body weight loss prompted a reduction of the high dose from 160 to 80 mg/kg. Soft or mucoid stools were observed at 40 and 80 mg/kg throughout the study. No effects were apparent at 10 mg/kg. Lens opacities, CNS effects, and testicular degeneration seen in dogs with other reductase inhibitors were not observed at 80 mg/kg for 13 weeks.


The acute nephrotoxicity of a new beta-lactam antibiotic (SCB 34343) was assessed in comparison to three other injectable cephalosporins (cephaloridine, cefazolin, cefotaxime) and one carbapenem (imipenem). Groups of rabbits were given single intravenous or subcutaneous injections of each test substance at doses of 80, 160, 320 or 640 mg/kg. One group of rabbits given an intravenous dose of sodium chloride served as control. Blood urea nitrogen (BUN) and creatinine levels were determined pretest and at 24 and 48 hours after dosing. All rabbits were sacrificed 48 hours after dosing and the kidneys were weighed and examined for gross and microscopic changes. BUN and creatinine values were unchanged after doses of 80 and 160 mg/kg of all antibiotics except cephaloridine. At higher doses (320 and 640 mg/kg) changes in one or both parameters were seen after each antibiotic except cefotaxime. Gross and microscopic changes in the kidneys substantiated the nephrotoxic potential of each antibiotic as characterized by increases in BUN and creatinine levels. These findings suggest a rank order of nephrotoxicity as follows: cefazolin > imipenem > SCB 34343 > cefotaxime > cephaloridine.


LY281389, 9-N-(propyl)erythromyclamylene, is a macrolide antibiotic with greater bioavailability than erythromycin. Beagle dogs (4/sex/group) were given 0.75, 0.25, or 75 mg/kg/day of LY281389 for 1 month. Additional dogs were used to assess the reversibility of toxic findings. Effects at the low-dose were limited to miosis and tachycardia. The mid- and high-dose groups had miosis, decreased food consumption, and tachycardia. Also, pathologic evaluation, including electron microscopy, identified vacuolar and membranous changes within the endothelial, smooth muscle, and parenchymal cells of a number of tissues. Some vacuoles were clear while others contained concentric lamellar bodies and irregular membranous electron dense material, particularly those in the liver and muscle. At the high-dose, a dog died in Week 3. Additional effects were decreased body weight gain, electrocardiogram changes, elevated transaminase and creatine phosphokinase activities, and accumulation of phospholipids in the liver. Relatively low concentrations of LY281389 were present in the plasma, while high levels were observed in all assayed tissues, especially lung, liver, and kidney. These observations are consistent with lysosomal drug storage, as has been demonstrated with a number of other cationic amphiphilic compounds.
SUBACUTE INTRAMUSCULAR TOXICITY STUDIES IN RATS AND DOGS ON THE ACETYLCHOLINESTERASE REACTIVATING AGENT HI-6. B. C. Levine, S. Tomlinson, and G. Schifferstein. Toxology Research Lab., Univ. of IL at Chicago, Chgo, IL; Path., Assoc., Inc., Chgo, IL; USAMRDC, Fort Detrick, Federick, M.D.

HI-6 is being developed as an AChE reactivating drug following organophosphorus exposure. The current studies examined the toxicity of HI-6 in Sprague-Dawley rats and Beagle dogs following IM injection for 14 days. Dose levels were 0, 50, 150 and 450 mg/kg/day for 10 rats/sec/dose and 0, 35, 70 and 140 mg/kg/day for 4 dogs/sec/dose. For rats, 2 males and 1 female at the high dose died. Reduced weight gain, decreased activity, tremors, hunched posture and poor grooming were seen for high dose survivors. Increased ALT and AST for both sexes at the mid and high dose levels suggested hepatotoxicity, although liver weights and histology were normal. Hematology parameters were unaffected except for slight dose-related increases in platelets for both sexes. Injection site inflammation was seen, however serum CK was not altered. For dogs, slight weight loss, vomiting, salivation and diarrhea were seen at the high dose. As with rats, dose-related increases in ALT and AST occurred at the mid and high doses. This was accompanied at the high dose by hepatomegaly and hepatocellular vacuolization. Injection site inflammation accompanied by dose-related elevations in serum CK-MM2 were also seen. ECGs and eye exams were nonremarkable. Supported by USAMRDC Contract No. DAMD17-87-C-7225.


Administration of CP to rats produced an immediate and sustained decrease in serum T4. This study examined the hypothesis that this effect results from increased hepatic metabolism of T4 and not from a direct effect on the thyroid gland. Male F344 rats were administered 30 mg/kg CP, or vehicle, by gavage for 1 to 14 days. Plasma clearance of 125I-T4, measured 2-6 h after dosing, increased progressively from a value 1.3 x control (p<0.001) after 1 dose of CP to a maximum 3.8 x control after 14 doses of CP. At this time hepatic microsomal 5'-deiodinase activity, was decreased (0.44 x control) while T4 UPT activity was markedly increased (5.8 x control). After 7 doses of CP, when T4 clearance was increased almost maximally, the rate and extent of excretion of radioactivity in faeces from 125I-T4 was greatly increased (p<0.001). This increase exceeded a decrease in urinary excretion of radioactivity resulting in a net increase in cumulative excretion of radioactivity derived from 125I-T4. This is consistent with the observed decrease in hepatic deiodinase and increase in T4 UPT activity and suggests that the lowering of serum T4 concentration following treatment with CP can be attributed to increased hepatic clearance of T4, mediated at least in part through selective induction of T4 UPT.


Administration of phenoxysobutyrates, such as CP, to rodents results in thyroid hyperplasia. This study was conducted to examine whether this effect is associated with an increase in serum TSH concentration. Male F344 rats were administered 30 mg/kg CP, or vehicle, by gavage for 1 to 14 days. Quantitative histomorphological examination of 0.245 mm2 section of thyroid was performed by image analysis. The number of mitotic figures, expressed as the total in each group, increased to a maximum after 5 doses of CP (27 v 1: CP v control). After 5 or more doses of CP there was a significant increase in follicular cell area (p<0.01). In CP-treated animals there was a transient decrease in serum T3 concentration after 1-5 doses, a progressive and sustained decrease in serum T4 concentration (minimum 17% control; p<0.001) and an increase in serum TSH concentration measured 6 h post-dose, particularly evident after 5 doses (p<0.01). This study demonstrates that administration of CP, like other fibrates, elicits a mild trophic response in the thyroid gland of the rat, probably attributable to the increase in serum TSH concentration.

RELATIONSHIP BETWEEN DOSE AND HEPATO-TOXICITY OF COCAINE AND DIAZEPAM IN MICE. S S Mehanna and M S Abdelsalem. Pharm/Tox. Dept., New Jersey Med. School, Newark, NJ and College of Medicine, Ain Shams Univ., Egypt

Pregnant mice were administered cocaine-HCl at 10 and 20 mg/kg via tail vein, and/or diazepam at 20 and 40 mg/kg by gavage from day 7 to 16 of gestation. Cocaine and diazepam alone or in combination induced a mild to moderate degree of fatty infiltration mainly affecting the periporal areas. The cocaine treated groups showed discernible infarction around the blood vessels in the periporal area. Both drugs in combination manifested the induction of peroxisomal proliferation. Dilation and vesiculation of the rough endoplasmic reticulum with some loss of its ribosomes was evident in the groups that received cocaine at 20 mg/kg, alone or in combination with diazepam. Fetal livers showed a dilation of the rough endoplasmic reticulum with some loss of its ribosomes together with a significant increase in peroxisomes at the high doses of the combination regimen. In conclusion, the increased toxicity of cocaine and diazepam in combination was evident.
536 IN VIVO INHIBITION OF PULMONARY AND MACROPHAGE PHOSPHOLIPASE IN DRUG-INDUCED PHOSPHOLIPIDOSIS. U P Kodavanti, R Sharma and H M Mendelec, Dept. Pharmacol. & Toxicol., Univ. Mississippi Med. Ctr., Jackson, MS

The purpose was to investigate whether cationic amphiphilic drugs (CAD) with varying ability to induce phospholipid storage disorder, inhibit lung lysosomal or macrophage phospholipases in vivo. Male Fisher-344 rats were given chlorpheniramine (CP), chloroquine (CQ), chlorpromazine (CPZ), imipramine (IM) or chlorimipramine (CIM), 20 mg/kg/day, for 2, 7, 21 or 21+21 days (recovery). Phospholipase A and C activities were measured in soluble lung lysosomal fraction and sonicated suspension of alveolar macrophages. Of all drugs, CP induced alveolar macrophage infiltration, caused phospholipidosis in the lung, in the alveolar lavage fluid, and in macrophages. Normal levels were found in all three fractions after the recovery. CP caused 75% and 85% inhibition of phospholipase A and C, respectively, in macrophages with complete recovery 21 days after discontinuation of drug treatment. The inhibition occurred as early as 7 days and was progressive in 21-days treatment group. CQ, CPZ and IM, which failed to induce phospholipidosis at the dose used, caused marginal stimulatory effect on macrophage phospholipase A and C in 2- and 7-days treatment groups. CP or other drugs had no effect on lung lysosomal phospholipase. CAD may induce pulmonary phospholipidosis by primarily inhibiting macrophage phospholipase with recovery upon discontinuation of drug treatment. (Supported by HL-20622)

537 MODULATION OF PHORBOL ESTER BINDING TO PROTEIN KINASE C BY ANTIARRHYTHMIC DRUGS. D Dessiiah and P S Vig, Dept. Neurol., Univ. Miss. Med. Ctr., Jackson, MS

Phorbol esters, the tumor promoters are known to mimic the effect of diacylglycerol in activating protein kinase C (PKC). PKC regulates a variety of intracellular and extracellular signals across the cell membrane. Like phorbol esters, the compounds that interact with the regulatory site of PKC may modulate its activity. Amiloradone (AM) and its major metabolite desethylamiloradone (DEA), the antiarrhythmic drugs are associated with neurologic and respiratory manifestations. These drugs are shown to interact with calmodulin. Since calmodulin inhibitors alter PKC activity, the present study was initiated to understand how these drugs interact with PKC and alter 4H-phorbol 12,13-dibutyrate (PDBu), binding. Both AM and DEA significantly altered the 4H-PDBu binding to PKC and the effect was biphasic. However, DEA was more potent in inhibiting the 4H-PDBu binding than AM. The scatterch analysis of 4H-PDBu binding to PKC revealed that at lower concentrations (5 μM), AM and DEA increased 4H-PDBu binding to PKC with decreased affinity. Whereas, at higher concentrations (>30 μM) these drugs decreased the 4H-PDBu binding. There was no significant increase in the PDBu stimulated PKC activity at lower concentrations of AM and DEA. However, DEA at 50 μM significantly inhibited PDBu stimulated enzyme. Altered kinetics of 4H-PDBu binding to PKC by AM and DEA suggests that these drugs may be binding at the regulatory domain of PKC, probably at two distinct sites.


rhDNase is being developed for the treatment of respiratory conditions associated with cystic fibrosis and other suppurative disorders of the airways. rhDNase was administered at daily aerosol concentrations of 30 mg/kg for 30 minutes and 83 mcg/L in monkeys for 28 days. These exposure regimens were equivalent to 2.2 mg/kg/day in both species, or approximately 20 times the projected clinical dose. Previous single dose inhalation exposure of rhDNase up to 4.6 mg/kg in rats and 5.6 mg/kg in monkeys revealed no signs of toxicity. There were no drug-related deaths, clinical signs, ophtalmic changes, or differences in body weight and food consumption. No significant clinical chemistry changes or gross pathological findings were observed. Mild to moderate gavulitis were noted in 11 of 20 high-dose rats. This finding was characterized by histiocytic accumulation in air spaces, a perivascular/intersitial mononuclear cell infiltration, and cuboidal epithelial cells lining the alveoli. Similar findings were not observed at 0.25 and 0.53 mg/kg/day. Results of the immunohistochemical staining of lung sections revealed no detectable deposits of immune complexes of rhDNase and immunoglobulin in association with the effect of the enzyme. High-dose monkeys exhibited mild bronchiolitis that was considered potentially drug-related since components of the lesions were qualitatively similar to those observed in rats. Evidence of an antibody response was observed in both species following inhalation exposure of rhDNase; however, it did not appear to correlate with pulmonary lesions. No histopathological findings were observed in the lungs of rats or monkeys after a 4-week treatment-free period. Based on the data, rhDNase was well tolerated following inhalation exposure. No findings were observed that would preclude the use of rhDNase in man.


The quinolones and their fluoro-derivatives are extensively used in antimicrobial chemotherapy. However, treatment of immature dogs and rats results in generalized and/or erosion of the articular cartilage of the weight-bearing joints. Although anthracyclys have rarely been observed following quinolone therapy in man, the toxicity observed in immature dogs and rats has caused restricted use of these drugs in children and pregnant women. Since these drugs would find wide usage in children, progress in the identification of new quinolone antibiotics which do not cause arthropathy is highly desirable. This task would be greatly facilitated by a rapid biochemical assay of cartilage toxicity which utilizes small quantities of test material and has greater sensitivity than current toxicity assays. Therefore, this study evaluated the utility of neonatal mice for quinolone-induced joint toxicity. Seven-day-old CF-1 mice were dosed subcutaneously with two quinolone known to produce joint toxicity, pipemidic acid (30, 400, or 3150 mg/kg) for 7 or 14 days or ciprofloxacin (60 or 200 mg/kg) for 4, 5, or 14 days, or the vehicle (0.5% carboxymethylcellulose sodium, 50 μl/kg). The animals were observed daily for clinical signs of toxicity, and following sacrifice the sternum and joints of the fore and hindlimbs including the elbow, shoulder, knee, hip, and multiple articulations in the foot were examined by standard light microscopic procedures. The clinical observation of lameness was observed only after high dose pipemidic acid (3150 mg/kg) treatment for 7 days. In addition, histopathological assessment of the various joints revealed a lesion consistent with that previously observed in juvenile rats and dogs only in mice treated with the highest dose of pipemidic acid. No evidence of toxicity was observed with ciprofloxacin which has been shown to cause joint lesions in adolescent dogs at concentrations similar to those used in this study. Due to the apparent insensitivity of this model, it is probably not useful as a screen to predict the potential of new quinolone derivatives to induce arthropathy.
540 COMPARATIVE TOXICITY OF 3'-DEOXY-3'-FLUOROTHYMIDINE (FLT) AND 3'-AZIDO-3'-DEOXYTHYMIDINE (AZT) IN MONKEYS. D L Novicki, L Boroje, R French, M Leal and R A Schroer. American Cyanamid Co., Medical Research Division, Pearl River, NY.

FLT and AZT are antiretroviral agents with activity against the AIDS virus. Cynomolgus monkeys were given 0.5, 2.5, 12.5 or 50 mg/kg FLT or 12.5 or 50 mg/kg AZT twice daily for 1 month by nasogastric intubation for total doses of 1, 5, 25 or 100 mg/kg/day and allowed to recover for 1 month. Evaluations included body weight, food consumption, hematology, postmortem examination and pharmacokinetics (PK). Drug-related mortality occurred in monkeys treated with 100 mg/kg/day of FLT. Dose- and time-related hematologic changes occurred in monkeys treated with FLT or AZT. Red blood cells decreased with both FLT and AZT. Platelets were decreased by 100 mg/kg FLT but were increased at other doses of FLT and by AZT. White blood cells were decreased by FLT. Postmortem findings in FLT-treated monkeys that died included hyperecellular bone marrow and extramedullary hematopoiesis. All hematologic changes returned to normal by the end of the 1 month recovery period.

PK data indicated that exposure to FLT was 2-3 times greater than AZT at equivalent doses. There was no accumulation of FLT or AZT and the half-life of FLT (1-1.6 h) was approximately twice that of AZT.

541 MECHANISM OF ADRIAMYCIN CYTOTOXICITY: S Awasthi, S Gupta, R Sharma, J A Boll, E Frankel and V C Awasthi. The University of Texas Medical Branch, Galveston, TX and University of Texas Southwestern Medical School, Dallas, TX.

Adriamycin (Adr) is an anthracycline antibiotic used as a chemotherapeutic agent for a wide variety of neoplasms. Even though binding to DNA is believed to be the mechanism of Adr cytotoxicity, the reasons for its selective toxicity to certain organs (e.g., heart) are not clear. Interaction of Adr with membranes and specific membrane lipids such as cardiolipin, strongly suggest that Adr may manifest its toxicity by interacting with cellular membranes. Present studies were designed to quantitate Adr in the membrane and soluble fractions of cells exposed to this drug and to study its effect on some of the membrane transport enzymes. Furthermore, the amounts of membrane associated Adr in parent sensitive and derived resistant tumor cells were compared to investigate if alterations in Adr membrane interaction could contribute to the observed resistance of tumor cells to Adr: upon repeated exposures. When erythrocytes were incubated with 1-40 \text{mM} Adr \text{in vitro}, the major fraction of Adr, taken up by the cells, was concentrated in the membrane. Based on the relative volumes of the cytosol and membrane of erythrocyte, the concentration of Adr was about 100 fold higher in membrane compared to cytosol when erythrocytes were incubated with 1-10\text{mM} Adr. At higher concentrations (40-50\text{mM}) this ratio decreased to about 30. At these concentrations the membrane enzyme dinitrophenyl glutathione ATPase (Dnp-SG ATPase) was inhibited by 20-25\% of control, indicating the effect of Adr on membrane function. In Adr-sensitive Chinese hamster fibroblast cell line V-79, the amount of Adr associated with membrane was found to be 4 fold higher as compared to that in Adr resistant cell line LZ derived from V-79. These studies suggest that Adr-membrane interactions may contribute to the mechanisms of Adriamycin cytotoxicity and in resistant cell lines these interactions may be reduced. (Supported by GM-32304, CA-27967 and CA-34269).

542 TOXICITY OF A PENCLOMIDINE (NSC-338720)-OIL/LECITHIN EMULSION IN RATS. R Dixit, R Lopez, T Douglas, N Indacochea-Redmond, Midwest Research Institute, Kansas City, MO; M A Stedham, Pathology Associates, Frederick, MD; A C Smith, J J Tomaszewski, National Cancer Institute, Bethesda, MD

Pencloamide (PEN), a synthetic a-picoline derivative, is a potential anticancer agent based, in part, on its superior antitumor activity in experimental breast tumor models. To support the preclinical development of PEN, studies were conducted in F344 rats to determine the systemic toxicity of the drug and its reversibility following either five daily i.v. injections (7.5, 42.5, and 75 mg/kg/day) or three i.v. injections (6.5, 32.5, and 85 mg/kg/ln) in a single day. Treatment of animals by either schedule resulted in dose-dependent signs of neurological toxicity (e.g. general muscular tremors and convulsions) which were rapid in onset and lasted about an hour after each dose. Within 72 hours following the completion of dosing in both studies, a marked dose-dependent leukopenia was observed. This correlated with the bone marrow atrophy seen in these animals, in addition to myelosuppressive effects, vehicle-induced (oil/lecithin emulsion) and possibly drug-potentiated alveolar capillary vacuolation and interstitial inflammation consisting of "fat emboli" were observed in the animals of the high dose group in both studies. Results suggest that hematopoietic suppression is the major dose-limiting toxicity of PEN, independent of dosing schedule. (Supported by NCI Contract No. N01-CM-87292).


CT-958 is a candidate anticancer drug that acts through DNA intercalation. As a benzothiopyran- indazole it lacks the redox cycling potential associated with the cardioactive anthracyclines. To evaluate the acute toxicity of CT-958 and identify potential target organs, single and multiple (daily X5) dose studies were conducted in male and female Wistar rats. CT-958 was administered in sterile saline by IV injection at single doses of 0, 1, 6, or 12.5 mg/kg with 5/sex necropsied on Days 4 and 29. Daily X5 doses were 0, 0.4, 1.5, or 4.5 mg/kg/day with 5/sex necropsied on Days 8 and 35. Hematology was evaluated weekly. There were no drug-related deaths. Dose-related reversible myelosuppression was considered dose limiting for both regimens. WBC counts were <50% of control at the high dose at Day 4 in single dose and at Day 8 in daily X5 dose regimens. Other transient dose-related effects included body weight gain suppression, lymphoid depletion of spleen and thymus, and bone marrow hypocellularity. No myocardial lesions were seen at any dose. In contrast to other target organ effects, degeneration of seminiferous tubular epithelium was not fully resolved by study termination. All of the effects seen were consistent with the cytotoxic nature of a DNA intercalator.

Pentostatin (2-deoxycoformycin), an adenosine deaminase inhibitor, is being developed as a treatment for hairy cell leukemia. To assess its acute toxicity, mice and dogs were administered single IV doses (mice - 100 to 400 mg/kg; dogs - 0.5, 2, 20, and 50 mg/kg). In mice, toxicity was dose-related and delayed. Deaths occurred on Days 9-15 and at all doses. Animals that died had small thymuses. Most surviving animals recovered clinically by Day 14. At termination (Day 29), several mice at 100-250 mg/kg had portal and periportal hepatocellular necrosis with lobular collapse and karyomegaly and cytomegaly. In dogs, clinical signs consistent with GI toxicity appeared for several days at 2 mg/kg. Both species had dose-related decreased body weights at one week (mice 8-29%; dogs 2-8%). Dogs also had dose-related lymphopenia (Days 1, 3, 7) and thrombocytopenia (Day 3), and elevated alk. phos. levels (Day 3). At interim necropsy (Day 8), dogs had dose-related thymic lymphoid depletion and increased bone marrow H&E ratios. There were no findings at terminal necropsy (Day 64). The results show that pentostatin induces a spectrum of effects that are consistent with its cytotoxic mechanism of action.


ABCD® (0.5 mg/kg, 1.0 mg/kg 2.0 mg/kg) or Fungizone® (0.4 mg/kg, positive control) was intravenously administered to Beagle dogs (8/sex/group) for 91 consecutive days followed by a 2 month washout period. Intravenous administration of ABCD® produced dose-related increases in blood urea nitrogen and serum creatinine, decreases in urine specific gravity and osmolality, and renal tubular nephrosis and nephrocalcinosis. Fungizone® (0.4 mg/kg) caused similar but slightly more severe effects by Day 91 than did higher doses of ABCD® (2.0 mg/kg).

All signs of toxicity were at least partially reversible; however, Fungizone® produced more persistent lesions than ABCD®. Compared to Fungizone®, ABCD® produced less severe effects at higher doses and produced no unique toxic effects.


PSA-II is a spontaneously arising fibrousarcoma in a C3H/SeJ mouse. The PSA-II tumor cell lines recurrent after irradiation have been established and cultured in vitro. The sensitivities of these primary tumor cells and the recurrent tumor cells to cis-platin, a chemotherapeutic agent that causes intra-strand crosslinking of DNA, have been investigated. Cells were treated with 6μM cis-platin for various times at 37°C and cell survival was determined by colony formation method. Survival data were fitted by exponential regression analysis and Do which is a treatment time to reduce surviving fraction from 1.0 to 0.37 was calculated. These Do values for different cell lines were as follows: PSA-II, 29.5 min; 457-0, 14.5 min; 457-5, 25.4 min; 457-7, 23.7 min; (457-R, 27.4 min). These results may indicate no induction of drug resistance by irradiation.

*This is based on a single experiment.


Zeniplatin is a platinum-containing antitumor compound. Single and multiple dose intravenous toxicity studies were done in rats and dogs comparing zeniplatin with cisplatin. A single dose of 10 mg/kg of cisplatin or cisplatin was lethal in the dog. In the rat, a single dose of 10 mg/kg of zeniplatin was lethal while lethality occurred at 5.0 mg/kg of cisplatin. When zeniplatin was given daily for 5 days at doses of 0.75 mg/kg/day, there was emesis and myelosuppression; cisplatin was lethal at this dose. When zeniplatin was given to dogs at doses of 1.25, 2.5 and 3.5 mg/kg, at 21 day intervals, there was emesis and myelosuppression at 2.5 and 3.5 mg/kg, and microscopic evidence of regenerating kidney tubules at 3.5 mg/kg. With cisplatin at 2.5 mg/kg there was emesis, myelosuppression and microscopic evidence of regenerating kidney tubules. When given daily to rats for 1 month, zeniplatin was lethal at a dose of 3 mg/kg/day. At this dose, there was decreased body weight gain, myelosuppression, increased BUN and liver enzymes. At a dose of 1.0 mg/kg/day there was only decreased body weight gain.
Adriamycin, an anthracycline aminoglycoside, is a potent and broad spectrum antineoplastic agent. However, its acute hematopoietic and gastrointestinal toxicity and delayed cardiomyopathy limits its therapeutic potential. The incidence of cardiotoxicity can be reduced by treatment with repeated small doses of adriamycin without impairment of the antitumor activity. Encapsulation of adriamycin in canine erythrocytes was studied as a potential slow delivery system. The canine red blood cell was shown to metabolize adriamycin to adriamycinol. In vitro efflux of adriamycin from untreated erythrocytes was 90% after 1h while 80% of the drug was retained in glutaraldehyde-treated cells. The plasma concentration of adriamycin in dogs 120h following administration of free and encapsulated drug were 0.1 and 10.0 ng/ml, respectively. Encapsulated adriamycin exhibits an initial phase of exponential decay (0-3h) and a transient zero-order drug release phase (6-48h). The 120h plasma level is 10-fold greater than that for the free drug. Adriamycin-loaded erythrocytes may provide a slow systemic drug delivery system that may reduce the severity of adriamycin-induced cardiotoxicity.

Hepsulfam is an alkylating agent that was developed as an analog of busulfan. It is currently known as DNA-protein and interstrand DNA cross links in cultured cells. The objective of this study was to compare the cytotoxicity of hepsulfam and busulfan for human tumor cells. Human tumor cell lines (leukemia: HL-60 and K562, lung cancer: SK-MES and CALU, colon cancer: COLO 320 and HT-29, and breast cancer: MCF-7) were used to compare the cytotoxicity of both antineoplastic agents at equimolar concentrations ranging from 25-250 μM. Primary human tumors were also tested for cytotoxicity to hepsulfam at concentrations of 2.7-270 μM. Test compounds were added for 1h or continuous exposure in both systems. Little or no toxicity was noted for hepsulfam following 1h exposure in either system. With continuous exposure, hepsulfam was more cytotoxic than busulfan at all concentrations in human tumor cell lines. In primary human tumors, the cytotoxicity of hepsulfam was greatest in breast, lung, ovarian, and gastric tumors out of 16 tumor types tested. With continuous exposure, a dose-dependent increase in cytotoxicity was observed in primary human tumors. In conclusion, hepsulfam was more cytotoxic than busulfan in cell lines derived from solid tumors and from leukemia cells. A dose-dependent increase in cytotoxicity was observed in primary human tumors following continuous hepsulfam exposure at levels that are clinically achievable. Sponsored by contract # N01-CM57737 from the NCI.

The toxicity of the quinazoline folate antagonist, CI-898, was investigated in mice using an intraperitoneal injection regimen. An initial subacute study, CI-898 was administered at 0, 5, 10, 20, and 40 mg/kg as one dosing cycle consisting of five consecutive daily injections and a 21-day recovery period. In the chronic study, doses of 0, 5, 10, and 20 mg/kg were given in six similar dosing and recovery cycles. Clinical findings consisted of injection site bruising or scarring at all dose levels after repeated dosing cycles, single or multiple convulsions at 40 mg/kg, and a sporadic low incidence of mortality in the single and repeated dosing cycle studies. Body weights were unaffected at any dose level. Red blood cell counts, hemoglobin and hematocrits were 82% to 92% of control at 10, 20, and 40 mg/kg after a single dosing cycle and 90% of control for females at 20 mg/kg after six dosing cycles. Hematopathologic changes identified in the testes at 5, 10, 20, and 40 mg/kg in the single cycle study and consisted of degeneration and necrosis of spermatocytes, and depletion of spermatids and spermatids; however, other rapidly dividing tissues were spared. This is in contrast to the rat where similar dosing regimens resulted in reduced peripheral red and white cell counts, thymic lymphoid depletion, bone marrow hypoproliferation and mucosal degeneration and necrosis in the cecum.
MURINE CARRIER ERYTHROCYTES CONTAINING SQUID DISIOPROPYL PHOSPHOFUORIDATE HYDROLASE. W D McGuinn, E P Cannon, C C Chui, and J L Way. Dept. of Med. Pharmacol. and Tox. Texas A&M Univ., College Station, TX

The entrapment of squid disiopropyl phosphofuoridate hydrolase (DFPase) within mouse red blood cells to detoxify cholinesterase (ChE) inhibitors such as disiopropyl fluorophosphate (DFP) is described. Resealed erythrocytes obtained by hypotonic dialysis containing DFPase provide a stable carrier system that can protect the activity of enzymes against rapid in vivo degradation to disiopropyl phosphate, a less toxic metabolite, and can minimize immunological reactions.

Squid DFPase was purified from the hepatopancreas of Atlantic squid by the method of Hoskins et al. (1984) with modifications. The activity of DFPase solutions was determined by measuring the rate of fluoride ion liberation using an ion selective electrode. Carrier murine erythrocytes containing squid DFPase were obtained by hypotonic dialysis. These ressealed annealed carrier erythrocytes were analyzed for DFPase activity. The rate of DFP hydrolysis observed with these cells was much greater than carrier cells not containing DFPase. (Supported by NIH fellowship F32 ES 05493 and NIEHS grant ES 65139.)
557 USE OF AN IN VITRO ACETYLCΟHOLINЕSTERASE ASSAY TO PREDICT THE EFFICACY OF PRETREATMENT COMPOUNDS IN VIVO AGAINST SOMAN INTOXICATION. J A Blank, D W Hobson, C T Olson, R Kiser, and G Dill. Battelle Memorial Institute, Columbus, OH.

Reversible acetylcholinesterase (AChE) inhibitors can enhance the therapeutic value of atropine and pralidoxime chloride (2-PAM) in treating intoxication with the organophosphonate pinacolyl methylphosphonofluoridate (soman). A comparison of chemical efficacy in inhibiting AChE in vitro with efficacy as an in vivo pretreatment for soman-exposure was performed. An automated in vitro method based upon the measurement of acetylthiocholine iodide hydrolysis over time was developed to measure AChE inhibition. For in vivo studies, ten mice were pretreated with a test chemical, exposed to soman, and then treated with atropine and 2-PAM. Survival of vehicle-treated, test chemical-pretreated mice was evaluated 24 hours following soman-exposure. Pyridostigmine was used as a positive control to assure discrimination of effective from ineffective pretreatments. The test chemical was deemed effective if the number of mice surviving the challenge was at least four greater than the vehicle-pretreated mice. Of the 40 test chemicals examined in the in vitro assay was able to predict 100 percent of the effective pretreatment chemicals in vivo. This in vitro assay can be used as a first level test to screen potential pretreatment compounds for soman intoxication. [Supported by USAMRDC DAMD17-69-C-9050]

559 SPECIES-SPECIFIC PHYSICAL CHEMICAL DETERMINANTS OF ORGANOPHOSPHORUS ANTICHOLINesterase Activity. K B Wallace and J R Kemp. Dept. of Pharmacology, University of Minnesota, Duluth, MN.

Species-selectivity of acute organophosphorus toxicity is well established and reflects, to a large degree, differences in the sensitivity of acetylcholinesterase (AChE) to inhibition by the active oxygen analog. This selectivity has been suggested to reflect species-related differences in both the degree of sterol hindrance and the nucleophilic strength within the esterase domain of the respective enzyme. Structure activity correlations were conducted to reveal distinct physical chemical correlations of anticholinesterase activity for a homologous series of dialkyl-p-nitrophenyl phosphates against brain AChE from rats, hens and rainbow trout. The dissociation constant (Kd) of the unbranched dialkyl-substituted organophosphates for rat and hen AChE, but not the trout enzyme, correlated with the octanol/water partition coefficient of the respective inhibitor and with their retention on various chromatographic columns. Molecular volume, not linear dimension, appears to be the critical factor affecting sterol hindrance. The first-order phosphorylation rate constant (k2) for trout brain AChE could be predicted from the UV or IR absorption spectra of the alkyl hydrolysis rate constant, or from either the 13C or 31P-NMR spectra. Conversely, none of these parameters yielded significant correlations with k2 for either rat or hen AChE. The results substantiate the proposed differences in the sterol and electronic properties of the respective enzymes and suggest that depending on the specific enzyme, selected physical chemical measurements may provide a convenient estimate of the inhibitory potency of organophosphate agents towards AChE. (This work was supported in part by a Grant-in-Aid from the University of Minnesota Graduate School.)

558 EVALUATION OF IN VITRO ASSAYS TO PREDICT THE IN VIVO EFFICACY OF ORGANOPHOSPHONATE TREATMENT COMPOUNDS. J A Blank, D W Hobson, R Kiser, and G Dill. Battelle Memorial Institute, Columbus, OH.

The efficacy of oximes, as well as other compounds, in treating organophosphonate (OP) intoxication in thought to be related to their ability to re activate OP-inhibited acetylcholinesterase (AChE). In this study, experiments comparing efficacy of 60 test chemicals in reactivating tabun-inhibited AChE in vitro with their efficacy as in vivo treatments for soman intoxication were performed. For in vivo efficacy studies, ten soman-exposed mice per group were treated with atropine and either test chemical or pralidoxime chloride (2-PAM). A test chemical was deemed efficacious if the number of mice surviving the challenge after 24 hr was at least four more than 2-PAM-treated mice. Results from these studies demonstrate that the in vitro assay identifies 80 percent of compounds effective in vivo and could reduce the number of animals needed for in vivo studies by 33 percent. When in vitro effects of the test chemicals on aging of soman-inhibited AChE are considered, the ability of these tests to predict efficacious treatment compounds in vivo is increased to 98 percent. These assays would be useful as a first level test to screen potential treatment compounds for soman intoxication. [Supported by USAMRDC DAMD17-69-C-9050]


A 6 week dietary study was required by the EPA to compare the NO Effect Levels (NOELs) of two different lots of technical diazinon. Our adoption of an improved process with fewer organophosphate-related impurities afforded an opportunity to meet the EPA mandate while testing the sensitivity of the experimental design. Diazinon (D) and diazinon-improved (DI) were admixed in the diet at 0, 0.2, 0.5, 2, 20, 100 and 300 ppm & fed to rats (10/sex/group). Plasma and red cells were assayed on three occasions and brains assayed once. D was 92.1% pure and contained 0.4% sulphonepp (S). DI was 97.4% pure and did not contain S. For D and DI, plasma NOELs were 2ppm for males and 0.5ppm for females and red cell NOELs were 2 ppm in both sexes. Brain NOELs were 100ppm in females for D & DI, but 300ppm in males for D and 100ppm for DI. The presence of S in D was not detected even in this matched bioassay setting. These data show the inherent difficulties in using bioassays for comparing test materials and suggest that data derived from such studies are of limited value for regulatory decisions.
TOXICOKINETICS OF PROPETAMPHOS IN THE MALE F344 RAT FOLLOWING INTRAVENOUS ADMINISTRATION. K D Washburn1,2, L T Burka1 and W C Dauterman2.
National Institute of Environmental Health Sciences1, Research Triangle Park, NC and North Carolina State University2, Raleigh, NC

Propetamphos is an organophosphate developed by Sandoz, Inc. as an insecticide effective against a wide range of household pests. Mammalian metabolism of propetamphos has been investigated by Sandoz, Inc. results have yet no toxicokinetic data exists in the literature. Therefore, the purpose of the current studies is to investigate the toxicokinetics of propetamphos in the male F344 rat. Propetamphos is highly bound to plasma proteins (free fraction = 0 to 0.09). Upon incubation of propetamphos with blood in vitro, the blood to plasma concentration ratio is 0.75 over a concentration range of 50 to 200 ng/ml of blood. In addition, rats have been dosed intravenously via the jugular vein (12 mg/kg or one tenth the oral LD50) to determine the clearance, apparent volume of distribution, half-life and rate constants that characterize the toxicokinetics of propetamphos in vivo. The rate of propetamphos is best described by a three compartment system with an approximate plasma clearance of 12 ml/min. The apparent volume of distribution is 200 ml and the overall elimination rate constant is 0.01 to 0.07 min-1. Propetamphos appears to be distributed throughout the body water and is rapidly cleared following intravenous administration.

CHOLESTERASE INHIBITION IN MICE FOLLOWING SUBCHRONIC DIETARY EXPOSURE TO THE INSECTICIDE PHOSPHAMIDON. K L Pavkov1, S M Macaskill1, J C Turnier1, M R Luedel1 and C R Saunders1.
Environmental Health Center, Agricultural Div., CIBA-GEIGY Corp., Farmington, CT and Greensboro, NC

Groups of 10 mice/sex were fed diets containing phosphamidon at concentrations of 0, 0.1, 0.3, 1.0, 50, 100 or 150 ppm. There were no clinical signs of toxicity. Body weight gains were significantly reduced in males receiving 100 ppm (67% of control) and 150 ppm (55% of control) and in females receiving 150 ppm (48% of control). At study termination, group mean body weights in the 150-ppm males and females were 89% and 87% of control, respectively. There were no biologically significant effects on hematology. Significant depression of cholesterease (ChE) activity was observed for serum, RBC and brain in both sexes. The serum ChE activities (percent of control) were 3%, 2%, and 19% for the 50-, 100- and 150-ppm dose levels in males. The RBC ChE activity values were 20%, 0% and 5% for the 50-, 100- and 150-ppm dose levels; brain ChE activities for the same dose levels were 65%, 61% and 57% of control. In females, the serum ChE activities (percent of control) were 85%, 87%, 20%, and 8% for the 0.3-, 1.0, 50, 100- and 150-ppm dose levels. The RBC ChE activity values were 20%, 6%, and 24% of control for the 50-, 100- and 150-ppm dose levels; brain ChE activities for the same dose levels were 56%, 48% and 49% of control. Segmental vacuolar degeneration occurred adjacent to the head of the epididymis of all male mice given 50, 100 or 150 ppm. The NOEL was 0.1 ppm. The MTD was 50 ppm.

RISK ASSESSMENT OF ALDICARB IN FOOD AND OTHER COMMODITIES. P E Breure, J P Standley and J J Jackson.
California Department of Health Services, Pesticide and Environmental Toxicology Section, Berkeley, CA

An assessment is performed of aldicarb ingestion from agricultural commodities. An acceptable daily intake (ADI) of 0.001 mg/kg has been recommended by various agencies and scientific bodies. The World Health Organization increased it to 0.005 mg/kg in 1982. These values have been based on a short-term feeding study in rats where a no-observed-effect level (NOEL) of 0.125 mg/kg-day was evident, or on a study with human volunteers, where the NOEL of 0.01 mg/kg-day, relative to plasma cholinesterase depression, was obtained by extrapolation and an uncertainty factor of 10 was applied to give an ADI of 0.001 mg/kg. Recently the US Environmental Protection Agency lowered the reference dose (RFD) for aldicarb to 0.2 mg/kg-day. This revision was based largely on further examination of the one-year dog study which resulted in the establishment of a lowest-observed-effect level (LOEL). Consequently a higher uncertainty factor should be used. Other factors considered in taking this action include data obtained from a study of aldicarb poisoning in watermelons in California in 1985, and food survey and field-trial data. It was concluded that the acute health risks from consumption of aldicarb in the diet exceeded the lowest-observed-effect level in the case of potatoes (concentration-range: 0 to 8.7 ppm; tolerance 1.0 ppm) and bananas (concentration-range: 0 to 0.02 ppm; tolerance 0.3 ppm) imported from South and Central America. An assessment of the existing tolerances for aldicarb in foods also indicates that total aldicarb intake from these foods would exceed both the WHO ADI of 0.005 mg/kg, the new EPA RFD of 0.2 mg/kg-day, and the lowest dose which produced symptoms estimated to be 0.2 mg/kg from the 1985 episode in California.
565 PROPHYLACTIC AND THERAPEUTIC POTENTIAL OF MEMANTINE IN ACUTE ALDICARB POISONING. R C Gupta and W L Kadel. Breathitt Veterinary Center, Murray State Univ. Hopkinsville, KY.

Administration of aldicarb in male Sprague-Dawley rats with sublethal acute dosage (0.4 mg/Kg, ip) developed the onset of hypercholinergic signs within 3-5 min. With increasing intensity the most severe acetylcholinotoxic signs (predominantly peripheral muscle fasciculations and convulsions) were evident within 10-15 min and lasted for about 90 min. A dosage of 0.6 mg/Kg was found lethal. Following sublethal dosage, a significant inhibition of AChE was noted as early as within 15 min in discrete brain regions, heart and diaphragm muscle. At 30 min, when AChE was maximally depressed, brain cortex was affected the most (22% of control) and striatum the least (72%). Time course on carboxylesterase (CarbE) revealed marked inhibition in brain regions, muscles, liver and serum, suggesting substantiative nonspecific binding of aldicarb. Propylxaxis with memantine (18 mg/Kg, ip) and atropine sulfate (16 mg/Kg, ip) 30 min and 15 min, respectively, prior to aldicarb provided complete protection. Therapeutic administration of these two antidotes in combination also significantly diminished the signs of intoxication. It is suggested that memantine antagonized the aldicarb toxicity by protection/reactivation of inhibited AChE and reversible blockade of hyperneuromuscular activity, in addition to cholinolytic effect of atropine.

566 HEALTH RISK ASSESSMENT OF AERIAL MALATHION APPLICATION. M J DiBartolomeis, J F Brown, A M Fap, and R J Jackson, Calif. Dept. of Health Services, Berkeley, CA.

A comprehensive health risk assessment was conducted on potential residential exposure to malathion and its co-products as applied in urban pest eradication programs. In August 1989, the Calif. Dept. of Food and Agriculture initiated an emergency Mediterranean fruit fly eradication program in Southern California consisting of nighttime aerial applications of protein bait containing malathion (2.8 ounces/acre), followed by releases of millions of sterile flies. Malathion inhibits blood and brain acetylcholinesterase (AChE) resulting in adverse neuronal effects. The evidence for the carcinogenicity of malathion is insufficient, but equivocal for a metabolite, malaoxon, which causes thyroid C-cell tumors in rats. There is limited evidence that malathion causes developmental toxicity in animals and genetic toxicity (e.g., chromosome aberrations). The most sensitive endpoint for toxicity is AChE inhibition (no-observed-adverse-effect-level = 230 μg/kg/d), and acute and chronic daily permissible exposure levels of 20 and 2 μg/kg, respectively, were derived from these data. Based on several exposure scenarios and upper-bound assumptions, human dose estimates ranged from < 0.06 to 250 μg/kg for acute, and < 4 x 10^5 to 2 μg/kg for chronic daily (lifetime) exposures. Malaoxon doses were significantly lower. It is concluded that significant adverse effects from AChE inhibition following acute or chronic exposures to aerially applied malathion bait would not be expected in the general population. However, AChE inhibition may be possible in sensitive subpopulations and allergic-type reactions may occur even at the low doses.


The state of California has conducted aerial spraying of the pesticide malathion in bait formulation as part of a Mediterranean fruit fly eradication effort. The Department of Health Services (DHS) has conducted a multipathway exposure assessment as part of an evaluation of public health impacts of aerial application of malathion bait in an urban area. Pathways of exposure considered were inhalation, dermal absorption, and ingestion. A number of scenarios were considered which represent activity levels for individuals ranging from sedentary to very active for adults and children. Inhalation exposures were quite small and represented only a small fraction of total exposure. Dermal absorption and ingestion exposures were predicted to contribute significantly to total exposure. Inhalation exposure estimates ranged from the 6 to 100 ng/kg-d for acute exposure estimates and from 7 to 60 ng/kg-d for chronic exposures. Dermal exposure estimates ranged from 2 to 240 μg/kg-d for acute exposures and from 0.8 to 80 μg/kg-d for chronic scenarios. Ingestion exposure ranged from 0.8 to 390 μg/kg-d for acute exposure scenarios and from 0.02 to 1.0 μg/kg-day for chronic exposure scenarios.


Malathion is widely used in California for mosquito abatement, agricultural pest control, and Mediterranean fruit fly eradication. Biomonitoring studies have been conducted to assess human bioavailability and excretory kinetics of malathion following dermal exposure to improve estimates of absorbed dose following worker and resident exposure. These studies supplement and extend those conducted by Malbach et al. and Wester et al. which used radiocarbon-labeled malathion in humans. Following dermal dosages of 50-2800 μg/cm^2 on the forearm, the biological clearance half-life in urine was 6-12 hours. Five metabolites of malathion were measured consisting of dimethyl-, dimethylthio-, and dimethylthio-phosphates as well as malathion - monocarboxylic and -dicarboxylic acids. Following oral dosage (0.05 mg/kg), these metabolites accounted for 95% of the dose while after dermal exposure they were 1-4% of the dose. The ratio of d1 to monocarboxylic acid ranged 0.1-0.7 to 1 but increased with time from dose suggesting sequential desaturation. The mono and dicarboxylic acids can be detected at 4 and 2 ppb, respectively and allow sensitive and selective detection of exposure. An observer dressed only in running shorts who was directly exposed to 18 helicopter overflights in Los Angeles absorbed 6-14 mg malathion. On this basis, a minimally-clothed adult bystander may absorb approximately 0.3 to 0.8 mg following a single overflight for Medfly eradication.
Malathion, a non-systemic organophosphate insecticide, is widely used in agricultural and public health practices. The disposition and metabolism of 14C-malathion was assessed in male and female Sprague-Dawley rats following single low (40 mg/kg), high (800 mg/kg), and multiple (40 mg/kg x 15 days) oral doses. Malathion was rapidly absorbed, biotransformed, and excreted predominantly in urine and to some extent in the feces. Urinary elimination of 14C was rapid and extensive in both sexes. Approximately 80% to 90% of the doses were eliminated in urine within 24 h except for the males of the high dose group, which excreted only 12% of the total dose at this time. Elimination in feces was limited, 4% to 10%, following low, high, and multiple doses. Cumulative excretion through both urine and feces (0 to 72 h) accounted for greater than 90% of the total initial dose in both sexes. Less than 1% of the administered dose was recovered in tissues and blood. Ten metabolites were identified by GC/MS. The major metabolites were identified as the \( \alpha \) and \( \beta \)-monoacetyl- and the diacetyl derivatives of malathion. The other minor metabolites were identified as malaoxon, desethyl malathion, \( \alpha\),\( \beta\)-dimethyl phosphorothioate acid, \( \alpha\),\( \beta\)-dimethyl phosphorothioic acid, \( \beta\)-mercaptosuccinic acid, furamido acid, and monomethyl furamate.

SITUATIONAL CHEMICAL EXPOSURE STUDIES PROVIDE HUMAN METABOLISM AND URINE CLEARANCE DATA FOR CHLORPYRIFOS (C), DIMETHATE (D) AND MALATHION (M). R J Krieger, T Thongsinhand, J H Ross, R Broberg, S Taylor, S Frederickson, S Begua, M Dong. California Department of Food and Agriculture, Worker Health and Safety Branch, Sacramento, CA.

Organophosphorus insecticide exposures were monitored by measuring alkyl phosphate esters (APs) in urine. Situational studies are unscheduled opportunities for monitoring that provide human metabolic and kinetic data concerning chemical exposures. Exposures included C following indoor fogging, D food residues, and M date dusting (NDL 25 ppb). Urines were collected for up to 4 days and analyzed for corresponding dialkyl-, dialkylthio- and dialkyldithio phosphates plus D/xon and M mono-/dithioc. Cumulative metabolites were plotted as F (time) and sigma minus analysis used to estimate half-life. Urine clearance of similar self-administered oral and dermal dosages was also measured. (1) 2 adults treated their 825 ft² apartment with 6 home-foggers containing C and followed normal weekend schedules for the following 48h. Urine from the corresponding period contained no APs (NDL < 40 ug/person). (2) Peas containing volatile levels of D (16 vs 2 ppm tolerance) were ingested (ca. 0.1 mg/kg). Clearance of APs was rapid t1/2 3-5h and consistent with oral and dermal findings. (3) M dust exposures of an observer in a date garden resulted in rapid appearance (2-3h) of APs and Xoxide. Availability of M was greater than expected. Clearance was rapid (t1/2 3-5h). Exposure assessments may be augmented by situational studies which represent human experience rather than hypothetical worst-case scenarios.

PARAOXON TOXICITY IS NOT POTENTIATED BY PRIOR REDUCTION IN BLOOD ACETYLCHESTERASE (AChE). S Padilla1, V C Mozer2, W S Brimijoin3, E Bennett4, L L Popa5, 1US EPA and 2XSI Tech. Services, Res.Tri.Park, NC, 3Mayo Clinic, Rochester, MN, and 4NLU, Monroe, LA.

The role of blood AChE in moderating the effects of organophosphate challenge in rats was tested. Adult male rats (n=42) were injected (iv) either with monoclonal antibodies (AB) to rat AChE (EC 3.1.1.7) or normal mouse IgGs (controls). Two days later, the rats were injected (sc) with paraaxon (PX) 0.17 or 0.34 mg/kg or with vehicle. Neurological integrity was assessed by a functional observational battery followed by motor activity, 3 to 4 hrs after dosing. Blood, and diaphragm tissues were then collected for determination of AChE activity. Ab treatment reduced whole blood and plasma AChE by 32% and 90%, respectively, but did not affect neurobehavioral parameters or the AChE of brain or diaphragm. PX challenge produced dose-dependent neurobehavioral changes and inhibition of brain and diaphragm AChE activity to the same extent in IgGs and Ab treated rats. Neurobehavioral changes were correlated with decreased brain AChE activity (274% of controls). Thus, significant loss in blood AChE alone produced no detectable neurobehavioral deficits and did not alter the subsequent responses to PX challenge. We conclude that lowered blood AChE has no major role in potentiating the effects of organophosphate exposure.
RELEASE OF PARAOXONASE BY RAT LIVERS PERFUSED IN SITU. LG Sultatos, JA Vitarius and Y-S Huang. Dept. Pharmacol. and Toxicol., UMDNJ, New Jersey Medical School, Newark, NJ.

The enzyme(s) capable of hydrolyzing paraoxon (0, 0-diethyl 0-p-nitrophenyl phosphate) have been identified in several tissues, including serum. However, the source of serum paraoxonase has not yet been identified. In the present study, rat livers were perfused in situ with a modified Kreb-Henseleit solution in a recirculating system for 2 h with a reservoir volume of 200 ml. Following perfusions, EDTA-sensitive hydrolysis of paraoxon ranged from 0.23 to 0.40 nmols paraoxon hydrolyzed/ml perfusate/min with a paraoxon concentration of 1 mM. EDTA-insensitive hydrolysis of paraoxon was extremely low or nonexistent. Although these studies do not exclude the possibility of secretion of paraoxonase by other tissues, they suggest that liver is a major source for paraoxonase found in the serum in the rat. (This work was supported by Grant ES04335 from NIHES).

576 AN INVESTIGATION OF HEPATIC ALIESTERASES AND THEIR PARAOXON SENSITIVITY IN 8-NAPHTHOFLAVONE TREATED RATS. A M Watson and J E Chambers. Dept. of Biological Sciences, Mississippi State University, MS.

Aliesterases are serine esterases which serve a protective role during organophosphate insecticide intoxication by providing alternate phosphorylation sites. The levels of hepatic aliesterase activity with several 4-nitrophenyl esters (valerate, propionate, butyrate, hexanoate, isobutyrate, isoheptanoate, and phenylacetate) and the sensitivity of aliesterases to inhibition by paraoxon, the activated metabolite of the common insecticide parathion, were investigated after the intraperitoneal administration of 8-naphtohflavone (BNF) to rats. Rats treated with BNF showed about 38-39% lower activity than the control rats when using 4-nitrophenyl valerate as the substrate. These aliesterases were also less sensitive to paraoxon inhibition. The $I_{50}$ values for the assay using 4-nitrophenyl valerate and 4-nitrophenyl propionate were about 9-fold higher in BNF-treated rats than control. This lower activity and reduced sensitivity of the hepatic aliesterases could leave the animal more susceptible to organophosphate poisoning. (Supported by ES04394 and ES00190).
577 ISOLATION AND CHARACTERIZATION OF A cDNA CLONE ENCODING RABBIT SERUM PARAOXONASE. C E Furlong, C Hassett, R J Richter, C Chapline*, J W Crabb* and C J O'Malley. Departments of Genetics, Medicine and Environmental Health, University of Washington, Seattle, WA, and *The W Alton Jones Cell Science Center, Lake Placid, NY.

Serum paraoxonase catalyzes the hydrolysis of organophosphate pesticides and other substrates, including nerve agents. At least two allelic forms of the enzyme exist in humans, one which hydrolyzes paraaxon with a high turnover number and a second which hydrolyzes paraaxon with a low turnover number. The polymorphism is substrate dependent and is not observed with other substrates such as chlorpyrifos oxon or phenylacetate. The rate of metabolism of organophosphate substrates of paraoxonase in a given individual depends on both the allelic forms of the enzyme present, as well as the serum levels of the protein(s). We have purified both rabbit and human paraoxonase to homogeneity. Sufficient amino acid sequence was determined from the rabbit enzyme to allow the design of two low redundancy oligonucleotide probes which were used to isolate from a rabbit liver library the cDNA that encodes paraoxonase. The clone is 1294 nucleotides in length and contains the entire coding region for paraoxonase (359 amino acids). The mature enzyme retains its secretion signal sequence, with only the amino-terminal methionine cleaved. Northern blot analysis indicates that this protein is synthesized in liver. Work on isolation of the human paraoxonase cDNA is in progress.

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578 ROLE OF SOCIALIZATION, STRESS AND SEX OF CHICKENS ON RESPONSE TO ANESTHESIA AND ON RESPONSE TO AN ORGANOPHOSPHATE NEUROTOXICANT. Andrea Odom, W Burnham Gross, and Marion Ehrich. Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA.

Socialization has been suggested to protect animals and man from infectious diseases and cancer, but reports of its influence on responses of chickens to drugs and toxicants are lacking. In this study roosters and hens were habituated to human beings (socialized) by being talked to, offered food and handled gently daily from hatch. After 21 weeks, half of the socialized group and half of the control group were subjected to stress (loud noises for 120 sec) twice daily. After 2 weeks, pentobarbital (37 mg/kg iv) sleeping time was determined. Roosters slept significantly longer than hens (mean sleeping times, 25 min and 15 min, respectively), and roosters that were socialized but not stressed slept significantly longer than other roosters (37 min and 21 min, respectively). Socialized and nonsocialized, stressed and unstressed roosters and hens were also administered a single oral dose of tri-glycyl phosphate, 360 mg/kg. Ataxia was significantly more pronounced earlier in roosters than in hens, although the sex difference disappeared by 18 days after dosing. This study indicated that socialization, rather than sex, contributed more to the exaggerated response of roosters to an anesthetic and to an organophosphate neurotoxicant.

579 USE OF A FUNCTIONAL OBSERVATIONAL BATTERY (FOB) TO DETECT EFFECTS OF CHOLINESTERASE INHIBITORS IN RATS WEEKS AFTER EXPOSURE. Marion Ehrich, Linda Shell, Michael Rosum, and B. S. Jortner. Virginia Tech. Blacksburg, VA.

Because residual effects in human beings exposed to cholinesterase inhibitors have been reported, the FOB was used to periodically assess neurotoxicity in adult male Long Evans rats during the 3 weeks between administration of single doses of triorthoisocyanurate TDP 1000 mg/kg po, disopropylphosphorothioate (DPP, 2000 mg/kg po), dichlorvos (30 mg/kg ip), or carbaryl (160 mg/kg ip) and pathological examination. Inhibition of brain acetylcholinesterase activity, determined 4-48 hours after toxicant administration, was 96%, 97%, 47%, 57%, and 65%, respectively. Except for rats given dichlorvos, all groups given cholinesterase inhibitors weighed significantly less than controls 2 weeks after the toxicant administration. Home cage activities and gait of the animals given TDP and malathion were also different from controls at that time. Mild to moderate myelinated fiber degeneration was seen in the rostral levels of the fasciculus gracilis in rats given DPP and TDP, more severe in the latter. 2/5 rats given DPP also had degeneration involving tibial nerve branches to the gastrocnemius muscle. No notable neuropathic lesions were seen in rats given malathion, dichlorvos or carbaryl. These results indicate that the FOB may detect some residual toxicity in rats exposed to high doses of cholinesterase inhibitors weeks earlier.

(Supported by USEPA 68D80098)

580 OPEN FIELD BEHAVIOR OF LEAD-TREATED MONKEYS: EFFECTS OF ARECOLINE. S A Ferguson and R E Bowman, National Center for Toxicological Research, Jefferson, AR and Harlow Primate Laboratory, Univ. of Wisconsin, Madison. WI. Sponsor: M G Paul

Nursery-reared rhesus monkeys were treated orally with no or moderate (mean=0.81 mg/kg/day) levels of lead during the first year postpartum only, reaching peak blood lead levels of 55 ug/dl at week 5, a mean of 36 ug/dl until 1 year, and 5 ug/dl from 26 months to age to date. Tests at 4 years of age in a nonhuman primate version of the open field revealed lead-induced increases in latency to enter the open field, increased environmental exploration and decreased activity. To determine a possible cholinergic involvement, the monkeys were retested at 5 years of age after IM injection of 0.02, 0.06, or 0.08 mg/kg arecoline, a cholinergic agonist. Although the lead-induced behavioral differences were still present, arecoline failed to interact with lead treatment suggesting cholinergic deficits are not involved. At all doses, however, arecoline decreased the number of sectors entered in the open field and exerted a marginal decrease in locomotion which appears similar to the fatigue described for arecoline-treated humans. Further studies of the effects of pharmacological manipulations on open field behavior of lead-treated monkeys are in progress.
581 LIFE-TIME EXPOSURE TO LEAD PRODUCES VISUAL DEFICITS IN ADULT MONKEYS. S G Gilbert and D C Rice. Toxicology Research Division, Health Protection Branch, Ottawa, ON, Canada.

Life-time exposure to low levels of lead produces a variety of cognitive deficits but detailed assessment of visual function has not been reported. Monkeys (Macaca fascicularis) were exposed from birth onward to lead at doses equivalent to 0, 500 or 2000 μg/kg/day. Blood lead levels peaked by 100 days of age at 55.3 and 115.0 μg/dl for monkeys in the 500 and 2000 μg/kg/day dose groups, respectively. Following withdrawal of infant formula, blood lead levels of both dose groups decreased to a steady state level near 33.0 μg/dl. At approximately 12 years of age, spatial and temporal visual function were examined under conditions of both high and low luminance. Visual stimuli were sinusoidal gratings displayed on oscilloscopes. Ophthalmological examination revealed that two treated monkeys were severely myopic. One of these monkeys was not tested, while the other monkey was tested with the stimuli 2 feet from the monkey’s eyes. Monkeys in both treated groups displayed deficits in spatial and/or temporal visual function with the pattern of deficit being variable between monkeys. In general, temporal visual function was more affected than spatial. These results suggest that, in the monkey, long term exposure to lead beginning at birth is associated with deficits in visual function.

582 DEVELOPMENTAL METHYLMERCURY EXPOSURE PRODUCES HEARING DEFICITS IN ADULT MONKEYS. D C Rice and S G Gilbert. Toxicology Research Division, Health Protection Branch, Ottawa, ON, Canada.

Deafness or hearing deficits have been reported in humans as a consequence of both adult and development methylmercury exposure, although detailed assessment of hearing function has not been reported. Monkeys (Macaca fascicularis) were dosed from birth to 7 years of age with 0 or 50 μg/kg/day of mercury as methylmercuric chloride. Blood mercury levels in the treated monkeys peaked at 1.2 ppm during infancy, and decreased to steady state levels of 0.6-0.9 ppm after withdrawal of infant formula. Blood mercury levels quickly decreased to background levels after cessation of dosing. When monkeys were 14 years old, hearing was assessed by determining the detection threshold for a number of frequencies spanning the hearing range of normal monkeys. Auditory stimuli were delivered via headphones fit against the monkey’s ears. Thresholds were determined separately for each ear using a psychophysical tracking task. The monkey was required to make hand contact with a bar, and release it upon detection of a tone. Each correct detection decreased the sound pressure by 3 dB with a probability of one-third, while each failure to detect increased the sound pressure by 3 dB. 33% of trials were catch trials, in which no tone was presented. Of the five monkeys exposed to methylmercury, two had normal pure tone detection thresholds, two had slightly elevated thresholds compared to the two control monkeys at middle frequencies (1000-4000 Hz), and one had a slight impairment at middle frequencies and a severe impairment at higher frequencies (10,000-12,000 Hz). These results suggest that exposure to methylmercury beginning postnatally may result in permanent hearing deficits as measured by pure tone detection thresholds.

583 EFFECTS OF PHYSOSTIGMINE ON RHEUS MONKEY PERFORMANCE IN AN OPERANT TEST BATTERY (OTB). M G Paulus and M P Gillam. Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR.

Complex behavioral tasks were used to study the effects of the anti-acetylcholinesterase physostigmine (PHYSO, 0.001-0.056 mg/kg) on several brain functions. The tasks in the multiple schedule, food-reinforced OTB were: Progressive Ratio (PR); Conditioned Position Responding (CPR); Incremental Repeated Acquisition (IRA); Delayed Matching-to-Sample (DMTS); and Temporal Response Differentiation (TRD). Performance in these tasks is thought to model motivation, color and position discrimination, learning, short-term memory, and time perception, respectively. Testing lasted 50 min and began 15 min after iv PHYSO or saline. Endpoints monitored included percent task completed (ptc), response rate (rr) and accuracy (acc). Doses below 0.03 mg/kg had no significant effects on any measure of behavior. PHYSO at 0.03 mg/kg caused disruptions in one or more of the performance of all tasks, as evidenced by significant decreases in ptc. PHYSO-induced decreases in ptc were accompanied by significant decreases in rr in all tasks and by decreases in acc in the TRD task. These data demonstrate that all of the OTB tasks are disrupted equally by enhancement of cholinergic activity.

584 USE OF A FUNCTIONAL OBSERVATIONAL BATTERY (FOB) TO DETECT NEUROTOXIC EFFECTS IN RATS GIVEN MULTIPLE DOSES OF ACRYLAMIDE OR 2,5-HEXANEDIONE. Linda Shell, Michael Rotun, B. S. Jortner, and Marion Ehrich. Virginia Tech, Blacksburg, VA.

The FOB is a series of clinical evaluations of rats that is useful in assessing neurotoxic effects, and was used in this study to evaluate the effects of 2 known neurotoxicants. Adult male Long Evans rats, 250-350 g, were administered 12, 25 or 50 mg/kg ip acrylamide 3 times weekly for 9 doses or 150, 225 or 350 mg/kg 2,5-hexanediol daily for 21 doses. Animals were examined using the FOB 1, 2 and 3 weeks after the initial administration of the toxicant. Parameters of the FOB significantly affected by both time and dosage of acrylamide and 2,5-hexanediol were righting reflex, agility on the rotorod, gait, general mobility, and body weight. With the exception of body weight, all of these were indices of detrimental effects of muscle tone and equilibrium. Activity of an enzyme indicating potential for neuropathy induced by another neurotoxicants (neurotoxic esterase) was significantly inhibited in rat brain and spinal cord 52 hours after initiation of acrylamide treatment. Lesions after the course of acrylamide administration consisted of dose-related axonopathy in peripheral nerve and spinal cord, with the greatest concentration in rostral levels of the fasciculus gracilis, and injury to cerebellar Purkinje cells. These results indicate that the FOB evaluation is a sensitive indicator of neurotoxic effects in rats given acrylamide or 2,5-hexanediol. (Supported by USEPA 68D00096)
Conditioned place preference (CPP) procedures are used with rodents to demonstrate drug-seeking behavior. To provide a model suitable for use with large numbers of mice in genetic studies of cocaine abuse and to provide a screening method for the pharmaceutical industry, we developed a CPP method which can determine a drug's abuse potential with only a few minutes of testing on 5 consecutive days. Mice are conditioned in an apparatus consisting of a central compartment and 2 goalboxes, each with distinct visual and tactile cues; infra-red beams allow monitoring of location. After administering drug or saline by ip injection, we enclose the subject in a goalbox for 20 minutes, repeating this procedure on 3 days. On the 5th day, we give no drug, and the subject is allowed to explore the apparatus for 15 minutes before being returned to its home cage. After 4 hours, another 15-minute session determines preference for the "drug" goalbox. With hydromorphone, significant (P < .008) CPP effects were obtained in each of 2 experiments with 24 (12 control and 12 treated) male DBA/2J mice. Fentanyl citrate also produced a significant CPP. With cocaine, neither male nor female mice showed reliable CPP effects. Strain and sex effects occur with other addictive drugs, suggesting genetic differences. This research is sponsored by the Advisory Committee for Research at SwRI.

Six behavioral neurotoxicity endpoints after positive control treatments were assessed in adult Sprague-Dawley rats as part of an ongoing program of development and validation of an adult neurotoxicity test battery designed to meet the TISA guidelines (1985). Motor activity (MA) was validated with d-amphetamine 1.p. 15 min pretreat (males 1.5 mg/kg, females 1.0 mg/kg, 10/sec/gp). Limb grip strength (GS/Ssec/gp) and hind limb splay (HS=5/mals/gp) were studied using 50 mg/kg acrylamide 1.p.. Nociceptive response (NR) was tested on a hot plate with 19.5 °C codazone s.c. 30 min posttreat (10/sec/gp). Startle response (SR) was tested for 20 males at 4 ages (6-19 weeks) using 1.0 mg/kg strychnine 1.p. 10 min pretreat. Step-through passive avoidance (PA) was tested with 1.0 mg/kg acetylcholine 1.p. 15 min pretreat on the learning trial and retention tested on 2 subsequent days (14-18/sec/group). Appropriate concurrent control groups were used for comparison in all studies. Results indicated: MA-saues showed greater increases in horizontal and vertical counts as well as movement than females; GS-saues showed the most dramatic effects for hindlimb measurements; HS-significant increases for splay distances were found; NR-males and females took 3 times longer to respond to the thermal stimulus; SR—both sexes had greater response amplitudes; PA—males and females crossed into the dark side in 1/3 trials as compared to controls on retention trials. These validation studies demonstrate the ability of the behavioral test procedures to detect pharmacologic and toxicologic effects.

Validation of the proposed revised EPA neurotoxicity guidelines was recently completed in our laboratory. Behavioral and neuropathological sequelae of substances with known nervous system effects were evaluated using the Functional Observational Battery (FOB) (798.6050), Motor Activity (798.6060) and Neuropathology (798.6460) testing guidelines. FOB data from acute parathion-treated rats (0, 1, 3 and 10 mg/kg, gavage) indicated increased salivation and incidence of clonic convulsions. Acrylamide (0, 12.5, 25 and 50 mg/kg/day, gavage) administered for 14 days produced a decrease in hindlimb grip strength (75% at the high dose). A dose-dependent increase in hindlimb footsplay was also observed (9.4 cm at 0 mg/kg to 14.4 cm at 50 mg/kg). Examination of selected sites from the brain, spinal cord and peripheral nerves of the acrylamide-treated rats revealed axonal degeneration in the most distal portions of the tibial and sural nerves. CNS and proximal peripheral nerves were unremarkable. Triadimefon (0, 30, 100 and 300 mg/kg, gavage) produced a dose-dependent increase (23, 49 and 76%, respectively) in activity during 30 min in a Figure 8 maze. Reduction in horizontal activity with chlorpromazine (0, 2.5, 5, and 10 mg/kg, i.p.) was most evident at the highest dose (8%).

We have been investigating the neurobehavioral effects of Polychlorinated biphenyls (PCBs) in a marine mollusk, Aplysia californica, with the aim of providing insight into the mechanism of PCB action on basic neural functions and behavior. In a preliminary experiment, exposure to PCBs via diet or injection resulted in a significant retardation of the righting reflex in Aplysia (Jahn-Pawar, Z.) and experiments conducted to determine the dose-effect relationship of this neurological deficit. Four groups (n=3) of Aplysia were injected respectively with 0, 25, 50, and 100 μg/kg dose of a standard Accorlor mixture (1016, 1221, 1254, and 1260, 1:1:1:1) in corn oil. The animals were placed in individual sea water flow through systems with aeration and gravel filtration and maintained in an environmental room under constant temperature (14 ± 1 °C), light-dark cycle (12:12), and food (seaweed, Gracilaria) supply (5 g/day) regimes. The mean righting reflex latency, measured daily (3 trials/animal/day) from day 0-26 post-injection, showed no significant difference between dose groups in day 0 and within the 0 dose group over 26 days. The latency increased in a dose-dependent fashion in the PCB injected animals in days 1-9 and declined in subsequent days. The mean latency of the 100 μg/kg dose group was 3 times longer than the control group in days 1-9. No significant latency differences were found between dose groups from days 22-26. This transient neurological effect may be due to a reduced PCB concentration in the nervous system following partitioning, metabolizing, or excretion of neuroactive congeners by the animal. Supported in part by Superfund grant #ER1ES0491:3-01(04).

Male and female Sprague-dawley rats were given either acetylcyphenone (ACP) or acetylpyridine (APD) for nine days by oral gavage. ACP was administered to rats/sex at a concentration of 250 mg/kg, while APD was administered to 2 groups of rats (5 rats/sex) at concentrations of 5 or 10 mg/kg. Evaluations of all rats for potential toxicity included clinical observations, food consumption, body weights, functional observation battery (FOB), gross observations, organ weights and microscopic observations of liver, kidney and brain. The FOB was performed prior to study initiation and 24 hrs after last administered dose. ACP treatment resulted in audible respiration, decreased food consumption, decreased body weight and body weight gain. No treatment related findings in the FOB, organ weights, gross or microscopic findings for the liver, kidney, brain or lungs were seen with rats treated with ACP. Treatment with APD did not result in clinical signs of toxicity, gross or microscopic findings, or alterations in food consumption or body weight. Increases in arousal, rectal temperature and decreased treadmill times were observed for some females in the 5mg/kg APD group. The biological significance of these findings cannot be clearly attributed to APD. In summary, ACP at 250 mg/kg produced moderate toxicity with no evidence of nervous system effects and APD at 5 or 10 mg/kg did not produce toxicity nor clear evidence of nervous system effects.

ENHANCEMENT OF CONDITIONED AVOIDANCE RESPONSE (CAR) BY ß-BLOCKERS, N-METHYL-D-ASPARTATE (NMDA) AND ACETYLCHOLINESTERASE IN MICE. H. Yen-Koo, B. Rosloff and J. Zhang. DRT, FDA, WASHINGTON, D.C. 20204

We have tested some CNS stimulants and hypnotogens that affect learning and memory and which might be useful in the treatment of Alzheimer's disease. Previously we reported that clonidine produces an enhancement of CAR (Yen-Koo, J. Agg. Behavior 17, No. 2, in press). In our procedure mice were trained in an automatic reflex condition at 1/4 hr. and 2 days after injection. Mice were dosed on days 1, 2, and/or 4. They were rechecked for memory retention up to 3 months. We tested two ß-blockers, atenolol and pindolol as well as NMDA (Headley & Grillner, TIPS, 11, 205, 1990) and 1-0H-THA (Shutake et al. J. Med. Chem. 22, 1305, 1989). Performance of CAR was cumulative during the training period. Pindolol treated mice showed significant CAR of 49-50% compared to control (10-15%). Atenolol treated mice scored 10-56% NMDA or 1-OH-THA treated mice scored 37-73% and 26-65% respectively. A combination of NMDA and 1-OH-THA produced potentiation (54-73%) lasting up to 3 months at advanced learning doses of (150-200 mg/kg) of both of these caused seizures which could be prevented by giving dizepam at 100 mg/kg i.p. These compounds, NMDA and 1-OH-THA, may alter brain GABA levels. However, the hippocampus area showed a slight damage in pyramidal cells in CA3 region. The enhanced learning and memory function of atenolol and pindolol treated mice may be due to a combination of inhibited adrenergic and enhanced cholinergic activities.


The neurotoxic potential of two industrially used alkyltin, vinyl tin adduct (VTA) and tributyl tin hydride (TBT), was evaluated in rats. Sprague-Dawley rats (15/sex/group) received daily oral dosages (5 or 20 mg/kg) of VTA or TBT for 2 weeks (wk). A third group received 1 mg/kg of trimethyl tin (TMT). Rats were evaluated, using a functional observational battery, prior to and during wk 1 and 2 of dosing; 5 rats/sex/group were tested during a 2 wk reversal (rev.) period. Morphologically examination was made in selected animals at the end of the treatment (tx) and rev. periods. For the VTA animals, rotarod performance was impaired during wk 2 of dosing. For the TBT animals, reduced locomotor activity (LMA) was seen during tx (20 mg/kg); reduced hindlimb extensor response was also noted during tx and rev. in a few males (5, 20 mg/kg). For TMT rats, increased LMA, rearing, and reactivity to stimuli were observed during the dosing and rev. periods. Histopathologically, lesions were seen only in the TMT group and consisted of necrosis of the neurons of the hippocampus and ventrolateral or pyriform cortex of the cerebral hemispheres. In conclusion, VTA was not neurotoxic in rats. For TBT, work is being undertaken to further characterize the reduced extensor response.

LEARNING DEFICITS IN RATS AFTER ORAL EXPOSURE TO STYRENE MONOMER. P.J. Bushnell. Neurotoxicology Division, US Environmental Protection Agency, Research Triangle Park, NC.

Occupational exposure to styrene monomer has been associated with deficits in human cognitive function. In two experiments, male Long-Evans rats were dosed orally with either corn oil vehicle or with corn oil containing 50% (v/v) styrene monomer (0.5 g/kg/day). In Exp 1, dosing occurred 5 d/wk for 8 weeks for control (n=6) and styrene (n=8) groups (total dose = 20 g/kg). In Exp II, 40 rats were dosed 5 d/wk for 8 weeks: controls (n=8) received corn oil for 8 weeks, while treated groups (n=8/gp) received styrene at 0.5 g/kg/day for 1, 3, 5 or 8 weeks, for total respective doses of 2.5, 7.5, 12.5 and 20 g/kg. Beginning 10 weeks after the last dose, all rats in Exp I were trained to perform a series of spatial discrimination reversals under an automatic schedule, in which the relative rates of response to two retractable response manipulanda were used to quantify reversal learning. In Exp II, 4 rats in each group were similarly trained, beginning 32 weeks after the last dose. Treated rats were impaired in acquisition of a reversal learning set (improvement in learning across successive reversals) in both experiments, despite higher response rates than controls. Reversal learning deficits after acquisition of the learning set were not related to the duration of dosing. In 2 or 3 animals in each group in Exp II showed abnormal response patterns and retarded acquisition of daily reversals. These results show that past exposure to styrene monomer can impair rats' learning ability later in life.
BEHAVIORAL TEST BATTERY REVEALS EFFECTS OF HEXACHLOROBENZENE IN MATERNALLY EXPOSED RAT PUPS. E S Golding and D H Taylor, Department of Zoology, Miami University, Oxford, OH; Sponsor: EW Fisher

Hexachlorobenzene (HCB) is a widespread environmental contaminant. Due to its highly lipophilic nature, HCB is stored in the body adipose tissue and is released with the milk during lactation. Female Sprague-Dawley rats were given 0 (control), 10, or 100 mg HCB/kg body weight, and dosing was completed two weeks prior to breeding. We evaluated the gestational and lactational transfer of HCB from the dams to fetuses and pups and determined that HCB is present in the developing rat brain. Subsequently, we assessed the developmental neurotoxicity of HCB using a battery of behavioral tests. The negative geotaxis response and olfactory homing were assessed in two male and two female pups from each litter between 6 and 11 days of age. The development of exploration and locomotion was assessed in whole litters 14-21 days of age. Acoustic startle response was assessed in both young and mature offspring. Learning (swim T-maze), exploratory and locomotor activity were assessed in mature offspring. The significant effects observed in the negative geotaxis and olfactory homing tests suggest hyperactivity in exposed young pups. Testing of mature offspring revealed significant effects only in the acoustic startle procedure indicating that HCB causes a reduced motor response. This study reveals the importance of testing more than one animal per litter, of using litter as the smallest unit of testing, and of following individuals throughout development. Our results demonstrate that HCB is a behavioral teratogen, and that the pre-bredding dosing protocol used here may find wide applicability in teratological assessments of similar halogenated compounds. This work suggests that human fetuses and suckling infants may be at risk from HCB.

PROLONGED EFFECTS IN RATS OF SHORT-TERM EXPOSURE TO 3',3'-IMINODIPROPIONITRILE (IDPN) USING A NEUROBEHAVIORAL SCREENING BATTERY. V C Moser, NSI Technology Services, Research Triangle Park, NC

A neurobehavioral screening battery (functional observational battery followed by motor activity assessment) was used to evaluate the effects of 3 days' exposure to either 0, 100, 200, or 400 mg/kg/day IDPN. Adult Long-Evans rats were tested before dosing, and 1, 14, 28, 56, and 91 days after the last dose. IDPN initially produced generalized CNS depression, weakness, and hypothermia. Thereafter, marked hyperactivity, increased excitability, neuromuscular weakness, equilibrium changes, and the "waltzing syndrome" (vertical and lateral head movements, circling, and retropulsion) emerged and persisted for 3 months. Males were more severely affected than females. Prolonged decreases in reactivity to the visual and auditory stimuli were obtained that may correspond to sensory deficits reported by others (Boyes and Moser, 87; Crofton et al., Neurosci. Abs. 15:688, 1989). Thus profound motor effects including hyperactivity, and sensory changes, could be detected using this screening battery following only 3 days' exposure to IDPN. These changes last at least 3 months and may be permanent.

VISUAL FUNCTION TESTING OF RATS EXPOSED TO 3',3'-IMINODIPROPIONITRILE (IDPN). W K Boyes and V C Moser, Neurotoxicology Division, USEPA and NSI Technology Services, Research Triangle Park, NC.

Strategies for neurotoxicity testing often include initial screening, such as a functional observational battery (FOB) and motor activity (MA) assessment, followed by detailed characterization studies. Exposure to 0, 100, 200, or 400 mg/kg/day IDPN for 3 consecutive days produced dose-related FOB and MA changes lasting throughout 91 days of testing, some of which were suggestive of visual dysfunction (Moser, 91). Following neurobehavioral testing, the rats were examined for visual function using flash- (3 intensities) and pattern- (3 pattern sizes by 3 contrast levels) elicited visual evoked potentials (VEPs). IDPN produced statistically significant changes in VEPs, particularly in the latency and amplitude of early-latency components of flash VEPs, thus verifying predictions made from the screening tests. However, the extent of the VEP changes produced by IDPN was not great. This suggests that the pronounced deficits in the sensorimotor responses of the FOB, which are dependent on sensory, integrative, and motor functions, were due to changes in addition to altered vision.


Hydroquinone (HQ) was given PO in water (gavage) to male and female S-D rats (10/sex/grp) at dose levels of 0, 20, 64, or 200 mg/kg/d, 5d/week for 13 weeks to study neurotoxicological and neurobehavioural potential of HQ. These studies, sponsored by the CMA HQ Panel, included both a FOB and neuropathology (NP) as required by a TSCA Sec. 4 Test Rule. The FOB was conducted at 1, 6, and 24 hrs and 7, 14, 30, 60, and 91 days. For NP the nervous system was fixed by intravascular perfusion with aldehydes and CNS and PNS tissues were examined following specific staining (CNS and PNS) or plastic embendtment (PNS). Doses of 64 or 200 mg/kg HQ resulted in increased incidences of tremors and reduced activity within 1 hr of dosing. Reduced activity was observed both within and outside of the home cage primarily at the 1 and 6 hr FOBs. Brain and kidney weights were not altered by HQ exposure, but bodyweight growth was reduced ~7% for the 200 mg/kg males. No neuropathological abnormalities were found. This study confirms the work of Christian et al. (1980) who found that 15 weeks of HQ exposure did not result in subchronic neurotoxic effects (motor activity).
Mice of two different strains, B6C3F1 and Swiss-Webster, were exposed to the benzodiazepine compound, oxazepam (625, 1250, 2500, 5000, and 10000 ppm) in feed for thirteen weeks. Neurobehavioral evaluations were performed during Treatment Weeks 2 and 12 to characterize the functional effects on the nervous system. Motor activity increases, perhaps reflecting the anxiolytic properties of the drug in humans, were seen with both strains and at both time points, although the effects were marginal at 12 weeks in the male Swiss-Webster mice. When observed, the motor activity increases were similar in magnitude at all dose levels. At 2 weeks, hindlimb and forelimb grip strength decreases (depressant effects) were seen at the highest two dose levels in both strains, and also at lower doses in the B6C3F1 mice. At 12 weeks, grip strength was normal in the B6C3F1 mice but still depressed in the high dose Swiss-Webster mice. In general, grip strength scores showed a clearer relationship to dose level. The grip strength deficits were not attributable to body weight decreases since the mean body weights of the control groups were similar to or less than those of the treated groups at both testing periods. (Supported by NIEHS Contract No. N01-ES-85213).

Trimethyltin (TMT) produces hearing loss in guinea pigs and rats, but similar functional data have not been reported for triethyltin (TET). Pigmented guinea pigs (n=5) were anaesthetized with urethane and the compound action potential (CAP) and the cochlear microphonic (CM) measured from the round window. CAP thresholds and a 1 µvolt CM isopotential curve were measured from 2-40KHz prior to and, again, 30 and 60 min following an i.p injection with TMT Cl (2mg/kg), TET bromide (12 or 24mg/kg) or vehicle. Loss of CAP sensitivity was observed both for the TMT and high dose of TET. No shift was observed in CM. CAP loss was not restricted only to high frequencies. The results suggest that these organotins rapidly and selectively impair function in the inner hair cell- the sensory receptors for sound- or the type 1 spiral ganglion cells. No evidence for outer hair cell dysfunction was observed. The data show that TET and TMT produce rapid impairments in cochlear function. (Supported by ES02852, ES03619, and ES07141).

Adult female, Fischer-344 rats were exposed to 275 mg/kg of tris(2-chloroethyl)phosphate (TRCP) by gavage. TRCP produced consistent signs of convulsive activity within 60-90 minutes after dosing and extensive loss of CA1 hippocampal pyramidal cells when examined 7 days after dosing. At the light microscopic level, toxic effects of TRCP on pyramidal cells in the CA3, and CA4 and granule cells in the dentate gyrus were less severe than on the CA1 cells. The seizures-related and neurohistological effects of TRCP were significantly attenuated by pretreatment with atropine or chloridezepoxide, suggesting that the hippocampal damage was related to the seizures produced by TRCP. In a second experiment designed to assess the potential health risk associated with TRCP, exposed rats were mildly impaired in the acquisition of a reference memory task in a water maze. However, TRCP-exposed rats were consistently impaired in performing a repeated acquisition task in the water maze. These data underscore the potential health risk associated with exposure to TRCP and support the conclusion that the hippocampus is intimately involved in spatial memory in rats.

We dosed 7 or 17 month old male rats with TMT (0.35, or 4.5 mg/kg i.p.). Despite similar blood TMT levels and increases of hippocampal glutamate in both age groups, only the older TMT-dosed rats exhibited passive avoidance deficits accompanied by neuronal necrosis and Alzheimer's type II gliosis. Glutamate-displaceable kainic acid (KA) binding sites were increased in TMT-treated 7-month old rats, but decreased in the TMT-lesioned, functionally impaired 17-month old rats. KA receptor upregulation may explain sensitization of aging neurons to exogenous toxic necrosis, since numbers of binding sites in untreated controls were higher in 17 than 7-month old rats. Neurotoxicological biomarkers of TMT exposure in 17-month old rats are similar to spontaneous biomarkers of more extreme old age. Therefore, a similar mechanism, such as exposure of increasingly sensitive neurons to endogenous and/or exogenous "excitotoxic" substances, may underly spontaneous age-related neurodegeneration.
CARbamates inhibit cholinesterases (ChE) in the central and peripheral nervous system; both sites may be important in determining the impact of acute exposures on neurobehavioral function. Separating the relative involvement of these two sites is important in understanding the mechanism of action of carbamates and other ChE inhibitors. These studies determined the effects and interactions of the following agents on motor activity (MA) in adult male LE rats: physostigmine (0.03-0.56 mg/kg), a centrally and peripherally acting ChE inhibitor; neostigmine (0.03-0.56 mg/kg), a peripherally acting inhibitor; scopalamine (0.1-3.0 mg/kg), a centrally and peripherally acting muscarinic antagonist; and methylscopolamine (0.1-3.0 mg/kg), a peripherally acting muscarinic antagonist. When given alone, physostigmine and neostigmine decreased MA in a dose-dependent manner. Scopolamine increased MA, whereas methylscopolamine had no effect. Pretreatment with scopalamine, but not with methylscopolamine, blocked the MA decrease produced by physostigmine. Together with the data on neostigmine effects with scopalamine or methylscopolamine pretreatment, these results provide a behavioral basis to identify central and peripheral actions of ChE inhibitors.


Previous studies have shown that chlordecone (CD), greatly amplifies CCl₄ hepatotoxicity and lethality. The present studies were designed to further evaluate whether hepatic failure is the cause of lethality during CD+CCl₄ toxicity. Hepatic failure was evaluated by biochemical, functional and histopathological parameters. Male S-D rats were maintained on control or CD diet (10 ppm for 15 days) and injected with CCl₄ (100 μl/kg, ip) on day 16th. Rats were killed at 0, 6, 12, 24, 36 and 48 h after CCl₄ administration. Plasma ammonia, bilirubin, AST, ALT, SDH, hepatic ATP and glycogen were measured. CCl₄ injection to CD pretreated rats resulted in 100% lethality within 72 h. A progressive hypoglycemia was observed with a 60% reduction in plasma glucose at the time of death. Hepatic glycogen content dropped precipitously. Similarly, hepatic ATP levels were suppressed to 80% of control. Plasma ammonia levels were elevated, and by 48 h, a 3-fold increase was observed. Plasma ALT, AST, SDH and bilirubin increased progressively until the death of CD+CCl₄ rats. CCl₄ injection to control rats showed only marginal changes in plasma enzymes without any alteration in bilirubin. Histopathologically, only liver showed progressive necrosis, vacuolation and fat accumulation, whereas, kidney did not show necrosis. These findings support the concept that hepatic failure is the cause of animal death in the interactive toxicity of CD+CCl₄. (Supported by AFOSR-88-0009).

Effect of phenobarbital (PB) and Mirex (M) on CCl₄ autoprotection. K N Thakore and H M Mehendale. Dept. Pharmacol. & Toxicol., Univ. Mississippi Med. Center, Jackson, MS.

The effect of PB and M pretreatment, which delays the early phase of hepatocellular regeneration caused by a protective dose (30 ml CCl₄/kg po), on the CCl₄ autoprotection was investigated. Rats maintained on normal (N), PB (225 ppm) or M (10 ppm) diet for 15 days received either corn oil (control) or protective dose on day 16. At 24, 48, 72 or 96 h after the protective dose, high dose (5 ml CCl₄/kg po) was given for 14 day lethality study. Liver microsomal cyt P-450 and associated enzymes, serum enzymes (AST, ALT and SDH) and histopathological changes were determined after the protective dose. Autoprotection was 100% in N rats, whereas only 45% in PB or M pretreated rats when the high dose was given 24 h after the protective dose. For 48, 72 and 96 h time points, autoprotection was only 50% in N rats, whereas it was 100% in PB and M pretreated rats. Liver microsomal enzymes were induced in rats after PB or M pretreatment but decreased at 24 h after the protective dose in all the groups and recovered to normal by 120 h. Liver injury seen in all the groups at 24 h after the protective dose disappeared by 120 h. The decrease in microsomal enzymes caused by the protective dose in N, PB and M groups did not correlate with lethality data. These results suggest that a delayed early phase CCl₄ stimulation of hepatocellular regeneration by PB and M pretreatment appears to accordingly delay optimal autoprotection. (Supported by AFOSR-88-0009).
Treatment of rats with the hepatotoxicant lipopolysaccharide (LPS) is associated with the accumulation of macrophages (MP) and endothelial cells (EC) in the liver. These NPC display enhanced functional capacity and release toxic O2 intermediates. Nitric oxide (NO) is another highly reactive molecule that has been implicated in tissue injury. In the present studies we characterized production of NO by hepatic MP and EC. Cells were isolated from livers of female SD rats by in situ perfusion followed by elutriation. We found that both MP and EC produced NO in response LPS or gamma interferon (G-IFN). Although tumor necrosis factor (TNF) by itself had no effect on NO production, the combination of TNF and G-IFN was synergistic. Hepatic MP were found to produce significantly more NO than EC. Treatment of rats with LPS resulted in increased production of NO by both EC and MP. MP were more sensitive to the effects of LPS than were EC. These data show that hepatotoxicant exposure is associated with increased NO production by NPC and suggest that this mediator may play a role in hepatotoxicity. (NIH GM34310)

Ethanol induction of adult hen liver microsomal cytochrome P-450 isozymes. M B Abou-Dania and R A Gupta, Duke Univ Med Ctr, Dept. of Pharm, Durham, NC.

Cytochrome P-450 in adult hen livers was induced by administering 15% ethanol in drinking water. The metabolism of selected substrates was studied in normal, ethanol, (phenobarbital) PB-, and (8-methylthiazole) BNF-induced hepatic microsomes. Aniline was the only substrate whose metabolism was enhanced on ethanol treatment when estimated on the basis of per nmol of cytochrome P-450. The Km value for aniline in ethanol-treated hens was close to normal and that for p-nitrophenol to BNF-induced microsomes. The Kt values of metapyrone for aniline and p-nitrophenol hydroxylases in ethanol-induced microsomes were also different from those in other normal or induced-microsomes. Among the inhibitors used, BNF differentiated aniline hydroxylase activity and p-hydroxyphenyl imidazole distinguished P-nitrophenol hydroxylase activity between ethanol- and BNF-induced P-450s. Immunoblot analysis showed that P-450 isoforms PB-A, BNF-B, and BNF-C were induced by ethanoel treatment. Studies suggested that along with the ethanol specific P-450 isoforms which were largely responsible for aniline and p-nitrophenol hydroxylation, ethanol also induced other P-450 isoforms that were induced by other known P-450 inducers.

Methylene dianiline, 4,4'-diaminodiphenylmethane, DAPM, used to produce polyurethane foams, spandex fibers and other polymers, is hepatotoxic and cholestatic in humans and predominantly injures bile duct cells in rats. Our objective was to determine the effect of DAPM on secretion of biliary constituents and biliary gamma-glutamyl transpeptidase (GGT) content and to study DAPM metabolism. Male SD rats (~350 g) were given 250 mg/kg DAPM po in 35% alcohol or 35% alcohol to controls. Four hr later, rats had biliary cannulas positioned under pentobarbital anesthesia and bile was collected for 2.5 hr. Serum, liver, spleen and thymus were evaluated for toxicity. Major observations were: By 4 hr, biliary protein was increased 5 fold while bile salt was decreased by half and glutathione was decreased ~10 fold. Of particular interest was a striking effect on biliary glucose which is normally ~9% of plasma value because of its reabsorption from the biliary tract. DAPM caused a 20 fold increase in biliary glucose by 4 hr which increased to 40 fold by 6 hr. Histochemical staining of bile ductal GGT was decreased and more diffuse in DAPM livers than controls. Serum ALT and alkaline phosphase showed modest increases while glucose was elevated 3 fold. In other animals examined 24 hr after DAPM, no bile could be collected. Reversed-phase HPLC analysis of liver homogenates and urine showed parent DAPM and at least 4 metabolites. Histology showed severe injury to bile duct cells and the cortex of the thymus. These results indicate that bile duct epithelial cells may play a major role in biliary glucose homeostasis.
609 A Murine Model for Halothane Hepatitis. K.L. Hastings, C. Thomas, A.P. Brown, and A.J. Gandolfi. Department of Anesthesiology, College of Medicine, University of Arizona, Tucson, AZ 85724.

Halothane hepatitis (HH) appears to result from an inappropriate immune response to liver neoantigens formed as a result of halothane metabolism. Although both rabbits and guinea pigs, when exposed to halothane, produce liver neoantigens and antibodies reactive with these neoantigens, neither animal exhibits symptoms consistent with halothane hepatitis. Male C3H/BL/6 mice exposed to 1% halothane in 40% O2 for 1 hr were demonstrated to produce significant quantities of bromide and trifluoroacetic acid, indicative of halothane biotransformation. Mice immunized with trifluoroacetylated albumin produce anti-halothane metabolite antibodies, but these antibodies have yet to be demonstrated in animals exposed to halothane. Immunohistochemical analysis of liver tissue from these mice demonstrated halothane associated neoantigens present on hepatocyte cell surfaces, apparently a necessary prerequisite for immune recognition. Liver microsomal protein from these mice were shown to contain numerous neoantigens with apparent molecular weights ranging from 115 kD to 43 kD. These neoantigens may provide appropriate immunogens for the experimental production of HH-like immunopathology. (NIH GM 34788)

610 THE PROTECTIVE EFFECTS OF OLEANCIC ACID AND a-HEDERIN ON CHEMICAL-INDUCED ACUTE NECROTIC LIVER INJURY IN MICE. Y P Liu, J Liu and C D Kjæsen. Univ Kansas Med Ctr, Kansas City, KS

We have previously shown that among ten cleaner-type triterpenoid compounds, oleanolic acid (OA) and a- hederin (a-H) were the most effective in protecting against acetoaminophen, carbon tetrachloride and cadmium induced acute liver injury. This study was designed to further examine the hepatoprotective effects of OA (200 µmol/kg, ip) vs a-H (50 µmol/kg, sc) on 10 other mechanistically different types of hepatotoxicants in mice. Acute necrotic liver injury was produced by bromobenzene (BB; 0.7 ml/kg, ip), chloroform (CHCl3; 1.0 ml/kg, ip), phenobarbital (PbH; 1.5 mg/kg, ip), ammonia (a-AM; 0.5 mg/kg, ip), thioacetamide (TA; 120 mg/kg, ip), dimethylnitrosamine (DMN; 45 mg/kg, ip), furosemide (FUR; 450 mg/kg, ip), colchicine (COL; 200 mg/kg, ip), allyl alcohol (ALOH; 90 mg/kg, ip) and D-galactosamine plus lipopolysaccharide (GALLPS; 700 mg/kg; 100 mg/kg, ip). Liver damage was assessed by quantitating serum activities of alanine aminotransferase and sorbitol dehydrogenase. OA and a-H dramatically decreased the toxicity produced by BB, PbH and GALLPS and significantly ameliorated the hepatotoxicity of TA and COL. However, these compounds had no effect on the toxicity of CHCl3, DMN, ALOH and a-AM. Whereas a-H protected against FUR-induced hepatotoxicity, it aggravated the hepatotoxicity of a-AM. These results demonstrate that OA and a-H protect against many, but not all hepatotoxicants, suggesting that their hepatoprotective effects may involve multiple mechanisms. (Supported by NIH Grant ES-01142).


[3H]Cocaine bound with high affinity to a microsomal protein in mammalian livers at 130-140 pmol/mg protein in rats and 0.8 to 8.9 pmol/mg protein in several other species. Its affinity for cocaine also varies greatly from Kg of 3-7 nM in rats, 100-200 nM in mice, 719 nM in human and 1496 nM in guinea pigs. This protein down regulates rapidly in rats upon acute and chronic administration of cocaine. Recovery rate following withdrawal of the drug is slow (30% in 4 days). Physostigmine at 0.1 µM does not have significant effect on cocaine toxicity or binding of [3H]cocaine to rat hepatocytes, which are very tolerant to cocaine. Appreciable cell death and depletion of glutathione are observed only after exposure to cocaine concentrations above 1 µM. Micromoles from hepatocytes have 10 fold lower affinity for cocaine than those from intact liver. (Supported by NIDA grants DA03680 (M.E.) and DA05827 (J.J.)).

612 ALTERING LIVER METABOLISM SHIFTS THE INTRALOBULAR SITE OF COCAINE-INDUCED INJURY. S M Roberts, L Roth, B C James and R D Harbison. Center for Environmental & Human Toxicology and College of Veterinary Medicine, University of Florida, Gainesville, FL.

Cocaine hepatotoxicity has been reported in humans and is well documented in mice. The hepatic cellular damage is mediated by toxic metabolites produced by the action of monoxygenases including cytochrome P-450. Previous studies have suggested that the zonal lesion produced by cocaine may be altered by pretreatment with hepatic enzyme inducing drugs, though this has not been well characterized. In this study, ICR male mice were pretreated with a number of hepatic enzyme inducers or inhibitors (viz. phenobarbital, β-naphthoflavone, β-ionone, diazinon, and pyrazole) prior to the administration of a single dose of cocaine (60 mg/kg, i.p.). The intralobular distribution of lesions at 24 hrs varied among pretreatment groups. Mice receiving cocaine with no pretreatment had midzonal to centrilobular lesions, while mice pretreated with phenobarbital had midzonal to peripheral lobular lesions. Centrilobular necrosis was observed in the liver of mice pretreated with β-naphthoflavone, pyrazole, or diazinon. Midzonal necrosis was observed in mice pretreated with β-ionone.
M-741 is a bis enamiro-immonium salt with neuromuscular blocking properties. In rats, at doses less than those required to produce skeletal muscle paralysis, M-741 exhibits potent hepatotoxicity following acute administration. Serum transaminase elevations were observed 24 hr following parenteral (IV or IP) administration of 1-4 mg/kg. At 2.0 mg/kg or higher, elevations were frequently 10-fold greater than the upper limit of normal. Histopathology demonstrated acute focal necrosis, primarily centrolobular to midzonal, in animals receiving a single IV dose of 2 mg/kg or greater. In time course studies, serum transaminase increases were observed within 3 hours of dosing. Since this data and previous in vitro hepatic slice data [Toxicologist 10(1): 265, 1990] implicated the liver as a target organ for toxicity, the effects of metabolism on toxicity were examined. Neither phenobarbital induction nor SKF 525A-induced inhibition of cytochromes P-450 altered the severity of acute toxicity. To investigate the role of glutathione, animals were pretreated with the glutathione synthesis inhibitor BSO; M-741 toxicity was not observed to be potentiated. Additional studies to help determine the mechanism of M-741 hepatotoxicity are currently in progress.

Inhibition of 1,2-dichlorobenzene hepatotoxicity by superoxide dismutase in male Fischer-344 rats. L Unnawadhan, S A Mobley and J G Sipes. Dept. of Pharmacol. & Tox., Univ. of Arizona, Tucson, AZ.

1,2-Dichlorobenzene (1,2-DCB) is a potent hepatotoxin in male Fischer-344 (F-344) rats. Since reactive oxygen species have been implicated in the toxicity of numerous chemicals, a possible role for oxygen radiations in the development of the hepatotoxicity of 1,2-DCB was considered. To investigate this possibility superoxide dismutase coupled to polyethylene glycol (SOD-PEG) was administered in an attempt to inhibit the hepatotoxicity of 1,2-DCB in F-344 rats. SOD-PEG (10,000 IU/kg, i.v.) was administered 2 hr before or after 1,2-DCB (3.6 mmol/kg, i.p.). SOD conferred considerable protection from 1,2-DCB induced liver injury, as assessed by the reductions in plasma ALT activity and by histopathological evaluation of hematoxylin/eosin stained liver sections. Plasma ALT activities in U/L were: 1481 ± 227 (n=5) for 1,2-DCB alone and 427 ± 89 (n=6) or 474 ± 227 (n=7) for SOD administered 2 hr before or 2 hr after 1,2-DCB, respectively. The results suggest that a pathway responsible for the generation of superoxide anion may be involved in the hepatotoxicity of 1,2-DCB in F-344 rats. (Supported by NIEHS No. 1-ES-85230).

Establishment of hepatotoxic threshold with a reduced use of anesthetics. JW Allis, JE Simmonds, DE House, BL Robinson and E Berman. HERL, US EPA, RTP, NC.

A unique approach for establishing the threshold for hepatotoxicity has been investigated in adult male F344 rats in which eighteen different doses were given, one dose per animal. Four chemicals, allyl alcohol, carbon tetrachloride, chloroform and 1,2-dichlorobenzene (DCB) were used. Endpoints measured were hepatic necrosis, relative liver weight, cytochrome P450 and for DCB only, serum alanine aminotransferase (ALT). The study was designed to establish a toxicity threshold using the hockey-stick statistical model. The power of this linear model was enhanced by maximizing the number of doses, hence one animal per dose. However, a satisfactory fit to the model was not obtained with any variable measured for these four chemicals and an alternate method using analysis of variance has been employed. In most cases, the initial decrease in P450 and/or increase in relative liver weight matched the lowest dose causing necrosis. For DCB, ALT also increased with necrosis. We suggest that a modification of our approach with an appropriate choice of endpoints can be used to obtain a good estimate of the toxicity threshold with a reduced number of experimental animals. (This abstract does not necessarily reflect EPA policy.)

Investigation of potential mechanism(s) of Se (selenium) induced protection against acetaminophen hepatotoxicity in the hamster. Yuan, C, Ary, T. and Schell, R.C. Dept. Pharm. Sci., N. Dak. State Univ., Fargo, ND 58105.

Experiments were conducted to assess the ability of selenium(Se) to prevent hepatotoxicity induced by acetaminophen (AAP) in the hamster. Results showed that the pretreatment with Se (6 μmol/kg, i.p.) for 48 hr prior to the administration of AAP (2.4 mmol/kg) produced a significant protection against the hepatotoxicity of AAP at 3 and 48 hr as evaluated by a decrease in serum enzyme activities (ALT, AST, SDH). Also, Se-pretreatment reduced the i.e. avo cova nt binding of AAP metabolites to hepatic proteins. Mechanistically, the protection by Se against AAP hepatotoxicity was not accompanied by a Se-induced increase in hepatic glutathione content. When hepatic glutathione biosynthesis was inhibited by buthionine sulfoximine (BSO), Se treatment provided partial amelioration of AAP-induced toxicity as assessed by serum enzymes. However, the marked loss of hepatic glutathione induced by simultaneous treatment with BSO and AAP was not prevented by Se. In other experiments, a significantly elevated hepatic UDP-glucuronic acid content and an increased liver microsomal UDP-glucuronol transferase activity were found in the Se-treated groups. These data suggested that the biochemical basis for Se protection against AAP hepatotoxicity was likely not mediated through the glutathione system but may be due to changes in the increased level of liver UDP-glucuronyl transferase and UDP-glucuronic acid system. (Supported by The Burroughs-Wellcome Fund and NIH GM 41920).
HEPATOTOXICITY OF IRON SALTS INVOLVES PARENCHYMAL NON-PARENCHYMAL CELL INTERACTIONS. S P Luu, R G Thurman and E C Kaufman. Lab for Cellular and Biochemical Toxicology, Rutgers Univ., Piscataway, N.J. 08854 and Lab for Hepatobiology and Toxicology, Univ. of North Carolina, Chapel Hill, NC 27599.

To explore mechanisms underlying injury to the periportal region of the liver lobule by iron salts, we examined the actions of FeSO₄ on the isolated perfused liver, hepatocytes and mixtures of hepatocytes and non-parenchymal cells. Perfusion of livers with FeSO₄ (1 mM) in Krebs Ringer bicarbonate buffer under an atmosphere of 95% O₂:5% CO₂ resulted in an immediate rise in perfusion pressure suggesting that FeSO₄ interacted with cells regulating hepatic microcirculation. Incubation of isolated hepatocytes with FeSO₄ (0.1 mM) caused a moderate decrease in ATP from 11.6 ± 0.7 to 8.7 ± 0.5 nmol·mg protein⁻¹; however, FeSO₄ did not alter the ATP content in non-parenchymal cells. When a mixture of non-parenchymal cells (0.2 mg protein per ml) and hepatocytes (1.0 mg protein per ml) were incubated with FeSO₄, the ATP content of hepatocytes decreased markedly to 6.9 ± 0.3 nmol·mg protein⁻¹. Collectively these data indicate that hepatotoxicity of iron salts may be due, at least in part, to release of toxic factor(s) from non-parenchymal cells. Interaction of FeSO₄ with non-parenchymal cells in the isolated liver may modify perfusion of the organ as well as the metabolic integrity of hepatocytes. Supported by NIEHS Grant ES-02759.

VITAMIN A PRETREATMENT POTENTIATES CARBON TETRACHLORIDE-INDUCED HEPATOTOXICITY IN RATS BUT ATTENUATES IT IN MICE. D L Earnest, S B Hooser, S A Mobley, and L G Sipes, Dept. Pharm/Tox and Int Med., University of Arizona, Tucson, AZ.

Vitamin A (VA) and other retinoids have immunomodulating effects and are being evaluated for use in treatment of cancer and immunodeficiency diseases. Previously, we have reported that pretreatment of rats with large doses of vitamin A (retinol) potentiates CCl₄-induced liver injury through activation of Kupffer cells. In the studies reported here, the effects of VA on the hepatotoxicity of CCl₄ in mice and rats was compared. Male, B6C3F1 mice, Balb C mice or Fischer 344 rats were given VA (retinol,250,000 IU/kg) or its vehicle po daily for 7 days. On day 8, they were given a minimally hepatotoxic dose of CCl₄ ip. 24 hrs later, the mice were killed, plasma collected for ALT activity, and liver sections fixed for histology. In rats, VA potentiated the hepatotoxicity of CCl₄ causing an 11-fold increase of plasma ALT. In mice, VA suppressed CCl₄ hepatotoxicity as indicated by a 4.5 and 6-fold decrease in plasma ALT activity in Balb C and B6C3F1 mice, respectively. Histologic examination of liver sections confirmed the ALT results. An explanation for opposite effects in rats and mice is currently unknown, but may involve species specific differences in VA modulation of the immune system.

EFFECT OF ROUTE OF ADMINISTRATION ON THE PHARMACOKINETICS AND ACUTE HEPATOTOXICITY OF CARBON TETRACHLORIDE (CCl₄). UY Sanzgiri, S Muralidhara, CE Dallas, and JY Bruckner. Dept. Pharmacol. & Toxicol., College of Pharmacy, University of Georgia, Athens, GA.

The objectives of this study were: (1) to compare the pharmacokinetics of equivalent inhaled and ingested doses of CCl₄ over the same time-frame; and (2) to contrast the hepatotoxic potency of the equivalent oral and respiratory doses of CCl₄. Unanesthetized male Sprague-Dawley rats (300-400 g) inhaled 1,000 ppm CCl₄ for 2 hr through a 1-way breathing valve. Serial samples of the inhaled and exhaled breath were collected and analyzed for CCl₄ content by gas chromatography, and the minute volume monitored, in order to determine the systemically absorbed dose. The absorbed dose was determined to be 17.8 ± 2.38 mg/kg (p ± SE, n = 5). Equivalent oral dosing was achieved by administering CCl₄ (179 mg/kg): (1) as an oral bolus in a 1% Emulphor® emulsion; and (2) by constant gastric infusion over a 2-hr period (same time-frame as inhalation) to rats with an indwelling caroid arterial cannula and gastric cannula. Serial blood samples were collected at time intervals of 2-60 min for up to 12 hr during and post-dosing to obtain blood concentration versus time profiles. Twenty-four hr post-dosing, blood and liver samples were taken for measurement of serum and microsomal enzymes. Peak blood CCl₄ concentrations following the oral bolus were markedly higher than in the gastric infusion and inhalation groups. In turn, the blood CCl₄ concentrations in animals inhaling CCl₄ were significantly higher than in animals receiving the chemical by gastric infusion. The toxicity indices were significantly higher in the oral bolus group than in the inhalation and gastric infusion groups. Our findings indicate that both the pattern and route of exposure can significantly affect the acute hepatotoxicity of CCl₄. (Supported by U.S. EPA CR-812367 and CR-816258)


Sponsor: F A Johns.

The long-term rat bio-assay with Banbuterol (a prodrug to terbutaline), revealed a tumorigenic response at a dosage of 202 mg/kg/day manifest as an increased incidence of thyroid follicular adenoma in both sexes. To examine the mechanism of this response, the effects of Banbuterol on thyroid function were studied in groups of rats treated for 13 weeks by the dietary route at dosages of 200 or 2000 mg/kg/day. Plasma triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) were measured at intervals. Thyroxine kinetics, thyroidal iodine uptake and hepatic microsomal enzyme studies were performed together with histological assessment of the thyroid, liver and pituitary. Data will be presented to illustrate an increased TSH drive from the pituitary, a more rapid clearance of plasma thyroxine levels mediated by hepatic microsomal enzyme induction, and the associated histological changes.
TIME-COURSE OF α-NAPHTHYLISOTHIOCYANATE (ANIT)-INDUCED CHANGES IN HEPATOBILIARY FUNCTION AND MORPHOLOGY. R S Goldstein, D Alberts and P C Meunier. Smithkline Beecham Pharmaceuticals, Departments of Investigative Toxicology and Experimental Pathology, King of Prussia, PA

Oral administration of ANIT to rats is known to produce damage to the biliary tree. These studies were designed to evaluate the temporal relationship between ANIT-induced changes in hepatobiliary function and morphology. Male Sprague-Dawley rats were administered a single dosage of vehicle or ANIT (150 mg/kg) by gavage. Hepatobiliary function and morphology was evaluated 16, 24, 48, 72 and 168 hrs following treatment. Bile flow, bile acid excretory rate and 14C-erythritol clearance were monitored in anesthetized rats; body temperature was maintained at 37°C. Hepatobiliary function dramatically decreased 24-72 hrs following ANIT; bile flow decreased by approximately 75% and bile acid excretory rate, by 50-60%. ANIT reduced erythritol clearance by only 30% at 24 hrs, suggesting that decreased canaliculair flow contributed only in part to ANIT cholestasis. These early functional deficits induced by ANIT were associated with morphologic evidence of biliary epithelial cell necrosis and pericholangitis. By 168 hrs post-treatment, biliary ductules were lined by hypertropic biliary epithelium and multifocal biliary epithelial cell hyperplasia was apparent; these morphologic changes were accompanied by complete restoration of hepatobiliary function. These data indicate that ANIT-induced damage to the biliary tree precedes the onset of biliary epithelial cell hyperplasia and hyperplasia, suggesting that the latter may represent a reparative or compensatory response.

CHOLESTEROL METABOLISM IN RAT LIVER: MULTIPLE PROTEIN CLASSES REGULATED BY MEVINOLIN, CHOLESTYRAMINE, OR CHOLESTEROL IN THE DIET. N L Anderson, J-P Hofmann and N G Anderson. Large Scale Biology Corporation, Rockville, MD; E Heuillet, L Richert and A Corlfield. Department of Toxicology, Rhone-Poulenc Sante, Vizy, France; R David and R Curren. Microbiological Associates Inc., Rockville, MD

We examined the gene expression response of rodent liver to treatments affecting endogenous cholesterol synthesis. Approximately 1,000 proteins were separated and quantitated in liver homogenates using 2-D electrophoresis with computer data reduction. Twenty-one proteins were found to be affected by at least one treatment, and these could be divided into several different co-regulated groups. One group (including protein spots assigned to the cytosolic HMG-CoA synthase) showed the expected increase in abundance with mevinolin (0.075%) or cholestyramine (1%) administered in the diet for 7 days, a synergistic further increase with mevinolin plus cholestyramine, and a dramatic decrease with high cholesterol diet. A second group of proteins showed regulation pattern inverse to the first. A third group, mainly comprised of mitochondrial proteins including pyruvate dehydrogenase, showed an effect of mevinolin alone, but little or no effect with any of the other treatments (including the combination of mevinolin and cholestyramine). Such an anti-synergistic effect of mevinolin and cholestyramine on the expression of some proteins was unexpected. Mevinolin alone may affect both cytosolic and mitochondrial acetyl-CoA metabolism, while cholestyramine and high cholesterol diets appear to affect primarily the cytosolic, cholesterol-synthesizing pathway. The technique used, regulation mapping of a large number of liver enzymes, yields novel information on the effects of cholesterol-lowering therapeutics.

COVALENT ASSOCIATION OF MICROCYSTIN-LR WITH A HEPATIC-CYTOPLASMIC PROTEIN. N A Robinson, C F Matson and J G Pace. Pathophysiology Division, U.S. Army Medical Research Institute of Infectious Diseases, Frederick, MD. Sponsor: PEI.Hohenester, Jr.

In vivo, microcystin-LR (MCYST-LR), a cyclic peptide hepatotoxin, binds to high-molecular weight, liver-cytosolic components. Proteinase digestion of heat-denatured cytosol released 80% of the bound radiolabel consisting of 22k parent toxin and two biotransformation products, with NPLG retention times (rt) of 9.4, 6.7 and 5.6 min, respectively. We studied the protein-toxin association and found that parent toxin and the 6.7-min biotransformation product covalently bound to a monomeric protein of apparent molecular weight 40,000. In vitro, binding to cytosolic fractions and biotransformation were time- and temperature-dependent and appeared to have no cofactor requirements. The binding protein(s) and biotransformation activity were present in liver, brain, kidney, heart, lung, intestines, testes, skeletal muscle and fat. Okadaic acid, a specific protein phosphatase inhibitor, showed a concentration-dependent inhibition of [3H]MCYST-LR binding to hepatic cytosol. Molecular weight and organ distribution of the binding protein(s), inhibition of binding by okadaic acid and MCYST-LR's effect on glycogen phosphorylase indicate that one of the binding proteins may be the catalytic subunit of protein phosphatase type 2A.

FRUCTOSE-1, 6-DIPHOSPHATE PROTECTION AGAINST HEPATIC INJURY CAUSED BY HYPOXIA IN RATS. L Kaphalia, N Samra, E Romagnoli and M T Maslen. Department of Pathology, University of Texas Medical Branch, Galveston, TX.

Fructose-1,6-diphosphate (FDP) has been found to protect against ischemic tissue injury produced in vivo by transient occlusion of renal, coronary and intestinal arteries. FDP, a high energy glycolytic intermediate, could provide an anaerobic energy source. Our objective was to determine if FDP had a protective effect against hepatic injury produced by systemic hypoxia. Male SD rats (~300 g) were pretreated with phenobarbital (1 mg/L drinking water) to produce liver hypertrophy, fasted overnight to deplete hepatic glycogen and glutathione, injected ip with either 350 mg/kg FDP in saline (FDP treated group) or saline (control group), exposed to 5% O2 for 45 min and sacrificed at 24 hr. Animals treated with FDP showed less liver injury as evidenced biochemically by a smaller increase in plasma transaminases (ALT and AST) and histologically and histochemically by less extensive alterations in centrolobular hepatocytes than saline treated controls. Animals treated with FDP also showed lower plasma uric acid values than the saline treated controls. Hypoxia-induced increases in serum uric acid reflect breakdown of tissue ADP to this stable end product. Thus the observed FDP-protective effect against hypoxia-induced liver damage could be related to maintenance of liver ATP. (Supported by a grant from the John Sealy Endowment Fund).
HYPERBILIRUBINEMIA MODEL IN NEONATAL RODENTS USING THE VITAMIN K ANALOGUE SYNKAYVITE. C E. Gilbert, E J Calabrese, E J Stanek, and R E. Levin School of Public Health, University of Massachusetts, Amherst, MA.

Experimental neonatal hyperbilirubinemia in the Sprague-Dawley rat has been developed through the intraperitoneal administration of the vitamin K analogue Synkayvite to neonates 24 hours after birth. Bilirubin levels, hematocrit, hemoglobin, methemoglobin, and reduced glutathione were analyzed at predetermined time intervals over the first fourteen days of life. Synkayvite administration increased bilirubin levels more than twice control neonates (0.661 mg/dl treated, 0.227 mg/dl controls) within one-half hour of injection. This elevation in treated animals initially declined to bilirubin values one and one-half those of controls, remained at this plateau approximately three days then declined to levels equal to controls (0.240 mg/dl treated, 0.233 mg/dl controls) and adult values 14 days following Synkayvite treatment. Synkayvite caused slight neonatal hemolysis reflected by no decrease in red blood cells, no hematocrit decrease and no hemoglobin increase. A neonate rat jaundice model reflecting the human neonate condition would be useful for studies into hyperbilirubinemia's physiological effects and to evaluate potential detrimental neuropsychological development effects.

PURIFICATION, CHARACTERIZATION AND GENE EXPRESSION OF β-NAPHTHOFLANOME (β-NF) INDUCIBLE CYTOCHROME P-450 IN RAT EPIDERMIS. S H. Hafiq1, I U Khan, and D R. Bickers. Dept. Dermatology and Medicine, Case Western Reserve University and Veterans Affairs Med. Ctr., Cleveland, OH.

Epidermal cytochrome P-450s (P-450s) are poorly defined. In this study topical application of β-NF (40 mg/kg) to neonatal rats (4-day-old) resulted in increases in P-450 content (2.5 fold) and monoxygenase (3-13 fold) activities. From these microsomes P-450 was purified by hydrophobic affinity column and HPLC and compared with adult rat liver P-450A1 purified identically. The purified P-450 had specific content of 1.53 nmol/mg protein and showed a major band at 54 kDa on SDS-PAGE which comigrated with hepatic P-450A1. Using Western blotting, the 54 kDa band immunologically cross-reacted with polyclonal and monoclonal (1-7-1) antibody to P-450A1. The purified preparation efficiently catalyzed benzo(a)pyrene hydroxylation in a reconstituted system which was inhibited by α-NF and by antibodies to P-450A1. Peptide fingerprint analysis of the purified epidermal and liver P-450A1 showed identical 1-7-1 reacting epitopes. N-ε-terminal sequence analysis of the purified epidermal P-450A1 showed similarity with P-450A1 sequence. Skin application of β-NF to rats resulted in increased gene expression in epidermis and liver as determined by Northern blotting and polymerase chain reaction using specific oligonucleotides. These results indicate that in rat epidermis P-450 induced by β-NF is enzymatically and immunochemically similar to rat liver P-450A1.

Effects of 13-Week Chloropentafluorobenzene Inhalation Exposure on Fischer 344 Rats and B6C3F1 Mice

E R. Kinkead1, E C. Kimmel1, H G. Wall1, J Grabau2, "M" System Services Corporation, Dayton, OH. 2Toxic Hazards Division, Armstrong Aerospace Medical Research Laboratory, Wright-Patterson AF, OH.

Chloropentafluorobenzene (CPFBB) has been identified as a candidate uptake simulant for persistent organic pollutants. Acute toxicity studies have shown that CPFBB has limited adverse effects on laboratory animals. A 21-day inhalation study of rats and mice to 2.5, 0.8 and 0.25 mg CPFBB/L resulted in reduced weight gain in the high concentration male and female rats and identified the liver as a target organ. This multi-concentration inhalation study was designed to detect a no-effect level associated with repeated exposure to CPFBB. Male and female rats and mice were exposed to 0.25, 0.05 or 0.01 mg CPFBB/L for 13 weeks. No effects on body weight or mortality occurred during the study. Increased ALP was noted in the high level mouse groups only. Liver pathology was limited to the high level mouse groups. A no-effect level of 50 mg CPFBB/m² was identified based on light microscopic, hematologic and in-life data. (Supported by DoC Contract No. F33615-85-C-0532)


The human CYP1A1 (cytochrome P-450) gene encodes an enzyme involved in the activation of procarcinogens such as benzo(a)pyrene to the ultimate reactive intermediate. About 10% and 90% of the human population exhibit high and low CYP1A1 inducibility, respectively. It has been reported that the high inducibility phenotype is at greater risk than the low phenotype for cigarette smoke-induced bronchogenic carcinoma. In one 3-generation family of 15 individuals, we show that the high CYP1A1 inducibility phenotype segregates concordantly with an infrequent polymorphic site located 450 bases downstream from the CYP1A1 gene. Our findings are consistent with a recent study from Japan showing an association between this polymorphism and an increased incidence of squamous cell lung cancer. Our data suggest that the CYP1A1 structural gene, or a region near this gene, might be correlated with the inducibility phenotype.

The enzyme assay for determination of the inducibility phenotype is a laborious procedure requiring about 40 cc of blood and 5 days of mitogen-activated lymphocyte cultures. An RFLP screening test, in combination with linkage analysis studies of each family, might be helpful in determining the CYP1A1 inducibility phenotype. Such tests might be useful in the future for predicting and possibly avoiding individual risk of environmentally-caused malignancy or toxicity caused by cigarette smoke and other combustion products.
639 INDUCTION OF CYPIA1 GENE EXPRESSION IN RAT H4-II-E HEPATOMA CELLS BY BENZO(e)PYRENE. WH Houser and M Vickers, Dept. of Pharm. & Tox., West Virginia University, Morgantown, WV.

In the rat, the expression of the CypIA1 gene is closely associated with aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin O-deethylase (EROD) enzyme activity. The polycyclic aromatic hydrocarbon (PAH)-induced expression of the CypIA1 gene appears to be regulated by several trans-acting factors, including the 8S (Ah receptor) and 4S PAH binding proteins. Our study was undertaken to evaluate the role of the 4S PAH binding protein in the induction of the CypIA1 gene utilizing benz[a]pyrene (B[a]P) and benzo[e]pyrene (B[e]P). B[e]P has been shown to bind to the 4S protein with high affinity and to the 8S with very low affinity. Our results show that both B[a]P and B[e]P induce EROD activity in rat H4-II-E cells in a dose dependent manner. Although B[e]P appears to be less efficacious than B[a]P in inducing CypIA1 gene expression, Northern and slot blot analysis indicates that both B[e]P and B[a]P induce the expression of the CypIA1 mRNA. Since B(e)P has previously been shown to bind only to the 4S PAH binding protein, these results suggest this protein may play a role in the trans-regulation of the CypIA1 gene in rat H4-II-E cells.

640 DEVELOPMENTAL REGULATION OF CYTOCHROME P450 IIC11 ENZYME ACTIVITY CORRELATES WITH mRNA LEVELS IN DES-IMPRINTED RATS. R C Zangari1,2, D R Buhler1,3, D L Springer2 1Toxicology Program, Oregon State University, Corvallis, OR. 2Battelle, Pacific Northwest Lab., Richland, WA.

Testosterone metabolism has been shown to be disrupted in rats neonatally exposed to diethylstilbestrol (DES) and other compounds. P450 IIC11 is known to metabolize testosterone to 2α- and 16α-hydroxylated products. To examine the effects of DES on adult P-450 regulation, male rats were subcutaneously injected with 50 mg/kg of DES on days 1, 3 and 5 after birth. At 24 weeks of age, hepatic microsomal metabolism of testosterone was assayed by HPLC, and P450 IIC11 mRNA levels were measured using northern techniques. Total testosterone metabolism was decreased to 62% of control values. Rates of testosterone 2α-, 16α-, and 6β-hydroxylation and androstenedione formation were 56 to 69% of controls. In contrast, 7α-hydroxylation was increased 58%. P450 IIC11 mRNA was decreased to 63% of controls. Within individual DES animals, mRNA levels were correlated with the 2α- and 16α-testosterone metabolites (r = 0.86 and 0.84, respectively). We conclude that neonatal exposure to DES alters the regulation of the hepatic cytochrome P450 system and, that in the case of IIC11, changes in enzyme activity correlate with changes at the mRNA level. Supported by Northwest College and University Association for Science under grant DE-FG06-89ER-75522 with the Department of Energy.

641 EVIDENCE FOR STRUCTURAL SIMILARITY BETWEEN TROUT P450 LMC5 AND HUMAN P450IIIA4. C.L. Miranda1,2, J-L. Wang1,2, M C Henderson1, X Zhao3, F P Guengerich1 and D R Buhler1,2 1Dept of Agricultural Chemistry, 1Marine Freshwater BioMedicai Center, Oregon State University, Corvallis, OR and 2Dept of Biochemistry and Center in Molecular Toxicology, Vanderbilt University School of Medicine, Nashville, TN.

Polyclonal antibodies (IgG) generated against trout cytochrome P450 LMC5 reacted strongly with P450IIIA1 from dexamethasone-induced rat liver microsomes and with P450IIIA4 from human liver microsomes in Western blots (immunoblots). In contrast, anti-P450 LMC1 IgG did not recognize these proteins in rat and human liver microsomes. Reciprocal immunoblots using anti-rat P450IIIA1 IgG or anti-human P450IIIA3 IgG showed that these antibodies do not recognize trout P450 LMC1 or LMC5. However, anti-human P450IIIA4 IgG was found to cross react strongly with P450 LMC1 and LMC5. Progesterone 6β-hydroxylase activity of trout liver microsomes, a reaction catalyzed by P450 LMC5, was markedly inhibited by anti-P450IIIA4 IgG and by gestodene, a mechanism-based inactivator of P450IIIA4. These results provide evidence for a structural and functional similarity between trout P450 LMC5 and human P450IIIA4. (Supported by NIH Grant Nos. ES-00210 and ES-03850).


The regulation of CYP1A1 expression is of interest due to the ability of cytochrome P450I1A1-dependent monooxygenase to activate several environmental procarcinogens. Previous studies from this laboratory have identified several cis-acting regulatory elements of human CYP1A1 including an upstream negative regulatory element (NRE) that is necessary to suppress basal CYP1A1 promoter activity. In the present study, we examined the position-, promoter-, and enhancer-dependence of the NRE. The element was placed in various positions relative to the SV40 and tk promoters and the SV40 and AhRE enhancers in CAT expression plasmids. HepG2 cells were transfected with the constructs and CAT activity in the cells was assayed. The NRE downregulated heterologous promoter and enhancer activities in a position-independent fashion. The NRE also downregulated the AhRE only in the absence of CYP1A1 induction. Thus, the NRE appears to function as a silencer in these constructs and in a manner similar to the intact CYP1A1 gene. (Supported by PHS grant ES-03832 from NIH)
CHARACTERIZATION OF A NOVEL POLYCYCLIC AROMATIC HYDROCARBON (PAH)-INDUCIBLE P450 FROM CSH1/10T1/2 CELLS. C R Jefcoat, M Christou, U Savas, L H Pottinger, C P Carstens, W E Fahl, and S Otto. Env. Tox. Center, Dept. Pharmacol., and Mc Ardle Lab., Univ. of Wisconsin, Madison, WI.

The major PAH-metabolizing cytochrome P450 in the mouse embryo fibroblast-derived 10T1/2 cell line (P450-EF) has been purified to near homogeneity from benz[a]anthracene (BA)-induced 10T1/2 cells. Surprisingly, induction of P450-EF by BA (20-fold) was routinely about 2-fold higher than that by TCDD, the most potent Ah receptor agonist, and was dependent on transcription and translation (blocked by actinomycin D and cycloheximide). A rabbit antibody to partially purified P450-EF (anti-EF) was much more effective than the corresponding chicken antibody at binding denatured P450-EF protein on Western blots. Conversely, only the chicken antibody was effective at inhibiting 7,12-dimethylbenz[a]anthracene metabolism catalyzed by microsomal P450-EF. Rabbit anti-EF did not recognize P450s of the IA, IB, IIC, IIIA, XI, or XXI families but reacted weakly with P450IIA1 and strongly with a hormonally regulated rat adrenal P450 recently purified in our laboratory. Immunoscreening of a λgt 11 library derived from BA-induced 10T1/2 mRNA (106 plaques), with rabbit anti-EF, yielded one positive clone. The lacZ-directed fusion protein of this clone (135 kD) was recognized by both anti-EF and anti-β-galactosidase. A 0.36 kb Eco RI fragment of recombinant λgt 11 DNA hybridized selectively to two BA-inducible mRNAs (2- and 3 kb) from 10T1/2 total cellular RNA.

USE OF THE POLYMERASE CHAIN REACTION (PCR) TO ASSESS CYTOCHROME P450 mRNA EXPRESSION LEVELS IN MAMMALIAN CELLS AND TISSUES. C J Omiecinski, P Costa, and C R Redlich. Dept. Environ. Health, Univ. of Washington, Seattle, WA.

The cytochrome P450 superfamily of genes code for an array of enzymes catalyzing the mixed function oxidation of a wide variety of environmental chemicals including proximate mutagens, carcinogens, and teratogens. In this study, we utilized discriminating oligonucleotide primers and highly sensitive PCR methodology to assess the adult and fetal expression of five differentially inducible P450 genes in rat and human tissues. For example, constitutive expression of CYP1A1; and 3A1, 2B1, and 282, were evident in day 15 rat fetal liver and increased markedly upon placental treatment with 3-methylcholanthrene or phenobarbital, respectively. Human expression of CYP1A1 and CYP2D6 mRNAs also were detected by PCR analyses, but not with Northern blotting, in embryonic organs as early as day 45 of gestation and in untreated adult lung, pulmonary macrophages, and blood lymphocytes. The authenticity of PCR products were verified by either direct DNA sequencing or hybridization to oligomer probes targeted to internal regions of the PCR product. These data clearly demonstrate the enhanced sensitivity and high selectivity offered by the PCR approach for assessing P450 gene products in a variety of tissues and cells where expression is not readily detectable with other methodologies. Supported by NIH Grants GM-32281, ES-04978 and ES-04696.

PERSISTENCE OF CYTOCHROME P450I A1 INDUCTION IN RAINBOW TROUT DURING CONTINUOUS EXPOSURE TO β-NAPHTHOFLAVONE. M L Haasch, E M Quardokus, L A Sutherland, M Goodrich and J L Lech. Dept. of Pharmacology and Toxicology, Medical College of Wisconsin and Marine and Freshwater Biomedical Research Center, Milwaukee, WI.

In order to determine if hepatic P450 induction is sustained during continuous exposure to an inducer, rainbow trout were exposed, under flow-through conditions, to β-naphthoflavone (β-NF), a known cytochrome P450I A1 inducer, in fish. Trout were exposed to β-NF concentrations of 0.05, 0.10 and 0.50 mg/L for 1, 3, 7, and 14d. At the above β-NF concentration-0- deethylase (EROD) activity was significantly increased but in reverse order to the β-NF concentration. Immunoreactive P450I A1 protein was also inversely related to the β-NF concentration. Hybridizable P450I A1 mRNA was increased in a concentration-dependent manner over time (1<3<7d), except at 1d where mRNA levels at all β-NF concentrations were approximately the same. Trout exposed to 0.0, 0.625, 1.25, 2.5, 5.0, and 10.0 μg/L β-NF had increased EROD activity, 3-, 5-, 16-, 21- and 58-fold for the respective concentrations by 3d. Immunoactive P450I A1 protein increased in a concentration-dependent manner 1.4-, 2.6-, 5.3-, 8.8-, and 14.4-fold, respectively, at 3d. Trout exposed to 0.05 mg/L for 2, 6, 12, 24, 32 and 48 hr had increased 38- to 163-fold EROD activity by 18 and 48 hr, respectively. Immunoactive protein increased up to 46-fold by 48 hr 0.05 mg/L β-NF treatment. These data indicate that P450I A1 mRNA remains elevated at least to 7d constant β-NF exposure and can be a valuable tool for biomonitoring of environmental contamination. Supported by ES 01080, ES 04184.


The bladder carcinogen benzidine (BZ) is metabolized to reactive metabolites by PHS and horseradish peroxidase (HRP) in the presence of a peroxide. However, only PHS activates BZ to mutagenic products. The aim of these studies was to determine the basis for the difference in BZ activation by PHS and HRP with emphasis on a role for bacterial BZ acetylation. In reactions with PHS and HRP the RP-HPLC profile of BZ oxidation products was identical. Thus, it is unlikely that PHS forms a unique stable mutagenic product from BZ. Time course studies of BZ metabolism showed that PHS and HRP rapidly oxidized BZ with 50% and 10% of the initial BZ remaining after one min, respectively. The unmetabolized BZ was rapidly N-acetylated by YG1012 bacteria, a strain possessing high acetyltransferase activity and extreme sensitivity to PHS-dependent BZ mutagenicity. Furthermore, preincubation of BZ with YG1012 resulted in a time-dependent increase in PHS-dependent mutagenicity. The amount of unmetabolized BZ in reactions with HRP is insufficient for YG1012 acetylation and mutagenic activation. Therefore, in mutagenicity studies with PHS, YG1012 converts unmetabolized BZ to N-acetyl-BZ, a promutagen, which can be activated by residual PHS activity resulting in mutagenicity. Identification of the metabolites of N-acetyl-BZ by PHS and HRP is ongoing.
The relative contribution of peroxidase-versus monoxygenase-dependent pathways in bioactivation of xenobiotics can be estimated by utilization of (+)-7,8-dihydrobenzo[a]pyrene (7,8-DHP) as a stereoselective probe in vitro and in vivo. Cytochrome P450-dependent monoxygenation produces predominantly the syn-9,10-epoxide, whereas, peroxidative-dependent co-oxidation yields mostly the anti-epoxide. Rainbow trout embryos (10 mg each, 100 per group) were microinjected with 1 μg of either H2O2, indomethacin or β-naphthoflavone (BNF). Ten days post-injection, the sac-sacs were microinjected with 0.5 μg of [3H]{(+)}-7,8-DHP. After 96 hr, the sac-sacs were killed, the yolk-sac removed and total DNA isolated from the trout. Sac-sac pretreated as embryos with BNF had 3-fold higher levels of [3H]{(+)}-7,8-DHP-derived DNA adducts than the other 3 groups. This increase in bioactivation correlated with a marked induction of P450 IA1, as determined by western blotting. Total DNA isolated from these groups will be analyzed for syn- and anti-9,10-isomer-derived adducts. Supported by NIH ES 04766.

DNA ADDUCTS INDUCED BY CYCLOPENTA-FUSED POLYCYCLIC AROMATIC HYDROCARBONS. I. In Vivo Binding of Cyclopental[cd]pyrene (CP) using the ultrasonisrle 32P-postlabeling assay for DNA adducts. Liver and lung tissues from male Sprague-Dawley rats treated with an ip. dose of 10 mg/kg CP were analyzed for DNA adduct formation. Our data indicate both liver and lung DNA adduct formation for CP with binding in the lung (the typical PAH target tissue) being 2.5 times greater vs. the liver (total binding= 228 attoatom/kg lung DNA vs 105 attoatom/kg liver DNA). CP has previously been shown to be a potent bacterial mutagen, a relatively weak mutagen in in vitro mammalian mutagenicity assays, and a weak carcinogen in mouse skin tumorigenic studies. However, its relatively high capacity (lung DNA binding = almost 1 per 107 nucleotides) to bind to DNA in vivo necessitates further molecular dosimetry studies and delineation of its mutagenic/carcinogenic properties. This is the first report from a series of ongoing in vivo and in vitro investigations into the reactivity of this class of unique, environmentally prevalent PAH.

CHARACTERIZATION OF BINDING OF BENZO[a]PYRENE TRIOIL SULFONATES TO DNA. J L Green and O A Read. Dep. of Pharm. & Tox., Univ. of Kansas Medical Center, Kansas City, KS.

Benzo[a]pyrene (BP) and sulfur dioxide (SO2) are ubiquitous environmental contaminants. SO2 is a cocarcinogen with BP. The reaction of sulfite, the physiological form of SO2, with (+)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene (BP-7,8-diol) and (+)-7,8-dihydrobenzo[a]pyrene (anti-BPDE) is known to produce BPT-9-sulfonate and BPT-10-sulfonate, respectively. Further, these BP-triol sulfonates enhance the mutagenicity of anti-BPDE in Salmonella typhimurium strain TA98 and are known to bind to cellular DNA at levels comparable to that of anti-BPDE. Spectral studies using fluorescence quenching agents indicate that the physical association is not a reversible, intercalative event. 32P-postlabeling analysis of purified calf thymus DNA modified with anti-BPDE, BPT-9-sulfonate and BPT-10-sulfonate support the covalent nature of the binding. Triol sulfonates-modified DNA samples each showed at least three discrete adduct spots that were unrelated to any anti-BPDE-derived adduct. HPLC analysis of acid hydrolysates of DNA modified with anti-BPDE released predominantly trans-anti-BPDE-tetraol, the expected hydrolys products of the BP-N2-quanine adduct. The DNA modified with BPT-9-sulfonate and BPT-10-sulfonate, however, failed to release any detectable tetraols upon hydrolysis. These results indicate that the adducts formed between BP-triol sulfonates and DNA are covalent in nature and predominantly distinct from those associated with anti-BPDE. The formation of covalent DNA adducts by BP-triol sulfonates may account, in part, for the apparent role of SO2 as a cocarcinogen with BP. Supported by NIH grants ES-04092 and ES-07079.


L-dopamine (3,4-dihydroxyphenylalaninehydrochloride) is mutagenic at the 9 location in mouse lymphoma L5178Y cells but is negative in the Salmonella assay. This discrepancy has been studied by comparing the interaction of dopamine with DNA from these cells, digested and filtered with Sephadex G-10, (ethylmethanesulphonate (EMS) and 3-methylcholanthrene (3-MC) as controls), with NIH/3210 rat liver S9 mix, using reverse phase HPLC, (CH3CN/phosphate buffer gradient to 50% CH3CN with formic acid). Abnormal nucleosides were resolved from normal ones (shown by nucleoside standards and 32P-NTP-β-S), and were induced by dopamine (4 hrs exposure) in L5178Y DNA only (9 abnormal peaks >5% of total peak area). EMS and 3-MC induced adducted/alterd nucleosides in mammalian and bacterial DNA (9 abnormal peaks >5% of total peak area). (32P)-labelled dopamine, or a metabolite, coeluted with L5178Y DNA, implying adduction. Abnormal peaks disappeared within 24 hours of exposure, suggesting repair, those for dopamine being reduced >98% by 2 hrs. Attempts are being made to characterise the abnormal nucleosides and to assess their relevance to the divergent mutagenicity observed.

The support of the Caledonian Research Foundation is gratefully acknowledged.

The toxic and carcinogenic properties of the widely used antioxidant butylated hydroxytoluene (BHT) are thought to be mediated through its metabolism. BHTOOH, a hydroperoxide metabolite of BHT, is in contrast to BHT a tumor promoter in mouse skin, a tissue that does not normally generate BHTOOH from BHT. BHTOOH is extensively metabolized by keratinocytes. The primary radical produced from BHTOOH is the BHT phenoxyl radical, which can then dismutate to form BHT quinone methide (BHT-QM), a reactive electrophile. Substitution of the 4-methyl group of BHTOOH with a deuterated methyl (4-CD-BHTOOH) or tert-butyl group (tBu-BHTOOH) produces compounds with a reduced ability or inability, respectively, to form BHT-QM. These analogues are, however, metabolized to a measurable degree by liver microsomes that are capable of metabolizing BHT-QM. These initial findings have been confirmed in an in vivo two stage tumor promotion study. Together, these data indicate that BHT-QM is mediating tumor promotion by BHTOOH and provide the first direct evidence that electrophilic intermediates can elicit this stage of carcinogenesis. Supported by NIH grants CA44530, ES07141, ES05131 and KO4 CA01230 (TWR).

652 EFFECTS OF 2,3,5-(TRIGLUTATHION-S-YL)HYDROQUINONE ON RAT RENAL MITOCHONDRIAL FUNCTION. Barbara A Hill, Serene S Lau, and Terrence J Monks. Div. of Pharmocol/Toxicol., College of Pharmacy, The Univ. of Texas at Austin, Austin, TX, 78712.

2,3,5-(Triglutathion-S-yl)hydroquinone (TgresyHQ) is a potent nephrotoxicant in the rat. There is substantial evidence which implicates mitochondria as a primary cellular target for aliphatic glutathione (GSH) conjugates. To determine if mitochondria could be a possible cellular target for aromatic GSH conjugates, the effects of tresyHQ on rat renal mitochondrial (RMR) function was investigated. At early time points after administration of tresyHQ (20 μM, i.v.) to rats (0.5-2 hrs), a significant elevation of state 4 respiration in RRM respiring on malate/α-ketoglutarate was observed. Thereafter (4-16 hrs) state 4 respiration returned to control values and state 3 respiration became significantly depressed. A total collapse in RRM function occurred at 24 hr. The effects of tresyHQ on RRM function preceded significant elevations in blood urea nitrogen, which occurred after 8 hr. In vitro exposure of RRM to tresyHQ for 5 min caused a striking concentration dependent (50-600 μM) increase in state 4 respiration but was without effect on state 3 respiration. The uncoupling effect of tresyHQ at 600 μM was similar to that seen with 100 μM 2,4-dinitrophenol, a classic uncoupler. Addition of exogenous γ-glutamyl transpeptidase (γ-GT) or inhibition of constituent γ-GT activity with AT-125 had no effect on the ability of tresyHQ to affect mitochondrial function. Moreover, HQ (0.5-2 mM) had no effect on RRM. It is therefore apparent that the GSH moiety is essential for the expression of tresyHQ mitochondrial toxicity since HQ alone had no effect on RRM function. These studies suggest that renal mitochondria may be an early target for tresyHQ induced nephrotoxicity. (ES 04662, GM 39338, PMA FND.)

653 Epoxide and Quinone Based Bromobenzene Adducts to Protein Sulfur Nucleophiles. Don Slaughter and Robert P. Hanzlik, Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas.

Bromobenzene (BB) hepatotoxicity is widely attributed to protein alkylation by chemically reactive metabolites. Both epoxides and quinones have been implicated in this regard. While this laboratory recently used acid hydrolysis to provide the first conclusive evidence that BB epoxides actually do alkylate proteins, acid hydrolysis is not suitable for the analysis of quinone adducts. To search for such adducts to cysteinyl or methionyl residues, we heated hepatic proteins from phenobarbital-induced BB-treated rats with a two-phase mixture of 16N KOH and CH3I ("alkaline permethylation"). HPLC(14C) and GC/MS analyses revealed bromo-thioanisoles in relative amounts comparable to the content of S-(bromophenyl)cysteines found by acid hydrolysis (p>0.05). These compounds accounted for <0.5% of total protein covalent binding. In addition, two dimethoxybromo-thioanisole isomers were observed. However, by far the major adduct (5-6% of total covalent binding) was 2,5-dimethoxythioanisole (i.e. a debrominated adduct). These results clearly show that alkylation of protein sulfur nucleophiles in vivo by BB derived quinones is 10-15 times more extensive than their alkylation by BB epoxides. A comparison of average deuterium retention by the isolated adducts following the administration of BB-14H with 3H14C retention ratios determined earlier for total liver protein covalent binding of dual-labeled [3H14C]-BB indicated that the overall pattern of BB metabolite binding to protein nucleophiles may closely parallel that seen here specifically for protein sulfur nucleophiles. (Supported by NIH Grant GM-21784 and a grant from Merck and Co.)

654 ANALYSIS OF AFLATOXIN-DNA ADDUCTS IN LIVER SPECIMENS. D P H Hsieh, M S Zhao, Q-X Li, L S Hsieh, and B D Hammock, Dept. Environmental Toxicology, University of California, Davis, CA.

Aflatoxin B1-DNA adducts (ADA) in liver specimens have been used as a marker to assess individual exposure to aflatoxin (AF). An ELISA using AFBA1 antisemur was found to be a relatively rapid method for molecular monitoring but to give false positive signals in some cases. Acid hydrolysates of DNA isolated from liver specimens of AF exposed animals and humans were analyzed with HPLC for AF residues to confirm ELISA results. HPLC analysis with UV and fluorescence detection indicated that 8,9-dihydro-8,9-dihydroxyaflatoxin B1 (AFB1-diol) is the compound largely responsible for the response to ELISA. When DNA was hydrolyzed by IN HCl at 100°C for 3 hr, about 10% of AF residues were present as AFB1-diol, suggesting that hydrolysis of ADA to release AFB1-diol is a sensitivity-limiting step in this assay procedure. Some AF-free fractions of HPLC eluate of ADA hydrolysate also gave considerable positive signals to ELISA. Under this experiment condition, the limit of detection by ELISA is 0.5 ng AFB1 equivalents/mg DNA and that by HPLC-fluorescence is 0.1 ng AFB1 equivalents/mg DNA. It is concluded ELISA followed by HPLC analysis of DNA from liver specimens offers a reliable method to assess AF exposure at an individual level, useful in molecular epidemiology. (Supported by NIHES ES04699, EPA CR81470901)
Dichloromethane (DCM): Metabolism to formaldehyde (FA) and formation of DNA-protein cross-links in mice and hamsters. M. Casanova, H.D. Heck and D F Deyo, CIT, Research Triangle Park, NC.

DCM induced liver and lung tumors in the B6C3F1 mouse (M) at 2000 and 4000 ppm but not in the Syrian golden hamster (H). DCM is weakly mutagenic in vitro. Though no DNA adducts have been reported, evidence was obtained for the formation of DNA-protein cross-links (DPC) in liver at 4000 ppm [Casanova et al. (1990), Toxicolology 10, 68]. Since FA is a metabolite of DCM and forms DPC, experiments were undertaken to elucidate the possible role of FA in the toxicity of DCM. Male mice and hamsters were pre-exposed for 2 days (6 hr/day) to 4000 ppm of DCM, then on the 3rd day were exposed to [14C]DCM (6 hr) in a closed, recirculating chamber. Chamber concentrations of [14C]DCM were allowed to decay from ~4700 to ~2400 ppm (TWA = 3124 ppm). Concentrations of DPC and amounts of 14C incorporated into DNA were determined using a published method [Casanova et al. (1989), FAAT 12, 397]. Significant (p < 0.01) species differences were observed in the concentration of DPC (pmoles/mg DNA) in liver [M = 14.7 ± 2.9; H = 1.3 ± 0.3] and lung [M = 3.8 ± 0.5; H = 1.8 ± 0.3] (mean ± SE, n = 5), and in the amount of 14C (pmole equivalents/mg DNA) metabolically incorporated into lung DNA [M = 1064 ± 166; H = 125 ± 19], while the difference in 14C metabolically incorporated into liver DNA [M = 63 ± 14; H = 115 ± 27] was marginally significant (p = 0.045). These data indicate that (1) FA derived from DCM forms DPC, (2) M generate much greater concentrations of DPC in liver than H, and (3) under these exposure conditions, M have much greater rates of cell turnover in lung than H. Concentrations of DPC and cell turnover rates may determine the carcinogenic potential of DCM.


Mouse strains react differently to exposure to benzene and its metabolites. We have previously shown that 3 metabolites of benzene, 1,4-benzoquinone (BQ), catechol (C), and hydroquinone (HQ), are toxic to the hematopoietic precursor CFU-e, in vitro, and that BQ and HQ are differentially toxic to the CFU-e's from C57Bl/6J (C57) and Swiss Webster (SW) mice. To determine whether combinations of metabolites may have additive or synergistic effects, bone marrow from each strain was exposed in vitro to all possible binary combinations of BQ, C, HQ, MA (mucous acid), and P (phenol). The concentrations employed were 40μM for P and 10 μM for the other metabolites. The most toxic combinations of metabolites to SW CFU-e were: BQ-HQ, which reduced SW CFU-e to 38% of control, C+HQ, which reduced SW CFU-e to 44% of control, and BQ+P, which reduced SW CFU-e to 48% of control. The most toxic combinations of benzene metabolites to C57 CFU-e were: HQ+MA, which reduced C57 CFU-e to 50% of control, BQ+HQ, which reduced C57 CFU-e to 53% of control, and C+HQ, which reduced C57 CFU-e to 61% of control. Six combinations caused significantly different responses between the two strains. BQ+HQ, BQ+MA, BQ+P, C+HQ, and C+4, were previously reported (Toxicology 10:58(1990)). Multiple regression was used to study the contributions of the components of binary mixtures of the benzene metabolites (METAB). Data obtained from standard curves of METAB and their mixtures are separable in regression analysis. T-testing of the data resulted in nonsignificant values for the mixture HQ+CQ indicating zero interaction and an additive response. The positive significant t-values obtained for the mixture HQ+BQ, indicate positive interaction or synergism. Since mutually exclusive agents share the same binding sites, and occupation of a site by one agent excludes its occupation by another, they cannot interact in producing the effect; combinations of these agents show zero interaction and are simply additive. This suggests that HQ and BQ are mutually exclusive and share the same binding site. Conversely, HQ and HQ are mutually non-exclusive and this requires the presence of at least two binding sites. These results support the hypothesis that the toxic effects of benzene are produced by several metabolites acting synergistically. This may require that benzene induce any chemical that has toxic metabolites, be viewed as a complex mixture problem. (Supported by ES2931)

Quantitative analysis of the synergistic interaction of benzene metabolites in mice. R I Gay and R Snyder. Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ.

Possible toxic interaction between mucosaldehyde (MDC) and hydroquinone (HQ) or benzoylhydroquinone (BQ) has been previously reported (Toxicology 10:58(1990)). Multiple regression was used to study the contributions of the components of binary mixtures of the benzene metabolites (METAB). Data obtained from standard curves of METAB and their mixtures are separable in regression analysis. T-testing of the data resulted in nonsignificant values for the mixture HQ+CQ indicating zero interaction and an additive response. The positive significant t-values obtained for the mixture HQ+BQ, indicate positive interaction or synergism. Since mutually exclusive agents share the same binding sites, and occupation of a site by one agent excludes its occupation by another, they cannot interact in producing the effect; combinations of these agents show zero interaction and are simply additive. This suggests that HQ and BQ are mutually exclusive and share the same binding site. Conversely, HQ and HQ are mutually non-exclusive and this requires the presence of at least two binding sites. These results support the hypothesis that the toxic effects of benzene are produced by several metabolites acting synergistically. This may require that benzene induce any chemical that has toxic metabolites, be viewed as a complex mixture problem. (Supported by ES2931)

Bioactivation and cytotoxicity of hydroquinone in human promyelocytic leukemia (HL60) cells. KB Meyer, VV Subrahmanyan, P Kalancha, and MT Smith. School of Public Health, University of California, Berkeley, CA.

Bone marrow, the target organ for benzene myelotoxicity and leukemogenicity, contains high levels of myeloperoxidase (MPO). Previous research from our laboratory has shown that MPO can activate hydroquinone (HQ), one of the major phenolic metabolites of benzene, to 1,4-benzoquinone (BQ), a potent alkylating species. Human promyelocytic leukemia (HL60) cells contain appreciable amounts of MPO. These cells have a low background mutation rate and can be readily cultured in vitro. These properties make HL60 cells an excellent in vitro model system to study the genotoxic effects of benzene metabolites. Here we report that HQ is metabolized by HL60 cells to BQ. Dose- and time-dependent cytotoxicity was observed in cells incubated with HQ and hydrogen peroxide. Hydrogen peroxide dose-dependent bioactivation of radiolabeled HQ and the subsequent binding to HL60 protein was also observed. Phenol and catechol, two other phenolic metabolites of benzene, had a stimulatory effect on HQ binding to cellular protein. Studies are currently in progress to determine the degree of genetic damage produced by HQ and hydrogen peroxide using the micronucleus assay in HL60 cells. Supported by NIH grant P42ES04705 and U.C. Toxic Substances Program.
IN VITRO METABOLISM OF trans,trans-MUCONALDEHYDE: IDENTIFICATION OF A NOVEL METABOLITE. D. Rose, D. Good, D. Caligiammi, J. Ruth and D. Pettenkofer. Molecular Toxicology and Environmental Health Sciences Program, School of Pharmacy, University of Colorado, Boulder, CO

Benzene-induced myelotoxicity has been attributed to the putative toxic metabolite trans,trans-muconaldehyde (MA), a highly reactive alpha, beta-unsaturated dialdehyde. Previous results have shown that both aldehyde dehydrogenase (ALDH)-catalyzed, NAD"-dependent oxidation and alcohol dehydrogenase (ADH)-catalyzed, NADH-dependent reduction are responsible for the detoxification of MA in vitro using purified yeast enzymes and subcellular fractions isolated from murine liver. Metabolites of MA were separated by reverse-phase HPLC and identified by co-chromatography with synthetic standards. In preparations containing purified yeast ALDH or ADH with the corresponding co-factor, the primary metabolite formed was the mono-oxidation or mono-reduction product, respectively. Oxidation and reduction products were formed in incubations containing both enzymes but only one co-factor (NAD" or NADH). Similarly, both oxidative and reductive metabolites were formed by mouse liver cytosol fortified with either NAD" or NADH. Unexpectedly, an additional major metabolite was detected in incubates containing both purified enzymes as well as those performed with hepatic cytosol. This metabolite was not formed when MA was incubated in the presence of mouse liver mitochondria or with either ALDH or ADH alone. In addition, formation of the unidentified metabolite was blocked by pyrazole, an ADH inhibitor. The novel metabolite was co-chromatographed with a synthetic hydroxy acid derivative of MA and also formed during ALDH-catalyzed oxidation of a synthetic sample of the hydroxy/aldehyde (mono-reduction) derivative of MA. Moreover, similar mass spectra were obtained following GC/MS analysis of the synthetic hydroxy acid derivative and the biologically-derived metabolite. These data suggest that the novel metabolite is the hydroxy acid derivative of MA, which appears to result from the interaction of oxidative and reductive routes of metabolism. (Supported by ES 04112 and AA 03277)

AGE-RELATED CHANGES IN BENZENE DISPOSITION IN C57BL/6N MICE DESCRIBED BY A PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL. M. A Medinsky, T. F. McMahon, and L S Birnbaum. CIIT, RTP, NC, USEPA, Washington, DC, and USEPA, RTP, NC

Experimental data obtained from disposition studies in 3- and 18-mo old mice given a single oral dose of 10 or 200 mg of 14C-benzene/kg were analyzed with a physiologically based pharmacokinetic model to determine the basis for age-related changes in benzene disposition. No significant age-related alterations were observed in total amount of benzene metabolized or in formation of benzene metabolites at 10 mg benzene/kg. At 200 mg benzene/kg, the total amount of benzene metabolized was less in 18- vs 3-mo old mice, with a concomitant increase in benzene exhaled. Decreases in the amounts of both hydroquinone conjugates and phenyl mercapturic acids excreted were also observed in 18- vs 3-mo old mice. Age-related changes in the amount of benzene metabolized were not due to changes in the kinetic constants for hepatic biotransformation of benzene, but to physiologic changes occurring with age, the most important being decreases in cardiac index (from 55 to 25 L/kg/hr) and fraction of blood flow to liver (from 25 to 12%) in the 18-mo old mice compared to the 3-mo old mice.

STRAIN DIFFERENCES IN THE IN VITRO METABOLISM OF BENZENE BY MOUSE LIVER MICROSONES. J A Bond and M A Medinsky. CIIT, Research Triangle Park, NC.

Benzene is a potent hematotoxin and leukemogen in humans. Studies on the hematotoxicity of benzene in mice identified the DBA/2 strain as being more sensitive than the C57BL/6 using either uptake of iron into erythrocytes or micronuclei formation as an index of toxicity. B6C3F1 mice exhibited a similar micronuclei response to C57BL/6 mice. Only the CBA/Ca strain develops myelogenous leukemia after exposure to benzene. Since the oxidized metabolites of benzene may be responsible for its toxicity, we hypothesized that these strain differences in response to benzene are the result of differences in metabolic capacity among the different strains. Metabolism of 14C-benzene was investigated in liver microsomes obtained from male B6C3F1, C57Bl/6, DBA/2, and CBA/Ca mice. Total capacity for metabolism of benzene was similar in all four strains. The primary metabolite produced was phenol. Capacity for formation of trihydroxybenzene, catechol, and hydroquinone was different among the four strains. Since phenol is not hematotoxic, these data suggest that the relative capacities for formation of polyphenolic metabolites (hydroxylation) and for detoxification (primarily conjugation) are important determinants in benzene toxicity.

TOXICO KINETICS STUDIES WITH HYDROQUINONE (HQ) IN FISCHER 344 RATS. J C Engleb,1 P J Deisinger2 S J Murphy, 2 and A M Blocker2 1Eastman Kodak Co., Rochester, NY; 2The Goodyear Tire & Rubber Co., Akron, OH; 3Ehboé-Pouilenc Inc., Research Triangle Park, NC.

Studies were conducted to determine the absorption, tissue distribution, excretion and metabolism of 14C-HQ in male and female rats following single oral gavage (25 and 350 mg/kg), repeated oral gavage (25 mg/kg/day, 15 days) or 24-hr dermal administration (25 or 150 mg/kg, 2 cm² occluded exposure). Absorption into the blood was rapid after oral administration; the Tmax values for 14C were obtained 10 to 48 min after dosing. In contrast, dermally applied HQ (5% aqueous solution) was poorly absorbed (generally undetected in blood). No obvious sex-related differences in disposition were observed. After oral dosing, 14C was excreted in the urine and feces primarily within the first 8 hr. Typically 87 to 94% of the 14C was excreted in the urine, 1 to 3% in the feces, and <1% was recovered in the tissues and carcasses, with highest concentration found in the liver and kidneys. HQ was extensively metabolized with typically <3% of the dose excreted as parent compound. The major urinary metabolites of HQ were a glucuronide and sulfate which represented 45-53% and 19-33%, respectively, of an oral dose. A <5 metabolite was identified as a mercapturic acid conjugate of HQ. Supported by the Chemical Manuf. Assoc. HQ Panel.

Benzene may be present as a contaminant in raw materials and can be used in certain pharmaceutical production processes. Low levels may be detected in final dosage forms of certain drug products. Based upon its potential to produce cancer, benzene was selected as one of several pharmaceutical impurities warranting a health-based residue limit employing the IRA procedure for setting such limits (Toxicology 10: 225). Benzene is an irritating, volatile, aromatic hydrocarbon known primarily for its effects on the hematopoietic and central nervous systems. While it has been shown to produce chromosomal aberrations and DNA adducts in some test systems, it was negative in in vitro short-term assays for genotoxic potential. In animal bioassays, benzene has produced a variety of tumor types, including leukemias. In epidemiologic studies of benzene workers, exposure to high concentrations was associated with an increased mortality from leukemia. Using both risk assessment and safety factor approaches, our evaluation suggests that in chronically-administered pharmaceuticals, a residue limit for benzene of 0.2 mg/day as a lifetime daily average would provide ample protection of human health from benzene-induced carcinogenic, chronic, and short-term adverse effects.

MECHANISM OF VINYL ACETATE-INDUCED CITOTOXICITY IN RAT NASAL EXPLANTS. W L. Taylor, and M S. Bogdanffy. Haskell Laboratory for Toxicology and Industrial Medicine, E I du Pont de Nemours & Co, Newark, DE.

Chronic inhalation exposure of rats to VA induces benign tumors of respiratory regions (RBS) and malignant tumors of olfactory regions (OLF) of rat nasal cavity. We propose the mechanism of action involves, but may not be limited to, nasal esterase-mediated hydrolysis of VA to the genotoxic agent acetaldehyde and the cytotoxic agent acetic acid. An in vitro system was utilized in this study to determine if the cytotoxic effects of VA are dependent on its metabolic activation by nasal carboxylesterases. Explants from regions of RBS and OLF were incubated in William's medium E containing VA. VA caused a concentration-related increase in explant acid phosphatase release, a biochemical marker of cytotoxicity. Pretreatment of rats with the carboxylesterase inhibitor bis-nitrophenyl phosphate (BNP) reduced VA metabolism approximately 60% and 20% in RBS and OLF, respectively. Similar pretreatment reduced cytotoxicity approximately 60% in RBS but did not protect OLF. In vivo incubation of RBS and OLF with semicarbazide, an aldehyde scavenger, had no effect on cytotoxicity. Acet acid alone was cytotoxic to both RBS and OLF. These results suggest the cytotoxic effects of VA in RBS are due to carboxylesterase-mediated formation of acetic acid but are presently inconclusive for OLF.


Glutaraldehyde is chemochemically similar to formaldehyde, a rodent nasal carcinogen. This study was undertaken to determine possible sites of glutaraldehyde-induced pathology and cell proliferation in the nasal passages of rats for comparison with data from formaldehyde. Male F-344 rats were exposed to glutaraldehyde at concentrations of 0, 62.5, 125, 250, 500 or 1000 ppb, 6 hr/d, for 1 or 40, 6 wk or 3 mo (50/wk). Each animal received a single i.p. injection of tritiated thymidine (6.7 Ci/mmole, 2μCi/gm) approximately 18 hr after the last exposure. Animals were killed 2 hr later and the nasal cavities prepared for histopathology and autoradiography. In animals exposed to 1000 ppb, histopathology revealed treatment-induced effects in specific regions of the anterior nose, including erosions, inflammation, squamous metaplasia and goblet cell hyperplasia. These lesions exhibited a progressively increasing severity through 6 wk, with squamous metaplasia predominant at 3 mo. In specific regions of the anterior nose, 1000 ppb glutaraldehyde-induced multifocal increases in cell proliferation (expressed as unit length labeling index) ranging from marginal (3x control) to severe (20x control), with clearest increases at 8 wk. The severity of all these responses exhibited a clear concentration-response relationship, being distinct at 1000 ppb, minimal to moderate at 500 ppb, and very minimal or absent at 250 ppb and below. While both glutaraldehyde and formaldehyde cause nasal lesions and increased rates of cell proliferation, glutaraldehyde is active in more anterior sites in the nasal cavity and at a lower concentration than formaldehyde. However, there was no evidence of the putative preneoplastic responses reported for formaldehyde following 3 mo exposure to glutaraldehyde.
667 ADAPTATION OF RAT NASAL EPITHELIUM TO OZONE EXPOSURE. J R Harkev., E C Averill, and J A Hotchkiss. Inhalation Toxicology Research Institute, Albuquerque, NM.

Inhalation of ozone, 0.8 ppm for 6 h, initiates DNA synthesis in rat nasal transitional epithelium (NTE) and repeated exposures induce increases in secretory cells and amounts of intraepithelial mucousubstances in NTE. The purpose of our study was to determine if NTE with increased amounts of mucousubstances is less susceptible to ozone-induced DNA synthesis than is normal NTE. Group 1 rats were exposed to 0.8 ppm ozone, 6 h/d, for 7 d, and then to filtered air for 8 d. Group 2 rats were exposed to 0.8 ppm ozone for 7 d, then to filtered air for 7 d, after which they received 1 d, 6 h/d, re-exposure to 0.8 ppm ozone. Group 3 rats were exposed to filtered air for 14 d, than to 0.8 ppm ozone, 6 h/d, for 1 d. Group 4 rats were exposed for 15 d to filtered air, 24 h after exposure, rats were injected with bromodeoxyuridine (BrdU) and killed 2 h later. Using an anti-BrdU antibody, S-phase cells in NTE were identified by immunohistochemistry. Mucousubstances were histochemically identified. Image analysis was used to determine total amounts of mucousubstances within NTE. Group 1 and 2 rats had 20 times more mucousubstance than Group 3 and 4 rats. Group 1, 2 and 4 rats had 1-2 S-phase cells/mm of basal lamina (BL), but Group 3 rats had 14-16 S-phase cells/mm of BL. This indicates that NTE with increased amounts of mucousubstances is resistant to ozone-induced DNA synthesis, adding evidence that secretory metaplasia is a protective adaptation. (Research sponsored by the U.S. DOE/DOER under Contract No. DE-AC04-76EV01013.)

669 NASAL TOXICITY IN B6CF1, MICE INHALING FURFURYL ALCOHOL FOR 2 OR 13 WEEKS. R A Miller, P W Mellick, C L Leach, B J Chou, R D Irwin, and J H Roycroft*. Battelle, Pacific Northwest Laboratories, Richland, WA and *NIEHS/NIH Research Triangle Park, NC.

Furfuryl alcohol is polymerized to form heat-stable resins resistant to acids, alkali, and solvents. The resins are used in metal casting molds and corrosion-resistant plastics, cements, and mortars. The ACGIH established the 8-hour time weighted average TLV at 10 ppm. To characterize the prochonic toxicity, B6CF1 mice (5/sex) were exposed to 0, 16, 31, 63, 125, or 250 ppm of furfuryl alcohol vapor in a 2-week repeated-dose study and mice (10/sex) were exposed to 0, 2, 4, 8, 16, or 32 ppm in a 13-week subchronic study. All mice exposed to 250 ppm died within the first 4 days of exposure. Dyspnce and decreased activity occurred at 63 ppm and above. Mice exposed to 63 or 125 ppm gained less weight than controls. Furfuryl alcohol-induced lesions were limited to necrosis and acute inflammation in the nasal respiratory and olfactory epithelium of mice that died. Mice that survived also had squamous metaplasia of the respiratory epithelium and degeneration of the olfactory epithelium. The severity of nasal lesions was dose-related and lesions were more prominent in the anterior nasal cavity. In the subchronic study there were no observed effects on mortality or body weight. The severity of nasal olfactory and respiratory epithelial lesions was dose-related. Olfactory lesions included degeneration, chronic inflammation and respiratory metaplasia. Cytosplastic hyaline droplets were observed in the respiratory and olfactory epithelium. Squamous metaplasia of the ducts of the submucosal glands was present in the anterior nasal section. A no-observed-effect level was not identified in either study.

668 REGIONAL DIFFERENCES IN THE ENZYMATIC HYDROLYSIS OF ETHYL ACRYLATE IN THE RAT UPPER RESPIRATORY TRACT. C B Frederick, J R Udinsky, and L Finch. Rohm and Haas Co., Spring House, PA.

Chronic inhalation exposure of rats and mice to high concentrations of ethyl acrylate (EA) results in inflammation and hyperplasia in the upper respiratory tract (URT) of rats and mice (R. R. Miller et al., 1983). The histopathological changes were localized primarily in the olfactory epithelium of the dorsal meatus of the URT. Histopathological changes were not evident in organs remote from the URT and a dose-related increase in neoplasia was not observed. In contrast, gavage dosing of ethyl acrylate induced forestomach tumors in rats and mice (NTP, 1986).

To estimate the rate of metabolism of EA for a physiologically-based pharmacokinetic (PBPK) model of the URT, the rate of carboxylesterase-catalyzed hydrolysis of EA has been measured with homogenates of epithelial tissue collected from the following regions of the URT: olfactory epithelium of the dorsal meatus (target tissue), olfactory epithelium covering the dorsal posterior septum (non-target tissue), and respiratory epithelium covering the anterior ventral septum (non-target tissue). Both regions of olfactory epithelium hydrolyzed EA with a maximum rate approximately twice that of the respiratory epithelium. These results suggest that other factors contribute to the local toxicity of EA in the URT.

670 NASAL TOXICITY IN F344 RATS INHALING FURFURYL ALCOHOL FOR 2 OR 13 WEEKS. P W Mellick, C L Leach, B J Chou, R D Irwin, and J H Roycroft*. Battelle, Pacific Northwest Laboratories, Richland, WA and *NIEHS/NIH Research Triangle Park, NC.

Furfuryl alcohol is a high volume production chemical with numerous uses in the foundry, plastic, and textile industries, and may be found in thermally processed foods. The ACGIH has established the 8-hour time weighted average TLV at 10 ppm. The purpose of these studies was to characterize the toxic effects of inhalation exposure to furfuryl alcohol for 2 or 13 weeks. In the 2-week study, groups of F344 rats (5/sex) were exposed to 0, 16, 31, 63, 125, or 250 ppm of furfuryl alcohol vapor; 10 male and female rats were exposed to 0, 2, 4, 8, 16, or 32 ppm in the 13-week study. All rats exposed to 250 ppm died after 1 or 2 days of exposure and had acute inflammatory lesions in the nasal respiratory and olfactory epithelium. Rats that survived the 2-week exposures had suppressive inflammation, necrosis, regeneration, and squamous metaplasia of respiratory epithelium, plus necrosis and degeneration of olfactory epithelium. Nasal lesions occurred in all exposed groups and the severity was concentration-related. All rats survived the 13-week study. Terinal body weights were reduced approximately 10% in females exposed to 32 ppm. There were no differences in absolute organ weights or organ to body weight ratios for thymus, heart, kidney, liver, lung, or testis. Clinical pathology evaluations revealed no chemical-induced effects on the renal, hepatic, or hematopoietic systems. Lesions in this study were limited to the nasal cavity and included squamous metaplasia, goblet cell hyperplasia, and inflammation in respiratory epithelium; epithelial hypertrophy of the nasal nasal, and respiratory metaplasia of olfactory epithelium.
MODELING NASAL UPTAKE OF VAPORS IN THE BEAGLE DOG DURING CYCLIC BREATHING. P Gerde and A R Dahl. Inhalation Toxicology Research Institute, Albuquerque, NM.

A model has been developed to simulate the nasal uptake of vapors in the Beagle dog during cyclic breathing. Input consists of morphological and physiological data for the dog, and physicochemical data for the vapors. The model simulates the nasal airflow as a slit-like duct, where air passes between the two airway walls whose thickness corresponds to the average air-capillary blood distance in the nasal airway. All resistance to mass transfer is assumed to lie on the liquid side - in the diffusion of vapors through the air/blood tissue-barrier, and in transport in the blood. The results agree well with previously derived experimental data. Nasal uptake of vapors during the inhalation part of the breathing cycle increases from a few percent for a compound with a bloodair partition coefficient of 1, to a more than 95% uptake for a compound with a partition coefficient of 2000. Desorption from the nasal tissues on exhalation increases from around 1% to around 30% over the same range of partition coefficients. Net nasal uptake for a complete breathing cycle then ranges from 1% to 70%. The model suggests that the rate of diffusion is a measure of the nasal tissues gives a temporary storage of vapors upon inhalation, followed by a desorption of vapors back to the air stream upon exhalation. Thus, compared to unidirectional flow, cyclic flow should result in higher nasal exposures and lower lung exposures to inhaled vapors. (Research supported by NIH/Grant ES04427, the Swedish Work Env. Fund, Grant 88-1255, and U.S. DOE/OTHER under Contract No. DE-AC04-76EV01013.)

ACTIVITY OF THE CYANIDE-METABOLIZING ENZYME RHODANASE IN HUMAN NASAL EPITHELIUM. J L Lewis, C E Rhodes, W C Griffith, P Gourdie*, and A R Dahl. Inhalation Toxicology Research Institute, Albuquerque, NM. (Istituto di Mutagenesi e Differenziazimento, Pisa, Italy.)

Exposure to hydrogen cyanide ranges from low concentrations released during cooking of cyanogenic fruits to high concentrations released during combustion of nitrogen-containing synthetic materials. Metabolism of cyanide within the nasal cavity may influence both its toxicity and its odorant properties. The latter are important in warning of environmental hydrogen cyanide. The enzyme rhodanese (thiosulfate:cyanide sulfurtransferase, EC 2.8.1.1) detoxifies cyanide to the odorless thiocyanate. Rhodanese activity is greater per milligram of mitochondrial protein in rat nasal tissue than in liver. We have examined rhodanese activity in mitochondrial preparations of respiratory epithelium from nasal maxilloturbinates obtained during surgical treatment for hypertrrophy of the inferior turbinates in seven human patients. Histopathological analyses of these tissues showed no abnormalities or neutrophil infiltration. Purity of the mitochondrial fraction was verified by comparison of cytochrome c oxidase (mitochondrial marker protein) and NADPH-cytochrome c reductase (microsomal marker) activities. The K_m from human maxilloturbinates is 16.5 mM KCN with a V_max of 0.170 rhodanese units/mg mitochondrial protein. Data from these human samples suggest that smokers have a reduced capacity to metabolize cyanide. This is consistent with anecdotal evidence that smokers have an enhanced ability to detect the odor of cyanide, but our small sample number does not allow an adequate test of this hypothesis. (Research supported by U.S. DOE/OTHER under Contract No. DE-AC04-76EV01013.)

INDUCTION OF PEROXISOMAL ENZYMES BY A TETRAZOLE-SUBSTITUTED 2-QUINOLINYL METHOXY LEUKOTRIENE D_1 ANTAGONIST. M Kelley, A Groth-Watson, J Knoble and D Kornbrust. Drug Safety Division, Rhone-Poulenc Rorer Central Research, Horsham, PA.

The induction of hepatic peroxisomal β-oxidation and the peroxisomal bifunctional enzyme (PBE) by the leukotriene D_1 antagonist, RG 7152, was evaluated in vivo in the mouse, rat, guinea pig, dog and rhesus monkey. The ability of RG 7152 to induce this enzyme system in rat extrahepatic tissues and in vitro in primary rat hepatocytes was also investigated. Western blot analysis for PBE and β-oxidation assays revealed significant induction by RG 7152 in liver homogenates from rats and mice but a minimal effect in guinea pigs, dogs and monkeys. The degree of induction in rat liver was less than that observed in a positive control group treated with clofibrate (CF). There was slight induction of PBE in rat kidney and small intestine by CF, whereas RG 7152 elicited a minimal response in the kidney and no effect in the small intestine. In vivo, RG 7152 produced a response that was greater than diethylhexyl phthalate, approximately equivalent to clofibrate acid but less than bezafibrate. These results demonstrate that in vivo pharmacologic doses of RG 7152 can induce peroxisomal enzymes in mouse and rat liver. However, the absence of biologically significant induction in the other species evaluated suggests that the effect may not be relevant to an assessment of human risk.

PEROXISOME PROLIFERATORS: OMEGA-3 FATTY ACIDS, ETHYL CHLOROPHENOXYSOBUTYRATE (CLOFIBRATE), and D1(2-ETHYLEXYL) PHTHALATE: THE INTERACTIVE POTENTIAL. A M Wysynski, L Baldwin, D Leonard, E Calabrese School of Public Health, University of Massachusetts, Amherst MA.

Omega-3 fatty acids, clofibrate, and d1(2-ethylhexyl) phthalate (DEHP) are known, independently, to cause peroxisome proliferation in rodents. In order to evaluate their potential for interaction, either clofibrate (0.3% w/w) or DEHP (0.25% w/w) were fed daily, in combination with omega-3 fatty acids, to male weanling Wistar rats for 10 days. Menhaden fish oil, the fatty acid source, constituted a low fat (5%) or high fat (20%) diet. The endpoint examined was the induction of hepatic peroxisomal beta oxidation. Measures of palmitoyl-CoA oxidase activity indicated an additive response when either clofibrate or DEHP are delivered with an omega-3 fatty acid rich diet (20% Menhaden oil). These trends are supported by densitometric analysis of the peroxisomal bifunctional enzyme (PBE) obtained through gel electrophoresis, and liver weight changes.

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Covalent binding of [1-14C]PFDA and PFOA to macromolecules was examined in rat tissues in vitro and in vivo. An autoradiogram of proteins separated by SDS-PAGE of suspended rat hepatocytes incubated with [1-14C]PFDA or PFOA revealed an association of 14C with several proteins, suggesting covalent binding. Furthermore, when graded concentrations of these perfluorinated acids were incubated with albumin or hemoglobin, dose-related increases in covalent binding were observed. Cysteine, but not methionine, added to the incubations protected both hemoglobin and albumin from covalent binding of the perfluorinated acids, suggesting that PDFA and PFOA are binding at a sulfhydryl group. PFDA and PFOA were also found to acylate proteins when administrated to rats in vivo. The liver, plasma and testes of male rats treated with [1-14C]PFDA or PFOA (9.4 μmol/kg) contained detectable levels of covalently bound 14C (0.1 - 1% of the tissue 14C content). Protein acylation by PFDA and PFOA may alter the turn-over rate or function of the bound protein, suggesting a possible mechanism for certain biological effects seen with these perfluorinated acids. (Supported by NIH grant GM 41131).

Effects of perfluorodecanoic acid (PFDA) on fatty acid utilization was examined in isolated rat hepatocyte suspensions and in rat liver mitochondria and microsomes at concentrations of PFDA which did not affect cell viability. PFDA inhibited the oxidation of palmitic acid but not octanoic or pyruvic acids when hepatocytes were incubated for 20 min with 1 mM PFDA. At this PFDA concentration the esterification of palmitic acid into triacylglycerols was also reduced. The activity of long-chain acyl-CoA synthetase (ACS), an enzyme essential for both oxidation and esterification of fatty acids, was reduced in hepatocytes incubated with PFDA. The nature of this inhibition was examined in isolated rat liver mitochondria and microsomes. The inhibition of ACS by PFDA was similar in both preparations with an EC50 of approximately 0.15 mM. In mitochondria incubated with PFDA, the Vmax of the ACS reaction was significantly reduced while the Km of the enzyme for palmitic acid was unaltered. The decrease in oxidation and esterification of palmitic acid by PFDA in vitro appears to be due to noncompetitive inhibition of ACS. (Supported by NIH grant GM 41131).
INTERACTION OF LY171883 AND OTHER PEROXISOME PROLIFERATORS WITH RAT HEPATIC FATTY ACID BINDING PROTEIN. J R Cannon, K M Indelicato, and P L Esko. Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN.

Fatty acid binding protein (FABP) is a 14 kdal protein found in hepatic cytosol which binds and transports fatty acids (FA) throughout the cell. The purpose of this investigation was to determine whether peroxisome proliferators (PP) bind to FABP and displace endogenous FA. FABP was purified to electrophoretic homogeneity from hepatic cytosol by standard column chromatography. LY171883 and its structural analog, 189585, in addition to the classical PP clofibrate, ciprofibrate and bezafibrate, displaced $3^H$-oleic acid binding to FABP (IC$_{50}$ = 135 ± 5.6, 24.3 ± 1.4, 114 ± 23.2, 32.4 ± 1.3, and 104 ± 0.6 μM, respectively). $3^H$-LY171883 co-eluted with FABP during the purification procedure, further demonstrating an interaction with FABP. $3^H$-LY171883 was conclusively shown to bind FABP (K$_D$ = 10.8 μM) using Scatchard-Rosenenthal analysis. $3^H$-LY171883 binding to FABP was inhibited by 189585 (IC$_{50}$ = 23.6 ± 1.3 μM) clofibrate (266 ± 6.8 μM), ciprofibrate (50.3 ± 13.8 μM) and bezafibrate (18.4 ± 0.6 μM). These findings indicate that PP can bind directly to FABP and, due to their structural similarity to FA, displace the endogenous ligands. PP-induced disruption of critical steps in FA metabolism, such as association of FA with binding proteins, may be involved in the development of peroxisome proliferation.

INDUCTION OF SISTER CHROMATID EXCHANGE, MICRONUCLEI, AND PEROXISOME PROLIFERATION IN PRIMARY CULTURES OF HEPATOCELLS BY THE PEROXISOME PROLIFERATOR, WY-14643. 1JJ Hwang, 2MTS Hisa, and 1RL Jirtle. 1Dept. of Radiology, Duke Univ. Med. Ctr., Durham, NC and 2The MITRE Corp., McLean, VA.

The ability of peroxisome proliferators to induce hepatocellular carcinoma has been known since 1981, but the mechanism of tumor formation is still poorly understood. In this study, we have used primary cultures of rat hepatocytes to address the question of whether the peroxisome proliferator, [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (WY-14643), causes genotoxic damage in hepatocytes as measured by sister chromatid exchange (SCE) and micronuclei formation. We found that the SCEs per chromosome increased significantly in a dose response manner from a background level of 0.65 to a maximum of 1.0 at 100 μM of WY-14643; 0.5 μM of WY-14643 did not increase SCE formation above the control level. Micronuclei formation was also increased in a dose response manner. The maximum increase in micronuclei was 1279% above control at 50 μM WY-14643. WY-14643 at a 0.5 μM concentration was also ineffective in causing micronuclei formation. This significant increase in SCE and micronuclei formation in hepatocytes was further found to be correlated with the induction of peroxisomes as measured by cellular palmitic acid CoA 6-oxidation activity. The results of these studies, for the first time, clearly demonstrate that the peroxisome proliferator, WY-14643, causes significant genotoxic damage in rat hepatocytes. (Supported by MITRE Corp.)

EFFECT OF THE PEROXISOME PROLIFERATOR LY171883 (LY) ON LIPID METABOLISM IN RATS FED FAT FREE DIET. P Foxworthy and P L Esko. Toxicology Division, Lilly Research Laboratories, Eli Lilly and Company, Greenfield IN.

One day administration of 0.1 or 0.3% LY in standard rat chow caused a dose-related increase in liver triglycerides (TG). In the 0.1% rats TG continued to increase throughout 90 days whereas in the 0.3% rats TG reversed to control values by day 3. The effect of LY on the more substantial increases in TG induced by fast feeding (FFD) was studied. Fourteen day administration of FFD caused a 6-fold increase in TG associated with a 3.3-fold increase in fatty acid synthetase. Co-administration of 0.1% LY caused a slightly higher accumulation of TG, while 0.3% LY prevented the accumulation of TG. Furthermore, the elevation of TG in rats fed FFD for 14 days was reversed by subsequent feeding of 0.3% LY. Fatty acid synthetase activity was comparably increased in all treatment groups indicating that 0.3% LY did not prevent the lipogenic response to FFD. In 0.3% rats, mitochondrial and peroxisomal 3-oxidation, carnitine palmitoyltransferase I, and plasma ketones were increased 5, 7.3, 1.9, and 3-fold, respectively. In the 0.1% dose group the increases were 2.7, 2.6, 1.4, and 1.7-fold. The data indicates that in the 0.3% dose group oxidative capacity was sufficiently increased such that excess fatty acids from lipogenesis were preferentially oxidized rather than esterified. In the 0.1% dose group oxidation was only mildly increased, so that fatty acids continued to enter the esterification pathway.
DOSE-DEPENDENT EFFECTS ON PEROxisomes AND CELL REPPLICATION WITH THE HepATOCARCINOGEN. WY-14,643. D S Marsman, N Wada and J A Popp, CIT, Research Triangle Park, NC.

The dose- and time-dependency of peroxisome proliferation and hepatocyte replication was evaluated in the liver of rats fed the peroxisome proliferator and hepatocarcinogen, WY-14,643. Male F344 rats were fed NIH07 (control) or the same diet blended with WY-14,643 at 5, 10, 50, 100 or 1000 ppm for 1.5, 3, 6 or 13 weeks. Hepatomegaly was induced by WY-14,643 at all doses and at all time points. Peroxisome proliferation was present in rats fed 5 ppm WY-14,643 as early as 1 week, as determined by the peroxisome-specific NADPH reduction of palmityl CoA (PCO) and the peroxisome-associated activity of carnitine acetyltransferase (CAT) 5- and 11-fold over control, respectively. The elevations of PCO and CAT were dose-dependent from 5 ppm to 50 ppm and then plateaued from 50 to 1000 ppm throughout the treatment period. Hepatocellular replication, evaluated by nuclear histosautoradiography (3H-thymidine labeling, 6-day incubation), was increased in all WY-14,643 dose groups after 1 week of treatment (5 ppm, 4-fold; 10 ppm, 5-fold; 50 ppm, 13-fold; 100 ppm, 12-fold and 1000 ppm, 13-fold over controls). However, in 5 and 10 ppm groups this cell replication returned to control levels by 3 weeks. In contrast, 50, 100 and 1000 ppm groups had sustained increases in cell replication up to 13 weeks (13 weeks: 6-fold, 7-fold and 9-fold over controls, respectively). We have demonstrated that WY-14,643 can induce peroxisome proliferation at 5 ppm, a dose 200 times lower than the dose shown to be highly hepatocarcinogenic in rats (100% incidence by 60 weeks). In contrast, 50 ppm was identified as the minimal dose which induced sustained cell replication in rat liver. These data show that peroxisome proliferation can be dissected from induced cell replication for correlating either peroxisome induction or cell replication with tumor formation.

SELECTIVE INDUCTION OF PEROXISOMAL ENZYME ACTIVITIES BY PERFLUOROOCTANOIC ACID (PFOA) IN AGED RATS. M Z Baud1 and L S Birnbaum2. 1University of Missouri-Kansas City, MO and 2Health Effects Research Laboratory, US EPA, Research Triangle Park, NC.

Several drugs and industrial chemicals cause a striking proliferation of hepatic peroxisomes in young and adult rodents. The morphological changes are accompanied by increases in peroxisomal enzyme activities. While aging can alter the susceptibility of the liver to the effects of xenobiotics, no reports are available on the effects of peroxisome proliferators in aged animals. Therefore, the hepatic enzymes of the nonmetabolizable peroxisome proliferator PFOA were examined in 10, 20, 50 and 100 week old male Fischer 344 rats. Rats were treated with either 150 mg PFOA/kg p.o. in 0.5 ml corn oil or corn oil alone and were sacrificed 48 hrs later. While hepatic peroxisomal β-oxidizing activities were significantly elevated (4-8 fold) in all age groups by PFOA, catalase activities were only enhanced (30-70%) in 10 and 20 week old rats compared to control rats. Thus, there appears to be an age-related decline in the induction of catalase by PFOA, while age has no effect on the induction of the fatty acid oxidizing enzymes. This could result in elevated hepatic levels of H2O2 in aged animals compared to young adults as a result of exposure to peroxisome proliferators. (Supported, in part, by ES-04778. This abstract does not necessarily reflect EPA policy.)

EFFECT OF CIPROFIBRATE ON THE IN VIVO AND IN VITRO RESPONSE OF THE RAT LIVER MICROSOMAL CA2+-ATPase ACTIVITY. A M Bennett and G M Williams. American Health Foundation, Valhalla, NY.

This study is based on the hypothesis that hypolipidemic peroxisome proliferator (HPP)-induced oxidative macromolecular damage contributes towards initiation of a pleiotropic response. Since C2+ is a potent mitogenic signal, the effects of ciprofibrate on hepatic C2+ homeostasis were investigated. The mitogenic stimulus exerted by the HPP's could involve impairment of the liver microsomal Ca2+ - ATPase, which is a major inositol-sensitive membrane channel in the endoplasmic. To test this hypothesis male Fisher F344 rats were given a single dose of ciprofibrate (200mg/kg) or corn oil vehicle by gavage and sacrificed at 22, 24, 48 and 72h later. Ca2+ - ATPase activity of hepatic microsomal fractions were measured by the release of Pi from ATP, in the presence and absence of C2+. No effect was observed on C2+ - ATPase activity at 12h. C2+ - ATPase activity was reduced to 36% and 17% of control at 24h and 48h respectively, and returned to within 86% of control by 72h. There was no evidence of non-specific enzyme inhibition, since the microsomal enzyme glucose-6-phosphatase was not significantly different between treated and control rats (p>0.05, n=8). Ciprofibrate also produced a dose dependent inhibition of the Ca2+ - ATPase in vitro, with an IC50 of approximately 9μM (n=4). Identity of Ca2+ - ATPase activity was validated using 2,5-di-tert-butydroxipine, a specific enzymatic inhibitor, which depressed Ca2+ - ATPase activity to 0% at 10μM (n=4). These results suggest that HPP-induced macromolecular interactions may cause C2+ homeostatic dysfunction. This could result in elevated and prolonged cytosolic C2+ transients, potentially HPP-induced mitogenicity (Supported by NIH grant CA3894-05S1).
Carcinogenic effects of 181 chemicals were investigated using a medium-term liver bioassay system. Rats were initially given a single dose (200 mg/kg) of diethylnitrosamine i.p. and from 2 weeks later were treated with test chemicals for 6 weeks and then killed, all rats being subjected to 2/3 hepatectomy at week 3. Modifying effects were evaluated in terms of numbers and areas of GST-P positive foci. Of the hepatocarcinogens, 21 out of 21 (100%) mutagens and 18 out of 20 (90%) non-mutagens gave positive results. Carcinogens with targets other than the liver gave less positive results (7/33; 21%). Since none of the chemicals reported to be non-carcinogenic demonstrated positivity, there were no complicating false-positives. Initiated rats were also maintained for 2 years with exposure to 6 representative test chemicals. Resultant hepatocellular carcinoma incidences were found to be closely correlated with the respective medium-term results. In conclusion, the present bioassay system thus has practical applications for rapid and economical screening of environmental carcinogens and modifiers of hepatocarcinogenesis.

Potential synergism between 5 heterocyclic amines at low doses was evaluated in the medium-term liver bioassay system for carcinogens. F344 male rats were subjected to a single injection of DEN(200mg/kg, ip), 2/3 partial hepatectomy at week 3 and six-week administration of the test compounds and killed at week 8. The chemicals used were Trp-P-1, Glu-P-2, Tq, MeIQ, MeIQx. Groups were given each chemical at the carcinogenic dose, or 1/5 or 1/25 of that dose. The two other groups received the 5 chemicals in combination, each at the 1/5 or 1/25 dose levels. Enhancement activity was assessed by quantitative analysis of GST-P positive foci. 1) Their numbers were significantly increased with all chemicals at the carcinogenic dose. Trp-P-1, Tq and MeIQx also showed an increase even at the 1/5 dose level. 2) Similar results were obtained regarding the foci areas at the highest dose levels, with the exception of Glu-P-2. Synergism between the chemicals was evident in the groups given simultaneously the 5 chemicals at both the 1/5 or the 1/25 dose levels. The numbers and areas of GST-P positive foci in the low dose combination groups were higher than the sum of foci observed for each single compound. Thus, carcinogenicity was predicted in all 5 heterocyclic amines tested, and the synergistic effects were more apparent at the low dose levels.

The effects of four antioxidants in combined administration at low doses were studied in a medium-term liver bioassay system (DEN-PH model) using glutathione S-transferase placental form positive (GST-P) foci in the liver as an endpoint marker. Male F344 rats were initially given a single dose of diethylnitrosamine (DEN, 200mg/kg, ip), and 2/3 partial hepatectomy at week 3. From week 2, the rats received the test chemicals for 4 weeks and were killed at week 8. Butylated hydroxyanisole (BHA, full dose 1%), tert-butyldihydroquinone (TBHQ, 1%), catechol (0.8%) and sesamol (0.5%) were used as test chemicals and incorporated into the diets at the full or at 1/4 of those doses alone or in combinations of 2 or 4 antioxidants. Three antioxidants (BHA, TBHQ and catechol) at full doses and the combined administration of 4 antioxidants significantly reduced both number and area of GST-P positive foci compared to the DEN alone group. Further, the incidence of foci in the group administered the 4 antioxidants at 1/4 of the full dose was lower than the sum of foci observed for each single compound also at 1/4 dose. Thus, the combined administration of antioxidants at lower doses showed a clear synergistic effect in the inhibition of the liver carcinogenesis.

CTFE trimer acid (TA) is a metabolite of the 6-carbon oligomer of Halocarbon 3.1 oil. It is a nonflammable hydraulic fluid composed of perhalogenated oligomers of varying chain length. Dosing of TA by oral gavage for 3 months resulted in a slight increase in the rate of peroxisomal β-oxidation, but no relative liver weight. No difference in β-oxidation was noted following an additional 3 months of dosing. The present study was designed to evaluate TA for both tumor initiating and promoting activity. Male F344 rats (4 weeks old) were partially hepatectomized. Groups of animals received a single dose of TA, as the initiator, followed by chronic phenoabarbital administration, a known tumor promoter, for 9 months; diethylnitrosamine, a known tumor initiator, was administered as a single dose to separate groups of animals. Three of these groups received different doses of TA as a promoter for 9 months. Quantitative stereological analysis was performed on foci from liver sections stained for a variety of histological and histochemical markers. TA does not possess tumor initiating ability. However, a significant increase in foci/cm² and foci/cm³ over those of control after 9 months of treatment clearly indicates that TA has promoting activity. (Supported by DoD Contract No. F33615-85-C-0336)

INTERACTION OF 3,4,3',4' AND 2,5,2',5' TETRACHLOROBIPHENYL IN A RAT HEPATOCARCINOSIS MODEL. L M Sargent and H C Pilot, McCrady Laboratory for Cancer Research, University of Wisconsin, Madison, WI.

Polychlorinated biphenyls occur as mixtures of planar and nonplanar congeners; therefore, studies of possible interactive effects are essential to understanding the mechanism of toxicity of these mixtures. To determine a possible interaction of the hepatocarcinogenic effects in vivo of 2,5,2',5'-tetrachlorobiphenyl (TCB) and 3,4,3',4'-TCB, rats were exposed to diethylnitrosamine (DEN) and to 0.1 ppm 3,4,3',4'-TCB alone and/or to 10 ppm 2,5,2',5'-TCB in the feed for one year following the Pilot protocol. Both liver and the peripheral blood were examined following treatment. TCB treatment caused an increase in altered hepatic foci (AHF) many of which were glutathione-S-transferase and γ-glutamyltranspeptidase positive. ATCase and glucose 6-phosphatase negative. And which were of equal distribution in DEN + phenoabarbital. ATCase + and ATPh-AHF correlated with foci areas of P-GP expression. The two PCB congeners interact in vivo to promote a synergistic increase in the number and total volume percent of AHF. The combination of the two PCBs caused a greater than additive decrease in the total number of lymphocyes and antibody producing. The thymocyte dependent (T)-helper cells isolated from the animals receiving the combination of PCBs demonstrated an abnormal subpopulation. The results indicate that the in vivo interaction of 2,5,2',5'-TCB and 3,4,3',4'-TCB, results in much greater toxicity in peripheral lymphocytes and in the liver than treatment with either congener alone.

LIVER CARCINOSIS IN B6C3F1 MALE MICE GIVEN 1,4-BIS[(3,5-DICHLOROPYRIMIDOXYLOXY)]BENZENE (TCPBOP8). B A Diwan, J R Henneman, J M Ward and J M Rice, BCDP, PRI/DynCorp and Laboratory of Comparative Carcinogenesis, NCI, Frederick, MD. Sponsor: R W Nims.

Male B6C3F1 mice (10/group) were given N-nitrosodiethyamine (NDEA; 90 mg/kg B.W.) or vehicle once i.p. at 12 weeks of age, preceded or followed by non-genotoxic TCPBOP8 (3 mg/kg B.W., i.p.) at weeks 5, 7.5, 9, 11; 14; 14, 16; or 14, 16, 18, 20. At 26 weeks, one mouse/group given NDEA, alone or preceded by TCPBOP8 4x or followed by TCPBOP 1x, had a single focus. In mice given NDEA followed by TCPBOP 2x, 5/10 had multiple foci (5.3/cm²); NDEA-TCPBOP 4x yielded multiple foci (33.2/cm²) in 10/10. At 40 weeks, in mice given NDEA only, 1/10 had 1 hepatocellular adenoma (HA). Of mice given only TCPBOP 4x, without NDEA, at either time interval, 5/10 had single HAs; 3/10 given only TCPBOP 2x and 0/10 given only TCPBOP 1x had single HAs. Single HAs also occurred in 5/10 mice given TCPBOP 4x followed by NDEA. TCPBOP given after NDEA dramatically increased tumor incidence and multiplicity in dose-dependent fashion: 1x, 7/10; 4.5 HAs/mouse; 2x, 10/10 (9.5 tumors/mouse); 4x, 10/10 (15 tumors/mouse). TCPBOP given 2x or 4x after NDEA accelerated tumor progression; these tumors included many hepatocellular carcinomas; some of which metastasized to lung. TCPBOP is both a non-genotoxic carcinogen and multitissue tumor promoter for mouse liver.
The hormonal effects and toxicity of Tamoxifen were studied in rats. Six week old female Sprague-Dawley rats were administered by gavage 3, 22 and 45 mg/kg Tamoxifen in 5 ml/kg carboxymethyl cellulose daily for 8 months. Necropsies were performed at 1, 3 and 6 months. Also, there was a one month recovery period in the high dose group after 6 months. There were no gross lesions in any group at 1 and 3 months. Endocrine parameters were determined for hepatic cytosolic (cER) and nuclear estrogen receptors (nER), alanine aminotransferase (ALAT), and serum ceruloplasmin (CPN) at 1 and 3 months. Hepatic cER levels were lower, nER and ALAT levels were higher than controls in all treatment groups at both 1 and 3 months, and CPN was significantly elevated only in the DES-treated group at 1 and 3 months. These results suggest that TAM has both estrogen agonistic and antagonistic properties. At 6 months, hepatic lesions were seen in the 45 mg/kg dose group only; 4 of 7 rats had hepatic masses which included hepatic adenomas and foci of cellular alteration. In the recovery group, 7 of 7 rats had multiple hepatic adenomas and foci of cellular alteration.


Short-term estradiol pretreatment was evaluated in a two-stage model for hepatocarcinogenesis using a single, 150 mg/kg injection of diethylnitrosamine (DEN) as the initiator. Ovariectomized (OVX) Sprague-Dawley rats were given 17-β estradiol (0.05 to 0.5 mg/kg) for three consecutive days prior to DEN administration and fed a choline deficient (CD) diet for 5 to 9 weeks. An additional group of estradiol-pretreated rats were given injections of 0.05 mg 17-β estradiol/kg three times weekly for five weeks during feeding of the CD diet. Compared with vehicle controls, pretreatment with estradiol enhanced the degree of focal and diffuse GGT+ staining observed in liver sections of DEN-dosed rats in a non-dose-dependent manner. GGT+ reactions in livers of estradiol-pretreated, DEN-dosed rats were similar with or without post-DEN administration of estradiol. Therefore, short-term estradiol pretreatment was as effective as a chronic posttreatment with estradiol in the enhancement of DEN-initiated GGT+ enzyme-altered foci in OVX Sprague-Dawley rats fed a CD diet.

DIFFERENTIAL EXPRESSION OF HEPATIC DETOXICATING ENZYMES IN HEAVY AND LIGHT (C3H x VF) F1 HYBRID MICE CORRELATES WITH INCREASED SUSCEPTIBILITY TO HEPATOCARCINOMA. JEA Leakey, CL Wolff, JJ Baxa and JC Lipscomb. Divisions of Reproductive and Developmental Toxicology, and Comparative Toxicology, National Center for Toxicological Research, Jefferson, AR and University of Arkansas for Medical Sciences, Little Rock, AR.

Higher body weight in phenobarbital (PB)-treated mice has been correlated with increased formation of multiple hepatocellular adenomas in A/J/A (C3H x VF) F1 hybrid male mice, which were fed 0.05% sodium PB in NIH-31 diet for 7 months. Heaviest and lightest mice in the untreated and PB groups were assayed for hepatic PB-responsive detoxicating enzymes. Constitutive expression of cytochrome P450IIA, 7-ethoxyresorufin-O-deethylase (EROD) and testosterone UDF-glucuronyl-transferase (TGT) activities was significantly decreased in light mice as compared to heavy mice. Total P450 and P450IIA content, and EROD and TGT activities were all preferentially induced by PB in light mice. In contrast, P450IIB-specific 7-pentoxyresorufin-O-dealkylase and glutathione-S-transferase N 1:1-dependent activities were both preferentially induced by PB in the heavy mice. Thus, expression of both constitutive and PB-inducible detoxicating enzyme forms differs between the heavy and light mouse subpopulations and differential susceptibility to PB promotion of hepatocellular adenomas is accompanied by, or results from, such differences in hepatic detoxicating enzyme regulation.
699 EFFECTS OF METHAPYRILENE ON HEPATIC PROTEINS OF RATS AND MICE. F C Richardson, D M Coppie, R A Bendele, and L Anderson. Toxicology Division Eli Lilly and Co., Greenfield, IN and Large Scale Biology Corp., Rockville MD. Sponsor: B Wierda.

Methapyrilene is a non-genotoxic carcinogen specific to the rat. Studies have been performed to determine the effect of methapyrilene on cellular proteins of rats and mice. Animals were exposed to a carcinogenic concentration of 1000 p.p.m. methapyrilene in the diet for 1 or 10 weeks and then either killed for sample collection or placed on control chow for 4 weeks prior to sample collection. Livers were collected and protein changes were determined by two-dimensional polyacrylamide gel electrophoresis using Isodalt™ methodology for analysis of protein gels. Proteins were stained with Coomassie Brilliant Blue. Results demonstrate that, within one week, methapyrilene affects the levels of at least 120 proteins, most of which are mitochondrial in origin. The vast majority of these effects were reversible in animals exposed for one week. Four mitochondrial proteins, two that appear to be the beta-F1-ATPase and carbomyl phosphate synthase, were uniquely modified, possibly by a portion of the methapyrilene molecule. Preliminary results indicate these changes also induced in the mouse, but to a lesser degree; suggesting that these modifications are not major factors in the specific carcinogenic effects of methapyrilene. Studies to define these protein changes are underway.

700 LACK OF CARCINOGENICITY OF DAMINOZIDE (ALAR) IN NEW RAPID IN VIVO BIOASSAYS. T Cabral, T Hoshya, K Hako, K Hasegawa, S Fukushima and N Ito, 1st Dept. Pathology, Nagoya City Univ. Med. Sch., Nagoya, Japan.

The carcinogenicity of Daminozide, a plant growth regulator used primarily in apple orchards, has been the subject of recent discussion by several national organizations because of contradictory test results. The aim of the present studies was to assess the carcinogenicity of Daminozide with two rapid in vivo bioassays in F344 male rats. 1.) Ito model (DEN-PH): rats were given i.p. 200 mg/kg bw of diethylnitrosamine (DEN) and two weeks later treated with Daminozide (10000 or 20000 ppm) in the diet for 6 weeks and then killed; all rats underwent partial hepatectomy at week 3. Hepatocarcinogenic potential was assessed by comparing the No. and area/cm² of induced GST-P-positive foci in the liver with values for controls given DEN alone. Daminozide was negative in our study. 2.) Wide-spectrum initiation model (DMD): after sequential treatment with DEN, N-methylnitrosourea and dihydroxy-di-N-propylnitrosamine, rats were fed 10000 ppm Daminozide in the diet. Sacrifice of all animals at week 20 and histopathological examination of the main organs again revealed a negative result. Notwithstanding the apparent lack of carcinogenicity we have now underway studies geared to evaluate the carcinogenicity of mixtures of Daminozide with 1,1-Dimethylhydrazine (UDMH), its major impurity.

701 INHIBITION OF ETHYL CARBAMATE (EC) INDUCED TUMORIGENESIS BY WINES FED TO MICE. G S Stoewsand, J L Anderson and *L Munson. Department of Food Science and Technology, Cornell University, Geneva, NY and *Department of Pathology, National Zoological Park, Smithsonian Institute, Washington, DC

EC, also known as urethane, is a naturally occurring carcinogen present in numerous fermented foods and beverages, e.g. soy sauce, bread, sherry, table wines, etc. EC precursors can be a variety of nitrogenuous compounds, such as urea, but the exact mechanism of EC formation is still obscure. Ethanol intake has been shown to inhibit the localization of EC in mouse tissues and delays EC metabolism (Waddell, et al 1987; Yamamoto, et al 1988), as well as reducing the development of EC induced pulmonary tumors (Kristiansen, et al 1990). We fed groups of C57, male, weanling mice either water, 12% ethanol, Concord red wine, Concord white wine, or Johannisseberg Riesling wine without or with EC (10 mg or 20 mg/kg/day) ad libitum. At the end of 41 weeks tissue histopathological examination showed a significantly lowered incidence and frequency of hepatocellular adenomas, with no development of hepatocellular carcinomas, in the wine drinking mice as compared to either the water or ethanol treated groups. The frequency of lung Clara cell adenomas were also significantly reduced in the mice on all wine and 10 mg/kg EC treatments. Wine nonflavonoid phenol compounds may be the cause of EC induced tumor reduction. (Supported by the NY State Wine and Grape Foundation).

702 HEALTH RISKS IN EASTERN EUROPE ASSOCIATED WITH ENVIRONMENTAL POLLUTION. B K Davis and J S Jamall*. Cal. Dept. Health Services, Sacramento, CA, *School of Medicine, Univ. Cal., Davis, CA, and *Dames & Moore, Sacramento, CA.

Recent revelations of severe environmental pollution in former East bloc nations prompted us to apply risk assessment methods to estimate the human health effects based on reported levels of contaminants. For example, lead contamination at 225 ppm for soils and 8.8 ppm for carrots has been reported for Krakow, Poland. Assuming a residential scenario, these levels translate into a daily lead intake of 750 µg for children under 6 years old. Using the methods of Ryu et al. (1983), this intake predicts an increase in blood lead level of 120 µg/dl. In comparison, 25 µg/dl is considered to be clinical lead poisoning for children (U.S. ATSDR, 1988). A second example is the SO2 level of 400 µg/m³ of air (5 times the U.S. standard) measured in Leuna, DDR. Using standard risk assessment assumptions, the expected intake of SO2 is 114 µg per kg of body weight per day. Similar approaches are used to quantify health risks for other contaminants and other media in various Eastern Europe locations.
Indoor emissions may occur as a result of volatilization of chemicals indoors during use of contaminated water (e.g., while showering). Based on air-water partition coefficients and ventilation equations, it can be predicted that indoor use of water contaminated with volatile compounds will pose as great or greater health risk to residents as drinking 2 liters/day (standard drinking water ingestion rate) of the same water. Indoor air sampling data supports the potential significance of inhalation exposure to contaminants in the water supply. Despite this potential, available guidance on how to quantify exposure to chemicals which volatilize from the water during home use is sketchy.

For the baseline risk assessment of a site involving contaminated ground water, the authors devised a methodology to quantify inhalation exposure while showering, operating a dishwasher, and operating a washing machine. The methodology involves assumptions and algorithms to estimate: 1) concentrations of chemicals in the breathing zone as a result of these activities; and 2) inhalation intake by potentially exposed individuals. The results provide insight into the relative contribution of inhalation exposure in the home.

The Air RISC was initiated in early 1988 by EPA's Office of Health and Environmental Assessment and the Office of Air Quality Planning and Standards as a technology transfer effort that would focus on providing information to state and local environmental agencies and to EPA Regional Offices in the areas of health, risk, and exposure assessment for air toxics. Provision of technical assistance to the state and local agencies is key to supporting their greater regulatory role. Information on risk assessment and risk communication is disseminated by Air RISC's two primary activities--technical assistance and guidance projects on air toxic, health, and educational issues and staffing a "hotline" to provide immediate response to state and local inquiries. Technical assistance and guidance projects have included a wide variety of activities in risk assessment, risk communication, and training in risk assessment methods. Since inception Air RISC has responded to a total of 1,342 inquiries--693 state, 239 regional, 66 federal, 202 local, and 142 other. The Air RISC technical assistance projects and rapid response "hotline" has served the intended agencies well. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)
SITE-INDEPENDENT RISK CHARACTERIZATION OF THREE POWER PRODUCTION TECHNOLOGIES. E J Hixon and F D Skinner. Radian Corporation, Austin, TX.

Potential health effects of power plant emissions and discharges are important considerations in the selection of appropriate plant sites and power production technologies. Both site and technology selection could be facilitated by a qualitative risk assessment process that allows the health and environmental impacts of different power production technologies to be compared in the context of one or more possible sites. Such a method for comparing trace element emissions and discharges to air, water, and solid waste has been developed. The method is independent of site, so specific data on distances to receptors, drinking water sources etc. is not required. The method allows health and environmental risk to be normalized for power output so that the effects of energy conversion efficiency can be included in the evaluation. The method was tested by comparing emissions from: a pulverized coal boiler using flue-gas desulfurization, a fluidized-bed combustion unit and a coal gasification combined cycle unit. The results indicate that coal gasification combined cycle has the most favorable emissions profile in terms of relative output of toxic and carcinogenic trace elements.


Dermal absorption of chemicals from soil or water has been studied for very few of the compounds found at hazardous waste sites. Mathematical models of dermal uptake provide the best alternative means of estimating uptake and risk from dermal exposure. Variations on the McKone physicochemical-based model of uptake of neutral organic chemicals (Risk Anal. 10:407, 1990) were compared to in vivo and in vitro data. Best correlation between the calculated uptake and the experimental data was obtained by adapting the model for movement of chemicals through both the aqueous and lipid phases of skin. Dermal uptake was estimated for several dozen chemicals commonly found at waste sites. In this series, absorption varied from <.001% to nearly 100%. Changing exposure parameters such as wind speed, thickness of the chemical-containing layer on skin, and duration of contact produced up to 10-fold changes in uptake. Considering the uncertainty in exposure conditions, the estimates derived from this model are probably as accurate as necessary. The predicted uptake values should be useful in estimating dermal hazard of chemicals.

DETERMINING HUMAN EXPOSURE TO POLLUTANTS MEASURED IN THE AMBIENT AIR EMITTED FROM A MUNICIPAL WASTE COMBUSTOR. M Eichelberger, P McGinnis, W Stiteler, R Nichols and P Sonich-Kullin. Syracuse Research Corporation, Cincinnati, OR, *Syracuse, NY., and **U S Environmental Protection Agency, ECAO, Cincinnati, OH.

A pilot study of chemicals in ambient air surrounding a municipal waste combustor (MWC) was undertaken in an attempt to determine human exposure. Several metals (e.g., lead and nickel) and organics (e.g., Bi(a)P and PCDD/PCDFs) were measured. The likelihood that the MWC was the source for these pollutants was evaluated using three approaches: 1) correlation of the amount of waste burned daily with particulate concentrations, 2) comparison of ambient air PCDD/PCDF congener profiles with those of potential sources (i.e., MWC stack emissions and residential wood burning systems), and 3) statistical analyses of the relationship between daily measured ambient air concentrations of pollutants and daily concentrations predicted from the Industrial Short-Term (ISST) air dispersion model. The daily measured concentrations were compared to the predicted concentrations by using two nonparametric procedures: a modified sign test and Friedman two-way analysis of variance. None of these analyses found a significant relationship between incinerator operation and measured pollutant concentrations. Advantages for each of these approaches of source apportionment are discussed.


Humans can be exposed to pollutants in combustor emissions via direct inhalation and indirect pathways. However, the relative importance of these pathways is often questioned by the conducting risk assessments of combustor emissions. An assessment was undertaken to determine the relative magnitude of inhalation and indirect exposures to emissions from a sewage sludge incinerator in Florida and a hazardous waste incinerator in Missouri. Actual data from each facility (facility configuration and emission characteristics) and meteorologic data from the cities where these combustors are located were used to determine deposition rates for two example chemicals, cadmium and benz(a)pyrene. These rates, as well as general, non-site-specific data concerning land use, population characteristics, soil properties, and environmental fate were used as inputs to exposure assessment models developed by the U.S. EPA. The resulting exposure values for three different exposure scenarios were compared. Total indirect pathways contributed 29% of exposure for both chemicals. Ingestion of crops was the most significant individual pathway, accounting for 19% for benz(a)pyrene and 40-88% of exposure for cadmium. These patterns were similar for all three scenarios examined.
DEVELOPING HEALTH-BASED CLEANUP CONCENTRATIONS FOR CONTAMINATED CONCRETE AT THE FMC PESTICIDE SITE IN YAKIMA, WA. SM Turnblom, T Holm-Hansen, PRC Environmental Management, Inc. (PRC), Seattle, WA, X Pharm-Mahini, PRC, San Francisco, CA, N Ceto, USEPA, Region 10, Seattle, WA.

Potential human health impacts of exposure to chemical contaminants at Superfund sites are evaluated by determining the extent of their carcinogenic and non-carcinogenic effects, using chemical-specific slope factors (SFs) or reference doses (RfDs), respectively, and appropriate population exposure data. Exposure estimates and associated health impacts can then be used to develop health-based cleanup concentrations. Guidelines exist for estimating exposure to contaminants in soil, water, and air, but not for contaminated materials such as concrete. At the FMC site, wipe sample concentrations were employed to develop health-based cleanup goals for dermal exposure to contaminated concrete. Conservative assumptions used to develop cleanup goals were 1) all removable chemical substances detected in wipe samples and available for adherence to or within direct dermal contact with the contaminated concrete, 2) human intake via direct dermal exposure can be calculated as an absorbed dose using chemical-specific or default value human dermal absorption factors, and 3) EPA-verified SFs and RfDs based on administered doses can be further adjusted for absorption efficiency using chemical-specific human oral absorption factors.

CHROMIUM CONTACT DERMATITIS - A HEALTH BASED RISK ASSESSMENT APPROACH. V.B Mylavarapu and True-Jenn Sun. New Jersey Department of Environmental Protection, Trenton, NJ.

Allergic contact dermatitis is a two step process: sensitization and elicitation. Sensitization is highly variable and is subjective to individual sensitivity to the agent. Elicitation occurs upon re-exposure to the same agent and is quantifiable. Chromium dermatitis is associated with occupational and environmental agents like cement, detergents, matches, dyed leather, chromium production and chromium plating to name a few. Much of the evidence for occupational chromium allergic contact dermatitis is from construction industry involving cement. Chromium in hexavalent (Cr VI) form can cause allergic contact dermatitis at very low doses. The quantity of Cr VI in cement is in the range of 1-40 µg/gm. Incidence of chromium allergic contact dermatitis among general population is about 2%. However, among residents of the chromium processing waste contaminated areas in Tokyo metropolitan region in Japan, the incidence of contact dermatitis is high, with highest incidence during summer time. Cr VI in the form of potassium dichromate yields positive patch test reaction among contact dermatitis patients. At lower potassium chromate concentration of 10 ppm about 10% of the sensitized individuals reacted positive to patch testing. Both trivalent and hexavalent chromium are skin allergens with Cr VI being about 50 times more sensitizing.

A HEALTH ASSESSMENT (INCLUDING ALLERGIC EFFECTS) OF CHROMIUM (Cr) RESIDUES FOLLOWING CLEANUP OF A LARGE DICHROMATE SPILL AT A PUBLIC FACILITY. K G Symms, Environmental Standards, Inc., Valley Forge, PA. Sponsor: D Rosenbaum.

An assessment of the health risk associated with potential exposures of the public to toxic compounds such as dichromates typically involves evaluation of ingestion and inhalation exposure pathways, quantiative estimates of consequent intakes, and comparisons with established acceptable intake values that are based upon systemic dose-response data. However, hexavalent chromium (CrVI) can be locally ulcerogenic following dermal contact and both CrVI and trivalent chromium (CrIII) (notably less toxic) can result in allergic sensitization and contact dermatitis. The dose-response relationship for sensitization and subsequent allergic response is highly variable and undefined, according to the ATESDR. Accordingly, cleanup goals for residual Cr on surfaces that the public, is likely to derrmally contact are often targeted to levels below the most sensitive limits of analytical detection. Nevertheless, compelling data from a number of studies concerning the dose-response relationship between dermal contact with Cr salts and the occurrence of dermatitis have emerged. Patch test provocation studies have revealed that the lowest minimum elicitation concentration of CrVI in solution is 885 ppm. A residual level of about 5 µg/cm² of CrVI on surfaces represents the most stringent health-based cleanup objective derived, which is adequately protective even for those individuals who respond allergically to very low amounts of Cr on the skin. In remediating dichromate contaminated surfaces, a non-toxic, mild reducing agent was utilized in the final rinse water which quenched CrVI to CrIII. Soluble CrIII is >30 times less potent than dichromate in provoking dermal reactions.


Exposure to PCDDs through the inhalation of contaminated particles may be underestimated due to enrichment of the compounds on respirable particles. In risk assessments, it has been necessary to use assumptions regarding the pulmonary bioavailability of PCDDs from particles such as fly ash and soil due to a limited database. The sub-10 µm fraction of PCDD-contaminated soil from a 2.45-T manufacturing site in Newark, NJ was isolated by chemical dispersion and gravity sedimentation and was enriched in TCDD from 2.3 ppm in the original soil to 74.4 ppm. Pulmonary absorption and bioavailability were evaluated in female Sprague-Dawley rats (200-250 g) following the intratracheal instillation of 15 mg Newark soil (74.4 ppm and 15 ppb TCDD), laboratory-recontaminated soil (74.4 ppm TCDD), and gallium oxide particles (control). Animals were sacrificed 4, 24, 48, or 96 hr after treatment. Enzyme induction time-dependent with 1150% and 90% increases in aryl hydrocarbon hydroxylase (AHH) and cytochrome P-450, respectively, following 48 or 96 hr exposure with Newark soil at the high dose. AHH induction from this soil was 65% of that from recontaminated soil, suggesting that bioavailability may be relatively high. This will be further evaluated by GC/MS analysis of liver samples. This approach should eliminate the need for default values for pulmonary bioavailability of TCDD from particulates and improve the accuracy of risk assessments of municipal waste incinerator or residential soil exposure (Supported by NJ DEP Grant C29510).
Most of the cyanide present in manufactured gas plant wastes is iron complexed cyanide, which is not absorbed in the gut to a large extent and is not high in toxicity. It is important for risk assessment to use an analytical method that yields an estimate of the amount of physiologically available cyanide in such soil or waste samples. We report here the results of a new method, the Physiologically Available Cyanide method, which estimates the amount of cyanide that can be absorbed in the stomach upon ingestion of cyanide containing soil. Three field samples of soil from former manufactured gas plant sites were analyzed in triplicate by: alkaline leach total, weak and dissociable, and physiologically available methods. A rigorous quality control program was carried out. The physiologically available cyanide method was carried out in a phosphate buffer that maintained a pH of 2.3. On average, the physiologically available method gave results that were 11% of the alkaline leach method, and the weak and dissociable method gave results that were 2% of the alkaline leach method. The implications for human health risk assessment is that total cyanide methods appear to overestimate the risks posed by cyanide in manufactured gas plant waste by an order of magnitude.

Phenanthrene is a major coal tar component found in hazardous waste disposal sites. The purpose of this study was to compare the dermal bioavailability of sandy (S) or clay (C) soil-adsorbed C-phenanthrene with the pure chemical (P). Decreased bioavailability of S was supported by a lower peak plasma concentration of radioactivity and smaller area under the plasma concentration time curve in S versus P and C. However, absorption and elimination half-lives between the groups were similar. Radioactivity was excreted primarily in urine and to a lesser extent in feces in all groups. Tissue concentrations of radioactivity 96 hr post administration of compound were highest in the skin application sites, duodenum and ileum of all groups while C decreased the bioavailability in ileum. In all treatment groups, phenanthrenequinone was the major metabolite detected in urine. Supported by NSF/Industry/University Center for Research in Hazardous and Toxic Substances.
RQs for chronic toxicity are health-related limits for emergency actions required under Section 101 of the Comprehensive Emergency Response, Compensation and Liability Act. Previously, using animal toxicity studies, RQs for lead and lead compounds and their moieties have been calculated. Toxicological studies in mammals, however, demonstrate that accompanying moieties of lead salts have a relatively low order of chronic toxicity in relation to the lead cation. Further, the literature shows that all lead compounds can contribute to the lead body burden. Therefore, using a linear model (U.S. EPA, 1986), an RQ for lead and lead compounds was calculated based on blood lead levels (10 µg/dl) in children associated with subclinical CNS effects. The relative fractional contribution of lead based on the molecular weight of the compound was used to calculate the contribution of individual lead moieties. This presentation will demonstrate that RQs developed for lead and lead compounds, based on animal data, are less protective than those developed from estimations of daily intake derived from the human model.


Chronic health effects associated with lead exposure have been related to elevated lead concentrations in the blood. As a result of recent toxicologic and epidemiologic research, the blood lead concentrations considered to pose a public health risk have decreased substantially recently, a fact which is not yet reflected in the toxicity values published by EPA. Because of this, estimates of blood lead levels are calculated to evaluate exposure to lead at hazardous waste sites.

Blood lead levels have generally been accepted as the best measure of the external dose of lead. A theoretically safe level of intake (TSLI) is calculated based on how much lead an individual’s daily exposure can be to reach but not exceed an acceptable blood lead level. A revised TSLI, calculated by subtracting out background exposures, is used to evaluate the potential health effects resulting from exposure to lead at a site.

Risk assessment of lead in ceramic tableware. A.M. Fau, M.J. DiBartolomeis, R.J. Jackson. California Department of Health Services, Berkeley, CA

An assessment of the health risk from potential intake of lead (Pb) leached from ceramic tableware was performed. The major health concern is effects on the nervous system, especially in children. Blood Pb levels are correlated with Pb intake. Pb concentrations of 10 mg/dl and higher in blood have been associated with deficits in intelligence, behavioral dysfunction, reduced stature, and enzyme inhibition (ferrochelatase, gamma-aminolevulinic acid dehydrase) in children. The Food and Drug Administration’s (FDA) provisional tolerable intake (PTI) for Pb for a 10-kg child is 6.18 µg/day. This is based on the concern blood Pb level of 10-25 µg/dl, a contribution of 50% of total blood Pb from food, an uncertainty factor of 5, a Pb intake from food of 4.7 µg/day, and a blood Pb increase of 0.10-0.2 µg/dl for every mg/day increase in dietary Pb. Monitoring of ceramic flatware in California has shown lead concentrations of 0.6-1.8 ppm in the acidic extract medium. Although these concentrations are lower than the current FDA action level of 7 ppm for hardware, the calculated ingestion of 150-650 mg of Pb exceeded the FDA PTI values. Therefore the ingested Pb from ceramic tableware may be in excess of a health protective dose based on the new toxicological and epidemiological data indicating lower effect levels of Pb in blood that were once thought not to be associated with adverse effects. There is pending regulation in California to evaluate the adequacy of the federal action levels for lead in ceramic foodware and to develop new regulatory levels.


Modifications were made in the Food and Drug Administration’s (FDA) quick color test for leachable lead. The UCOLT can screen large amounts of ceramic ware in a short time frame while also providing information on the health effects of lead. Lead was leached from ceramic ware using citric acid and identified using rhodizonic acid as a chromagen in this quick, qualitative test. The results of tests on ceramic ware in use by Davis and Sacramento, CA, residents are reported. Of 92 items, approximately 6.6% were clearly positive for leachable lead. Approximately 67% of the positive samples were handmade in Mexico. The UCOLT is sensitive to two parts per million (ppm) leachable lead, which is within the FDA limits of 2-7.5 ppm. Ceramic ware with detectable leachable lead levels may pose a health risk to individuals using such items for food storage or eating. The UCOLT can be completed in 20-30 minutes allowing rapid feedback of the test results to participants. Participants are also given an informational pamphlet on the health effects and sources of exposure for lead. The pamphlet is available in English and Spanish.

The EPA often regulates toxic chemicals causing non-cancer health effects using Reference Doses (RfD). RfD are lifetime daily oral chemical exposures that are unlikely to produce adverse health effects. RfD are calculated by identifying the critical effect (the first adverse effect that occurs as the dose rate increases) and dividing the appropriate dose rate by uncertainty factors (UF). UF reflect the scientific uncertainties inherent in toxicity data bases: the greater the uncertainties, the greater the UF. In establishing regulatory guidelines for nitrates, EPA used a 'weight-of-evidence' approach in which the critical effect and corresponding dose rate were derived from a composite of human epidemiologic and clinical studies. This approach was needed because 1) the sample size for each study was small; 2) the critical effect, reversible clinical methemoglobinemia, was observed to occur only in infants 0-3 months old; and 3) although the range of doses tested was large, the no-observable-effect-level (NOEL) 10 mg/L nitrate-nitrogen in drinking water, appeared to be consistent across studies with adequate exposure data. The RfD was calculated to be 1.6 mg/kg/day, using a UF of 1. The process by which the RfD for nitrate was derived and its implications for quantitative risk assessment using exposure data are discussed. The opinions expressed in this abstract are those of the authors and do not necessarily reflect the policies of the U.S. Environmental Protection Agency.

RISK ASSESSMENT OF MANGANESE (Mn) IN DRINKING WATER, J C Du' S P Velazquez' and C Sonich-Mulin'. US EPA, Office of Drinking Water, Washington, DC; Eviron- mental Criteria and Assessment Office, Cincinnati, OH.

Manganese is essential for human health, with a daily intake of 2 to 5 mg suggested by NAS to be adequate. A typical localized American diet provides most of the required Mn, with very little being contributed from drinking water. Mn toxicity in humans has been well documented following inhalation exposure, primarily to miners. Because efficient homeostatic control is exerted over ingested Mn, exposure via the oral route is not generally considered to pose a health threat. Several approaches for the risk assessment of Mn were considered. The only data currently available that are suitable for use in the risk assessment for Mn come from total dietary studies in humans. Based on several of these studies, U.S. EPA verified a reference dose (RfD) of 0.1 mg/kg bw/day. The basis for the RfD and its applications to drinking water will be discussed with particular emphasis on issues of bioavailability.


The EPA sets regulatory guidelines for a variety of toxic chemicals. These guidelines are derived using References Doses (RfD). RfD are estimates of lifetime daily chemical exposures that are not likely to cause adverse effects and are calculated using noncancer endpoints divided by uncertainty factors. Since most toxicity studies do not consider that some chemicals are necessary for normal health, problems may arise when setting RfD for ETEs. Either deficient or excess intake of ETEs may lead to adverse effects. For example, selenium (Se) deficiency can cause cardiomyopathy, while large doses can result in hair loss and nervous system lesions. One possible method of examining this issue is to compare RfD to Recommended Daily Allowances (RDA) or Estimated Safe and Adequate Daily Dietary Intakes (ESADDI) which are set by the National Academy of Science (NAS) to establish adequate nutritional intakes. For Se the RfD is 3 µg/kg/day, while the RDA is 0.9 µg/ kg/day. With chromium, NAS has set an ESADDI of 3 µg/kg/day and the EPA has set an RfD of 1000 µg/ kg/day. When setting regulatory guidelines for ETEs, we believe that both nutritional and toxicity data should be considered in deriving RfD. The opinions expressed in this abstract are those of the authors and do not necessarily reflect the policies of the U.S. Environmental Protection Agency.

COMPARISON OF FISH CONSUMPTION ADVISORIES BASED ON RISK ASSESSMENT WITH THOSE BASED ON FDA ACTION LEVELS. W A Robison and W J Birge. Dept. for Environmental Protection, Franklin, KY and Graduate Center for Toxicology, Univ. of Kentucky, Lexington, KY. Sponsor: L W Robertson.

Many states are faced with issuing consumption advisories for areas which contain contaminated sport fish stocks. Currently, two major methods are used to determine whether an advisory should be placed in effect: 1) application of FDA action levels to contaminant concentrations found in edible portions of the fish; and 2) use of a risk assessment based approach. FDA action levels for fish are available for only a few contaminants of concern in fish. The FDA regulatory levels were developed on a national basis and apply to interstate commerce of fish and fish products. They were not intended to protect local populations of recreational or subsistence fishers that likely consume contaminated fish or shellfish at a rate greater than the national average. Risk assessment may be applied to a greater number of contaminants and may also be used with chemical contaminants found at concentrations below FDA action levels. However, assumptions regarding the population at risk, extrapolation of sample data, and species differences may not be clear. The advantages and limitations of each approach will be discussed using four separate consumption advisories issued for PCBs and chlordane in fish. These case studies involve river systems in Kentucky and surrounding states.

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THE EFFECTS OF SOME COMMON VOLATILE ORGANIC DUMPSITE CHEMICALS (DSCs) ON RAT NEUTROPHIL (PMN) FUNCTION IN VITRO. E M Shobe, P E Ganey, and J A Roth. Dept. of Pharmacol./Toxicol. and Institute for Envirion. Toxicol., Michigan State Univ., E. Lansing, MI.

The activation of PMNs to release reactive oxygen metabolites or hydrolytic enzymes has been implicated in some mechanisms of toxicity. This study characterized the ability of six DSCs to alter the production of superoxide anion or release of β-glucuronidase by PMNs in vitro. The DSCs tested were trichloroethylene, 1,1,1-trichloroethene, carbon tetrachloride, benzene, toluene, and methylene chloride. Glycolen-solubilized pentane PMNs from rats were incubated in vitro at 37°C with DSCs in the range of 0.5 to 5 ppm. DSCs did not significantly alter superoxide production or β-glucuronidase release. In addition, the DSCs were not cytotoxic to the PMNs as indicated by a lack of LDH release. Synergistic effects between the DSCs and known PMN activators were also investigated: the DSCs were again found to have no effect on superoxide production or β-glucuronidase release. These results indicate that the common DSCs used in this study do not affect membrane-associated functions of PMNs in vitro. (Supported by ESO4911)

DEVELOPMENT OF RECOMMENDED PUBLIC HEALTH LEVELS FOR DRINKING WATER IN CALIFORNIA. Y Y Wang, R H F Lam, J P Brown, A M Fan, and A M Miles. Calif. Dept. of Health Services, Berkeley, CA.

The California Safe Drinking Water Act of 1989 requires the development of recommended public health levels (RPHLS) in addition to maximum contaminant levels (MCLs) for drinking water contaminants. RPHLS will provide additional health protection for approximately 75% of the State's population. RPHLS are based solely on health effects and MCLs are to be set as close as practical to RPHLS. The Act also requires MCLs to be reviewed every five years after adoption. As of October 1990, 42 MCLs for organics and 11 for inorganics have been promulgated. Initially, 45 RPHLS (38 organics and 7 inorganics) will be proposed. Ten of the RPHLS will be lower than existing MCLs: benzene, chloroform, 1,2-dibromo-3-chloropropane, 1,3-dichloropropene, ethylene dibromide, hepachlor epoxide, perchloroethylene (PCE), toluene, trichloroethylene (TCE), and vinyl chloride. RPHLS can not be enforced if the chemical can not be detected at that level. Analytical method detection limits (MDLs) need to be at least 5 to 10 times lower than the enforceable levels so that monitoring may be effectively conducted. This paper compares the RPHLS to the MDLs for data gap investigation. A preliminary analysis indicates that among the 10 chemicals, only benzene, PCE, and TCE have methods available with adequate MDLs for monitoring purposes.

EVALUATION OF POTENTIAL SOURCES OF 1,2,8,9-TCCD IN AQUATIC BOTA FROM NEWARK BAY. R Harris, B Finley, R Wemming and D Paustenbach, Chemicst, a Division of McLaren/Hart, Irvine, CA. Sponsor: D J Paustenbach

A recent report indicated that elevated levels of 1,2,8,9-TCCD had been measured in the hepatopancreas of several lobsters (Honarius americanus) and blue crabs (Callinectes sapidus) located in and around Newark Bay. Although a recent fingerprinting study has indicated that there are multiple sources of PBDDs and PCBs in Newark Bay, it has been suggested that the presence of 1,2,8,9-TCCD in the biota samples is related to a former manufacturing facility located several miles upstream on the Passaic River. This paper presents a review of the PCB/PDCF literature with respect to the sources of 1,2,8,9-TCCD in the environment. The analysis indicates that 1,2,8,9-TCCD is associated almost exclusively with combustion products of wood and waste incinerators rather than chemical synthesis or manufacturing (as originally suggested). Further, 1,2,8,9-TCCD does not appear to be a product of chemical reactions that produce PBDDs/PCDFs such as pulp and paper mill processes and the synthesis of 2,4,5-T and 2,4-D. Accordingly, it is likely that the presence of 1,2,8,9-TCCD in sediments and in aquatic biota collected from Newark Bay is due to combustion sources rather than the former manufacturing facility.

INTERIM REPORT OF THE 65 WEEK FINDINGS OF THE CHRONIC TOXICITY/CARCINOGENICITY STUDIES FOR DENVER'S POTABLE REUSE DEMONSTRATION PROJECT. G Wolfe, B Mosbach, K Widdowson, A Condle, Hazleton Washington, Vienna, VA; D Denver Water Dept., Denver, CO; and U S Army, Dugway, UT.

Chronic Toxicity/Carcinogenicity studies are being conducted in F-344 and B6C3F1 mice to evaluate the relative health effects of highly treated reclaimed water derived from secondary wastewater compared to Denver's high-quality drinking water. Water samples of reclaimed water (RD) and ultralfiltrated water (UF, rat only) and Denver's drinking water (FH) are concentrated 5000 X using XAD resins. Samples are then diluted to 500 X or 150 X using distilled water. After dilution of the FH sample, chloroform, bromodichloromethane, and 1,1-dichloropropene are added to represent volatile organic carbons (VOC's) found prior to and lost during the XAD resin concentration process. No VOC's are added to the RD or UF samples since no VOC's are consistently identified in the RD or UF samples. The first 65 weeks of the 104 week studies have been completed and no findings of toxicological significance have been noted in survival, body weights, food and water consumption, clinical observations, and clinical, gross, and microscopic pathology. There has been a slight decrease (5 - 15%) in weekly water consumption in the rat and mouse FH groups which is believed to be the result of the V0C's and not of toxicological significance.

An evaluation of copper (Cu) is undertaken when levels of 4-60 mg/l were found in the drinking water ('blue water') of new homes in Contra Costa County, CA. The secondary maximum contaminant level (MCL) for Cu is 1.0 mg/l and the proposed MCL is 1.3 mg/l. The proposed MCL is derived from a human study on healthy individuals who are supposed to be able to increase biliary copper excretion significantly when subjected to increased copper intake. High risk groups (infants and children, carriers of Wilson's disease, patients with liver disorders including primary biliary cirrhosis, glucose-6-phosphate dehydrogenase deficient individuals and kidney dialysis patients) were not considered. The daily intake of copper contaminated drinking water at the levels mentioned above by normal individuals can cause gastrointestinal irritation, nausea, abdominal cramps, vomiting, and diarrhea. As an essential element, the dietary intake of Cu is 0.5 - 3.0 mg/day.

The source of 'blue water' has been attributed to the green colored corrosion products found inside both household copper pippings and the copper laterals. The corrosion products were electrolyte-chlorine-induced created by solubility flux (which contains zinc chloride and/or ammonium chloride) and/or the hypochlorite used to disinfect the laterals. The problem arose because initial optimum conditions for the formation of the protective cuprous oxide scale inside the pipes were not established due to unintentional water stagnation in the laterals and household piping systems. Residents with > 1.3 mg/l Cu in their drinking water are advised to use bottled water.

NATURALLY OCCURRING SEAFOOD TOXINS. F.E. Ahmad, Food and Nutrition Board, Institute of Medicine (IOM), National Academy of Sciences, Washington, DC.

A committee of the IOM evaluated the extent of public health risks to the US population from consumption of fishery products containing natural toxins. It reported that toxic fish and shellfish are indistinguishable from nontoxic animals by sensory inspection, and that toxins are not destroyed by cooking or processing. Incidents of illness reported to CDC for 78-87 were limited to cigutera, scrobroid fish poisoning (SP) and paralytic shellfish poisoning (PSP). Intoxications reported earlier included puffer fish poisoning (PPP) and neurotoxic shellfish poisoning (NSP). Diarrhetic shellfish poisoning (DSP) and amnesic shellfish poisoning (ASP) represent potential risks. Except for NSP, natural intoxications are highly regional and species associated. Ciguatera is due to consuming certain species of fish from tropical waters. It was responsible for nearly half of reported outbreaks to CDC in 78-87; scrobroid poisoning caused similar number of outbreaks, but was more widespread in occurrence. Tuna, mahimahi and blue fish were implicated as the major cause of poisoning. The disease is generally mild and self resolving and symptoms can be ameliorated by antihistamine drugs. FSP was reported as a minor cause of illness in 78-87, with only two deaths. Although no other natural seafood intoxication has been reported recently in US, the potential for their occurrence is real. Recommendations for control were given.

EFFECTS OF LAGENIDIUM PEST CONTROL AGENT ON FRESHWATER ORGANISMS. L.B. Nestrud, L.R. Anderson and J.A. Dellingier. Center for Lake Superior Environmental Studies, University of Wisconsin, Superior, WI.

Genetically engineered microbes may be used as pest control agents, and therefore, must be tested for environmental toxicity. Protocols were developed for testing zoospore producing fungi using Lagenidium giganteum as an example. Non-target species were exposed to the zoospores in acute (4 d) and chronic (7 d) protocols. Species tested included the standard tests for cladocerans (Ceriodaphnia dubia, Daphnia pulex, and D. magna) and fathead minnow (Pimephales promelas). New methods were developed for exposing mosquitoe (Anedes sp.), cyclopoid copepods, cironomids (Chironomus sp.), oligochaetes (Lumbricus sp.), snails (Physa sp.), and ostracods. Twelve test procedures were completed and each included concurrent bioassays with the target species, mosquitoees (LC50s ranged from 9000 z/ml zoospores/ml water) at 24 h to 250 z/ml at 96 h). Fathead minnow tests showed no apparent effect on adults or larval development.

Chironomids were infected by the zoospores at 5000 z/ml. Ceriodaphnia acute tests yielded LC50s as low as 24,000 z/ml, and the chronic tests yielded LC50s as low as 5,500 z/ml. Daphnia were also susceptible non-target organisms with LC50s as low as 9,000 z/ml for D. pulex and 8,000 z/ml for D. magna. No effects were noted at 50,000 z/ml for the other test organisms.

(Total Threshold Limit Concentrations (TTLCs) provide a legal basis in California for classification of waste as being hazardous. TTLCs were derived from fish bioaccumulation data, and are not health-based values. However, TTLCs are often used for remediation of organochlorine pesticides in farmland being developed for residential use. With DDT, use of TTLCs is especially problematic because DDT occurs in soil above the TTLG of 1 ppm statewide. Risk estimates for DDT, with soil as the exclusive medium of exposure, were calculated using dermal exposure and soil ingestion rates of 450 mg/day or 100 mg/day, respectively (Sedman, Environmental Health Perspectives, 79:291-313, 1989; USEPA, Risk Assessment Guidance for Superfund, Interim Final, 12/89). The results show that remediation of DDT to the TTLG of 1 ppm is overconservative, with a risk of 6x10^-6. Consequently, remediation of DDT to the TTLG can waste resources and reduce housing costs with no benefit to public health. In contrast, remediation of aldrin, dieldrin, chlordane, heptachlor, lindane, mirex, or toxaphene to their TTLGs is not health protective, with risks up to 2x10^-4 (dieldrin). Therefore, TTLGs are inappropriate for soil remediation, due to waste of resources or non-attigation of significant health risks. The necessity of TTLGs clearly shows that health-based risk assessments are necessary guidance tools for soil remediation.

A total of 300 workers exposed to pesticides in the pesticide formulating industries and 300 workers not involved in Egypt were examined. A significant number of clinical signs and symptoms were reported in the exposed groups. Among these were peripheral neuritis, the severity of which was related to the duration of exposure to pesticides. 26% of those exposed were also found to have definite EEG changes, as compared to 2% in the control population, with a significant number showing optic nerve abnormalities. Psychiatric analyses were present in a significant number of exposed workers as compared with the control group. A significant number of pesticide workers exhibited asthmatic manifestations (20% compared to 5% in the normal population) and about one-third presented pulmonary function changes. Other findings related to atherosclerosis, hypertension with cardiac asthma, enlargement of the liver, some of them with tender liver more characteristic of pesticide intoxication than the fibrotic sharp liver of bilharziasis. About 90% of the exposed groups presented with abnormal liver functions and significant number with ECHO changes. The disorders vary with the extent of exposure, the type of pesticide and the health status.

737 INDUCTION OF HEAT SHOCK PROTEINS IN ISOLATED HEPATOCYTES OF WINTER FLounder, PSEUDOLEPONECTES AMERICANUS. SM Nelson, Science Applications International Corporation, and SM Baks, US Environmental Protection Agency, Narragansett, RI.

Production of heat shock, or stress, proteins (hsp's) have been proposed as a biomarker to measure environmental stress. We have shown that isolated hepatocytes from the winter flounder, Pseudopleuronectes americanus, produce heat shock proteins in response to stress. Hepatocytes were isolated from fish acclimated at 15° and 22°. Cultures, in suspension, were incubated at 15°, 22° and 30° for 1 hour before the addition of 5-methionine. Cells were removed 1 hour and 2 hours post labelling, checked for viability and lysed. Analysis of the cell lysates by SDS-polyacrylamide gel electrophoresis and autoradiography indicated the induction of a 70 kilodalton class of hsp at 30° in fish acclimated at both temperatures. These experiments indicate that isolated winter flounder hepatocytes exhibit an adaptive response to stress. This system may provide a sensitive method to investigate potential effects of environmental contaminants in eco-toxicological assessments.

736 PROPERTIES OF 5-AMINOLEVULINATE DEHYDRATAS FROM FISH LIVER. B A Connor 1, B A Foley 2, B G Sasse 3, Program in Toxicology, The University of Maryland at Baltimore, Baltimore, MD and The Rockefeller University, Department of Medicine, New York, NY 10021.

Selective inhibition of mammalian 5-aminolevulinic acid dehydratase (ALAD) porphobilinogen synthetase, EC 4.2.1.24 by lead (Pb) serves as a potentially important biological marker of chemical exposure and injury. Water-borne exposure of Pb has been shown to inhibit ALAD in the blood and liver of several fishes. This study was undertaken to evaluate the relative susceptibility of fish hepatic ALAD to Pb inhibition. In vitro, as determined by a combination of biochemical and immunological techniques. A method for the colorimetric determination of ALAD was applied to fish hepatic tissue. The reaction rate and assay sensitivity increased as a function of temperature and duration of incubation. Lineeweaver-Burk analysis of fish hepatic ALAD activities indicated a Km of 4x10^(-4) M ALA and a Vmax of 2.33 nmol Pb/h/mg of protein. The IC50 (concentration causing half-maximal inhibition) for Pb inhibition of fish liver ALAD activity appears to be higher than those values reported for mammals by a factor of 10. Further in vitro studies demonstrated no activation of fish hepatic ALAD by zinc and only slight inhibition by EDTA. Rabbit polyclonal antibodies directed to purified mouse and human erythrocyte ALAD did not cross-react with the fish enzyme, suggesting that the fish enzyme is immunologically distinct from the mouse and human ALAD. The kinetic data and findings concerning activation and inhibition presented above support a similar conclusion that fish hepatic ALAD is biochemically distinct from the mammalian enzyme.

738 INTERACTIVE EFFECTS OF PENTACHLOROPHENOL AND A NATURAL STRESS FACTOR IN THE RED ABALONE AS MEASURED BY IN VIVO 31P NMR SPECTROSCOPY. R S Tiedema 1, R Kauten 1, and D G Crosby 1. 1Aquatic Toxicology Program, Institute of Marine Sciences, University of California, Santa Cruz, CA; 2Department of Food Science and Technology, University of California, Davis, CA; 3Department of Environmental Toxicology, University of California, Davis, CA.

In vivo NMR spectroscopy represents a powerful tool for investigating both the sublethal effects of toxicants on intact aquatic organisms and the interactive effects of natural stress factors. Using a teflon and plexiglass chamber, we exposed abalones (Haliotis rufescens) to both pentachlorophenol (PCP), in flowing seawater at a sublethal concentration (1.2 mg L^-1), and periods of air emergence. While PCP is both a general biocide and an uncoupler of mitochondrial oxidative phosphorylation, air emergence simulated a natural stress imposed on intertidal organisms by daily tidal changes. Phosphagen changes in foot muscle were followed during 3-h exposure, 40-min emergence, and 1-h recovery periods using 31P surface probe localized NMR spectroscopy; the foot is easiest to focus on and necessary for survival. During exposure, intracellular phosphoarginine (PA) levels decreased by 50%, being offset by a rise in inorganic phosphate (Pi). Also, the spectral Pi peak shifted, indicating intracellular acidification. During subsequent air emergence, both PA declined and Pi increased rapidly, indicating an additive effect. During recovery PA, Pi, and pH rapidly returned to pre-emergence levels, then slowly returned to pre-exposure levels. Interactions between pollutants and natural stresses may enhance deleterious environmental actions, explaining the unanticipated effects that are often observed.
An experimental protocol was developed using altered environmental oxygen concentrations to increase gill water flow while maintaining control levels of gill blood flow. Subsequently, gill blood flow was increased as water flow decreased. This protocol was used as a probe for measuring the influence of ventilation volume (Vg) and cardiac output (Q) on chemical flux at the gills of rainbow trout. Gill flux rates of a low Log Octanol/Water Partition Coefficient (Log P) compound, butanol, and a high Log P compound, decanol, were measured in vivo under varying gill blood and water flows. Changes in Q and Vg were measured directly and continually during control, hypoxia, and post-hypoxia. Butanol gill flux rates increased over control flux levels with elevations in Q during post-hypoxia, but butanol flux did not change during hypoxia when Vg was elevated. Decanol gill flux increased to the greatest extent during hypoxia with maximum increase in Vg. The trout gill flux of the low Log P compound was gill blood-flow limited while the flux of the high Log P compound was gill water-flow limited.

The toxicity of a commercial mixture of polychlorinated biphenyls (PCBs), Aroclor 1248, to embryos-larval stages of the fathead minnow (Pimephales promelas) was investigated under static-renewal conditions. Solutions (10 to 100 µg PCBs/L) were changed daily and utilized 100 mg/L of acetonitrile as a carrier solvent. Exposures began within 12 h of fertilization and continued for 8 days (approx. 4 d post-hatch). Initial experiments indicated that the use of small solution volumes (40 and 80 ml) produced less toxicity than use of a larger volume (400 ml). Analysis of water confirmed that PCB loss due to adsorption, volatilization, and fish uptake was greater (% basis) for the smaller test volumes. Consequently, all subsequent tests used 400 ml volumes to avoid underestimation of toxicity. Aroclor 1248 at concentrations up to 100 µg/L produced no embryonic mortality. First signs of toxicity were noted 2 d post-hatch when hyperactivity and abnormal swimming behavior were observed. Narcosis and death followed later at the highest PCB concentrations. Considerable teratogenicity was observed in test organisms. LC50 values exceeded the water solubility of Aroclor 1248 (~52 µg/L) but EC50 (teratogenic LC10 values were below this level. In addition, effects of PCBs on fish growth (length) was determined at test termination following preserved specimens.

Tissue slices are a useful model for examining in vitro metabolism and toxicity due to their relative ease of preparation and the ability to combine histopathological with biochemical endpoints of toxicity. Aquatic species are being used increasingly in toxicity studies as bioindicators for environmental pollutants and for mechanistic research. In the present studies, rainbow trout liver slices were prepared and treated with allyl formate (AF), a known trout hepatotoxicant, and one of its metabolites, allyl alcohol (AA). Exposure to both AF (0.1 mM) and AA (0.1 mM), its major metabolite, for four hours caused GSH levels to be depleted to 34% and 16% of control levels at four hours, respectively. In contrast, following exposure to either AF or AA at concentrations as high as 1.0 mM, slice ATP levels remained comparable with control levels at four hours. Corresponding histopathology indicated the appearance of isolated pyknotic nuclei in the slices treated with AF (1.0 mM) and AA (1.0 mM) at incubation times as early as four hours. Subsequent studies are being carried out to longer time points in order to allow development of histopathological lesions and associated biochemical changes. In addition, various chemical tools will be utilized in order to alter the toxic response and further explore the mechanisms of AF and AA toxicity.

The biological function of copper metallothionein (CuMT) is poorly understood. In the blue crab, a direct correlation exists between levels of CuMT and Cu(II)-hemocyanin during the molt cycle (Engle and Brouwer, Biol. Bull. 173, 239–251, 1987). This phenomenon makes it possible to study the involvement of Cu(II) in metallothionein activation and metal detoxification. To further understand the role of MT in metal-regulatory processes, we have determined how many different Mr isoforms are present in blue crabs and how these different isoforms respond to elevated dietary levels of copper and cadmium. Anion-exchange HPLC showed two MT isoforms in control (CuMT-1, CuMT-2) and cadmium-treated crabs (CuMT-1, CuMT-2) while three forms (CuMT-1a, CuMT-1b, CuMT-2) were present in copper-treated animals. CuMT-2 was induced to a much greater extent than CuMT-1a or CuMT-1b. In the case of cadmium, however, the induction of CuMT-1 was much more significant than that of CuMT-2. Purification of the metal-free MTs by reverse phase HPLC and amino acid analysis showed that each form from metal-treated animals had a unique amino acid composition suggesting that regulation of crab metallothionein isoforms may be metal-specific.
Isolated fish hepatocytes are being explored as non-mammalian models for toxicological studies and to investigate the impact of environmental contaminants on aquatic species. Although fish hepatocytes are relatively easy to maintain in suspension culture, attachment of these cells to tissue culture surfaces has been difficult to attain. Methods were developed that resulted in firm attachment of winter flounder hepatocytes to 96 well tissue culture plates treated with flounder biomatrix prepared from homogenized flounder livers. Hepatocytes were isolated by a two stage perfusion and cells were suspended in Dulbecco’s Modified Eagle Media and Williams’ Media E (1:1) supplemented with antibiotic/antimycotic and insulin, transferrin and selenium. Cells were plated at densities from $1 \times 10^5$ to $3 \times 10^5$ cells/well. Viability was assessed by the neutral red uptake assay. These experiments indicated that plated primary cultures of hepatocytes can be established from winter flounder and that they can be used in cytotoxicity assays such as the neutral red assay.

Immunosuppression in B6C3F1 mice following oral exposure to 7,12-dimethylbenz[a]anthracene (DMBA) has been demonstrated by this laboratory. To evaluate the potential contribution of distribution and macromolecular binding to immunosuppression, the biodistribution and covariant binding of DMBA in selected tissues of B6C3F1 mice was determined following a single oral gavage of 5 μCi $^{3}H$-DMBA in corn oil. The elimination of $^{3}H$-DMBA from mice was relatively rapid. At 24 hours, greater than 85% of the radioactivity could be recovered in the urine and feces. Plasma radioactivity peaked at 6 hrs, while major organs showed maximum levels 6-12 hrs after the oral gavage. Lymphoid tissues accumulated the highest levels of $^{3}H$-DMBA per gram of tissue. A concentration of 1.5 ± 0.3 μCi/g and 1.3 ± 0.4 μCi/g tissue was observed in the mesenteric lymph nodes and the Peyer’s patches respectively. Radioactivity in the bone marrow did not accumulate until after 9 hours and then diminished by 24 hours. Covalent binding measured in nmol $^{3}H$ per mg protein, was found to peak at 9 hours in all tissues with a range of 4.9 ± 0.5 nmol/mg in the liver to 1.4 ± 0.4 nmol/mg protein in the spleen. Furthermore, increased binding was found in the spleen relative to the lung and liver at all time periods. Thus, lymphoid organs appear to be important targets of DMBA distribution and binding in B6C3F1 mice. The role of covariant binding in the immunosuppressive effects of DMBA is being further investigated.
A major concern with extrapolation of immunotoxicological data from experimental animals to man is the source of immunocompetent tissue used for evaluation of immune status. In man, blood is the primary tissue available while for experimental animals, spleen and lymph node cells are used. To determine the relationship between function in immune cells of blood and spleen, studies were conducted in Fischer rats using CTX as a prototype immunosuppressant. Rats were treated with CTX for either 4 days at doses between 2 and 35 mg/kg or 14 days at doses between 0.1 and 4 mg/kg. Immune status was assessed by measuring mitogen-induced lymphoproliferation (LP), NK cell activity and enumerating the percent and number of immune cells using monoclonal antibodies. Overall, LP measured in blood cells was equal to or more sensitive to suppression by CTX than spleen cells. NK cell activity of spleen cells was slightly more sensitive to CTX than peripheral blood cells. Surface marker studies demonstrated that while total cell numbers were decreased, in the spleen and peripheral blood, the percentages of cells with markers from C57Bl/10 (T cells, Vβ8 helper) and C57Bl/6 T cytotoxic were not affected. The studies to date indicate that the cells from the blood provide similar information as those from the spleen.

**IMMUNOTOXICITY ASSOCIATED WITH A THERMO-TOLERANT STRAIN OF PEMICILLUM CITRINUM.** Y C Yang, D N McMurray, B A Clement, J P Stack, and J D Phillips. Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX.

*Pemucillium citrinum* has been frequently isolated from flours, cereals, dried beans, peanuts, pecans, salami, country cured ham, and other foodstuffs. Although *P. citrinum* contamination is most commonly associated with colder climates, a thermostolerant strain of this fungus was recovered almost from peanuts collected in the peanut growing regions of Senegal, West Africa. In preliminary studies, cultures of this isolate were found to be significantly immunotoxic. The culture medium resulted in significant inhibition of 3H-thymidine incorporation into DNA using the in vitro lymphocyte blastogenesis assay with concanavalin A, phytohemagglutinin, and lipopolysaccharide as mitogens. *P. citrinum* culture filtrate (0.2 ml) administered to mice by gavage every other day for 10 days, did not significantly alter immune function. However, a challenge with Listeria monocytogenes resulted in a higher infection rate in treated mice compared to untreated controls, which indicates a cell-mediated immune alteration. The immunotoxicity was found to be closely associated with a strong antibiotic activity. A bacterial assay was used during the fractionation and isolation of the toxic principle. The toxic agent was chemically identified as citrinin (confirmed by GC/MS and FTIR). Subsequent studies with pure citrinin resulted in a predictable immunotoxic response (Supported by USAID Project 02-50305-2 and TARA H6215).

**FUNCTIONAL AND PHYSICAL ATTRIBUTES OF ANTI-MOUSE INTERFERON-Y RECEPTOR ANTIBODY.** R D LeClair, R B Lorsbach, A Y Cheng, M Basu, and S W Russell. University of Kansas Medical Center, Departments of Pharmacology / Toxicology / Therapeutics, and the Cancer Center, Kansas City, KS

Sponsor: A Parkinson

Due to the myriad biological activities attributable to IFN-γ there is great interest in its potential therapeutic uses, as well as that of agonists and antagonists. Therefore, antibodies specifically interacting with the mouse IFN-γ receptor were produced. Reactivity was confirmed (1) using a recombinant secreted form of the mouse IFN-γ receptor in the solid phase of an ELISA, (2) by demonstrating their capacity to immunoprecipitate the secreted receptor, and (3) by flow cytometric analysis. Three antibodies, a goat polyclonal and two rat monoclonals, inhibited binding of [35P]-IFN-γ to recombinant receptor expressed in rat cells. The polyclonal and one of the two monoclonal antibodies repressed biological function induced by IFN-γ, as assessed in a macrophage-mediated cytotoxicity assay. The third, a monoclonal IgM, did not diminish the induction of cytotoxicity by IFN-γ. Eight other rat monoclonal antibodies reactive with the receptor did not inhibit either [35P]-IFN-γ binding or macrophage activation by IFN-γ. One of these monoclonals and the polyclonal antibody repressed activation of macrophages by lipopolysaccharide alone. No significant cross-reactivity with human or rat cells was observed, as evaluated by flow cytometry and western blotting. Trafficking of and signal transduction by the native and transfected receptor are currently being investigated with these antibodies. Supported in part by NIH Grant CA38779, Office of the Surgeon General, U.S. Army, Contract DAKF19-78-H-0039, and the Wilkinson Endowment for Cancer Research.
571 USE OF STAPHYLOCOCCAL ENTEROTOXIN A-INDUCED INTERLEUKIN 2 PRODUCTION AS AN INDICATOR OF IMMUNOTOXICITY. LL Reid, K L Hastings, AJ Gandolfi, M Van Ert. Department of Anesthesiology, University of Arizona, Tucson, AZ.

Suppression of mitogen-induced splenocyte interleukin 2 (IL-2) production can be used as an indicator of immunotoxicity. Staphylococcal enterotoxin A (SEA) is the most potent in vitro inducer of IL-2 production described to date. An in vitro system was used to measure impairment of SEA-induced IL-2 production using splenocytes from female C57BL/6 mice dosed with either cyclosporin A (50 mg/kg/day, 14 days), benzene (220, 440, 880 mg/kg/day, 14 days), or vehicle. Splenocytes were stimulated with either Con A or SEA and proliferation determined by 3H-thymidine biotogenesis assay. Benzene and cyclosporin dosed animals demonstrated significant decreases in splenocyte proliferation. Inhibition of IL-2 production was determined by incubating splenocyte culture supernatants with IL-2 dependent cytotoxic T-cells (CTLL-2), pulsing with 3H-thymidine, harvesting the cells, and determining incorporated label by liquid scintillation counting. Cell proliferation was inhibited by benzene in a dose-dependent manner. This effect was more clearly demonstrated in SEA-induced splenocytes, indicating a more sensitive assay than seen with Con A-treated splenocytes. (NIH GM 34788; T01 OH 07223).

572 PENTAMIDINE ISETHIONATE MODULATES THE CONTACT HYPERSENSITIVITY RESPONSE IN MICE. B L Blaylock, Y Kouchi, E C Comment, F Corsini, G J Rosenthal and M J Lustig. Immunotoxicology Group, National Institute of Environmental Health Sciences, Research Triangle Park, NC

Pentamidine isethionate, a diamidino compound, has been shown to down-regulate interleukin 1 (IL-1) release. We have demonstrated that pentamidine, applied topically, reduces the ear swelling reaction to oxazolone challenge in BCCSF1 mice. This application must be given 1 hr before the challenge dose and must be applied within one hour of challenge. The application must also be topical, as intravenous injection had no effect on reduction of ear swelling. In dose response studies, we achieved 50% reduction in ear swelling with 20 mg pentamidine per ear. When compared to dexamethasone, a 25-fold greater pentamidine concentration was needed to produce the same degree of suppression as dexamethasone. Pentamidine did not affect the transport of antigen from the challenge site to the draining lymph node as measured by fluorescein isothiocyanate transport. However, there was a 30-40% reduction in la expression on Langerhans cells from pentamidine-treated mouse ears compared to control. These studies demonstrate the potential usefulness of topically-applied pentamidine in controlling hypersensitivity reactions. Studies are ongoing to directly determine the mechanism of action and the relationship of IL-1 to that mechanism.

574 COVALENT BINDING OF A HALOTHANE METABOLITE TO A 30 KD PROTEIN IN GUINEA PIG LIVER SLICES. AP Brown, KL Hastings, AE Cress, AJ Gandolfi, K Brendel. Arizona Health Sciences Center, Univ Arizona, Tucson, AZ.

The anesthetic halothane (H) is bioactivated to an intermediate that trifluoroacetates liver protein possibly resulting in altered protein antigenicity which can lead to a hypersensitivity response in the liver. An in vitro system was used to study the formation of adducts to protein following exposure to H. Liver slices (1 cm x 300 m) from adult male Hariley guinea pigs (600-800 g; n=3) were exposed to 3H-C=H (0.5 μCi, 1-7 mM) in 95% O2 for 12 hr. The slices were then sonicated and proteins were collected by precipitation. Proteins were resolved on polyacrylamide gels and the protein bands examined for radioactivity by scintillation counting and autoradiography. Greater than 80% of detectable radioactivity was localized in the 20-30 kD range with the majority bound to a 30 kD protein. SDS-PAGE analysis of the microsomal membrane fraction from these slices did not detect a protein of that MW nor any radioactivity in the 20-30 kD range indicating the 30 kD protein is non-microsomal. Western immunoblot analysis using antibodies against trifluoroacetylated albumin demonstrated weak recognition of the 30 kD protein while the well documented antigens in the 50-100 kD range were well recognized. It is of interest that the major neoantigens have only low amount of covalently bound radioactivity while this 30 kD protein has the majority of the covalently bound radioactivity but little neoantigenic properties. (NIH GM34788)
TRIPHENYLHYDROXIDE (TPTH) IMMUNOTOXICITY - I: ESTABLISHMENT OF A NOEL IN MICE BASED UPON THE IMMUNE SYSTEM AS A TARGET ORGAN.


To identify changes in immunity associated with dietary exposure to TPTh, two experiments were conducted. In the first experiment, groups of male and female Wistar rats were fed diets containing 0, 0.1, 0.5, 2.5, 50, or 125 ppm TPTh for 28 consecutive days. Immune function assays were performed on groups of 10 rats/group on day 29 and on 5 rats/group after a 28 day recovery. The net effects of TPTh exposure in both sexes were limited to reductions in body weight at 25 and 50 ppm dose levels. At the 125 ppm dose, there was a reversible suppression of splenic T- and B-cell numbers in both males and females. There was a marked difference in effects on serum immunoglobulin (Ig) levels between sexes. TPTh had no effect on serum IgG levels in males but suppressed the IgM levels in females at doses of 25 ppm or greater. Positive control mice treated with cyclophosphamide exhibited significantly lower lymphoid organ weights, cellularity, and numbers of T- and B-cells. Reversible reductions in relative spleen weight in both sexes (50 ppm TPTh or greater) and lower serum IgG levels in females without other immune effects were the most sensitive indicators of exposure. On this basis, a NOEL for 28 day dietary exposure to TPTh was determined to be 25 ppm for male and 5 ppm for female mice.

TRIPHENYLHYDROXIDE (TPTH) IMMUNOTOXICITY - II: ESTABLISHMENT OF A NOEL IN RATS BASED UPON THE IMMUNE SYSTEM AS A TARGET ORGAN.


To identify changes in immunity associated with dietary exposure to TPTh, two experiments were conducted. In the first experiment, groups of male and female Wistar rats were fed diets containing 0, 0.1, 0.5, 2.5, 50, or 125 ppm TPTh for 28 consecutive days. Immune function assays were performed on groups of 10 rats/group on day 29 and on 5 rats/group after a 28 day recovery. TPTh had no effect on body weight, food consumption and induced no gross toxicity in any animals. Except for a reversible 17% decrease in spleen weight at the highest dose, TPTh had no significant effect on lymphoid organ weight in either sex. Splenic T- and B-cell numbers were unaffected by TPTh at any dose level in either sex. Rats treated with diocetyltn dichloride (positive control) exhibited significant lymphoid depletion and reductions in splenic lymphocyte numbers. In male rats, peripheral blood white cell counts were decreased only at the 125 ppm TPTh dose. Platelet counts were increased at 25 ppm TPTh or greater. In female rats, the most sensitive immune-related indicator of exposure was a reversible decrease in serum IgG levels at doses of 50 ppm TPTh or greater. Based upon platelet counts, the NOEL for male rats was 5 ppm with effects on the immune system seen only at 25 ppm. Based upon IgG levels, the NOEL in female rats for 28 day dietary exposure to TPTh is 25 ppm.

THE IMMUNOTOXICITY AND MYELOOTOXICITY OF 2’,3’-DIDEOXYXIDEHYDROTHYMIDINE (D4T) IN MICE. GM Shoop, DA Clark, KA Gabreth, and GI Rosenthal.

The nucleoside analog, 2’,3’-dideoxyxidedyhydrothymidine (D4T) has been shown to be a potent inhibitor of HIV replication in vitro. Clinical trials are under way to determine its efficacy in human subjects. The present study was undertaken to assess the immunotoxicity and myelotoxicity of D4T in C57BL/6 and DBA/2 mice. Female mice were administered D4T orally, daily for 30 days. The dose levels were 125, 250, and 500 mg/kg/day. After exposure the following immune function assays were performed: peripheral blood hematologic, spleen antibody-forming cell response to sheep red blood cells, the cytotoxic T lymphocyte assay, splenic cell subunit analysis, lymphocyte proliferative response to mitogens and allogeneic spleen cells, the natural killer cell assay, and bone marrow cellularity, and myeloid and erythroid, progenitor cells. Mice exposed to 500 mg/kg showed increased spleen and liver weights. Peripheral blood hematologic showed slight, but statistically significant, erythroplasia (88% of control) and decreased hematocrit (94% of control), at the high dose level for both strains of mice. Effects were also seen on bone marrow function. For the C57BL/6 mice, nucleated cell numbers were decreased, down to 60% of control, for the high dose level. Erythroid colonies per 10^4 cells were decreased, down to 53% of control, for the high dose level. No effects were seen on the myeloid colonies for the C57BL/6 mice. For the DBA/2 mice, no effects were seen on any of the bone marrow function parameters. No effects were seen with any of the other immune function assays. [NIH contract NO1-ES-95238]

IMMUNOTOXICOLOGIC EVALUATION OF A PURINE NUCLEOSIDE PHOSPHORYLASE INHIBITOR IN THE CYNOBOLUS MONKEY. M R Bleaving and J D Alvey.

Pathology and Experimental Toxicology, Parke-Davis Pharmaceutical Research, Ann Arbor, MI.

Children congenitally deficient in purine nucleoside phosphorylase (PNP) exhibit profound impairment of cell-mediated immune function. The potential immunotoxic effects of CI-972, a chemical inhibitor of PNP, were therefore of interest in the drug's preclinical development. Cynomolgus monkeys (Macaca fascicularis) were administered 0, 50, 100, or 250 mg/kg/day of CI-972 orally for two periods of 14 days. After the first dosing period, peripheral blood lymphocytes were evaluated for CD-4^+ , CD-8^+ , and CD-20^+ cells, and response to concanavalin A and pokeweed mitogen. Dosing was discontinued for 4 weeks and each animal experimentally sensitized with diphtheria toxoid, tetanus toxoid, Candida albicans, and Trychophyton mentagrophytes. On Day 12 of the second dosing period, percutaneous challenge was performed using the same four antigens. Delayed-type hypersensitivity (DTH) responses were measured 24 and 48 hours later. Monkeys treated with CI-972 were able to initiate normal responses following in vitro (mitogen-induced proliferation) and in vivo (DTH) stimulation. The percent and absolute numbers of T- and B-lymphocytes were also comparable to control. Based on these three measurements of immune function, CI-972 was not immunotoxic.

A variety of chemicals, including Nicl2, induce hypothermia in laboratory rodents. Such animals choose a cool ambient temperature (Tc) if possible and can survive exposure to higher chemical doses than animals prevented from lowering core temperature (Tc). This study investigated the effects of preventing hypothermia on the immunotoxicity of Nicl2. C57BL/6J mice were injected i.m. with saline or 18 mg Nicl2/kg and maintained at room temperature (RT) or 32°C for 24 h. Tc was measured at 0, 1.5 and 24-h. Saline-injected mice had normal Tc at all time points, regardless of Tc. Maintaining mice at 32°C prevented development of hypothermia in Nicl2-treated mice; however, mice kept at the elevated Tc were moribund 1.5 h post-injection whereas Nicl2-injected mice at RT had a normal appearance. Body, spleen and thymus weights were not affected by either Nicl2 or Tc. Natural killer (NK) cell activity was suppressed by Nicl2 injection at 22°C and 32°C, whereas proliferative responses of mitogen-stimulated T-cells were elevated in Nicl2-injected, RT mice but were not affected at 32°C. These results suggest that blocking development of Nicl2-induced hypothermia can alter certain effects of Nicl2 on the immune system. (This is an abstract of a proposed presentation and does not reflect EPA policy.)

760 LIPOPOLYSACCHARIDE (LPS) INDUCES INTRANUCLEOSOMAL CLEAVAGE OF THYMIC DNA, T C Caffrey and D J Thomas. Dept of Pediatrics, Univ Nebraska Med Ctr, Omaha, NE.

Treatment of adult female NAW/wl mice with Salmonella typhimurium LPS causes transient decreases in body and thymus weight. The reduction in thymus weight parallels double stranded DNA fragmentation in a process characterized by the production of multimers of 200 bp DNA fragments. This pattern is consistent with intranucleosomal cleavage of DNA. Fragmentation of thymic DNA is maximal at 24 hours after LPS treatment; at 48 hours post LPS treatment, there is no residual evidence of thymic DNA fragmentation. Some fragmentation of splenic DNA accompanies LPS treatment; however, liver DNA is not fragmented. Zn pretreatment of mice antagonizes LPS-induced fragmentation of thymic DNA. Notably, maximal protection of thymic DNA against LPS-induced fragmentation occurs at 72 hours after Zn treatment (24 hrs post LPS treatment). At this time point, Zn antagonizes thymic DNA fragmentation in a dose dependent manner. Zn treatment alone does not alter the integrity of thymic DNA. Parallel studies of Cd as an antagonist of LPS fragmentation have demonstrated a similar pattern of protection. Induction of an acute phase response with casein also protects against LPS-induced fragmentation, suggesting that the induction of systemic inflammation response is protective.

761 ACUTE MONOCYTOSEAL (MCT) IMMUNOTOXICITY IS UNALTERED BY PHENOBARBITAL AND CAN BE REPRODUCED IN VITRO INDEPENDENT OF METABOLISM. J A DEY, R L REED, D R BUEHLER, N I KERVIK. College of Vet Med. and Dept. of Ag. Chem., Oregon State Univ., Corvallis, OR.

Acute MCT exposure (50-400 mg/kg, po) in C57BL/6 (B6) mice inhibits their antibody response to sheep erythrocytes (SRBC) in a dose-dependent manner. In rats, acute MCT-induced pneumotoxicity can be enhanced through P450 enzyme induction with phenobarbital (PB). To investigate the role metabolism may play in the acute immunotoxicity, B6 mice were injected with PB for 4 days (80 mg/kg, ip) prior to a single oral dose of MCT (100 or 200 mg/kg). Results indicated that PB pretreatment did not significantly enhance the immunosuppressive effect of MCT alone. Subsequently, it was shown that the in vitro antibody response to SRBC could be suppressed in a dose-dependent manner by the addition of 1 to 3 mM MCT. HPLC analysis of the supernatant from parallel cultures incubated with 2 mM MCT showed no evidence of MCT metabolism. In contrast to other aspects of MCT toxicity, these results question the role of MCT metabolism in the acute in vivo immunotoxicity of MCT. This research was supported by PHS 302600304.

762 BINDING OF 1-NAPHTHYL-N4-(14)C-METHYLCARBAMATE ("C-METHYL CA) AND 1-14C-NAPHTHYL-N4-METHYLCARBAMATE ("C-NAPHTHYL CA) TO CTL2 LEUKOCYTES. S. Behar, H J Mar, E F Viththum and G F. Castile. The Dep of Pharmaceutical Sciences, Univ. of Nebraska Medical Cnr., Omaha, NE and "The Institute of Agriculture and Natural Resources, Univ. of Nebraska, Lincoln, NE.

Previously we showed that the anticholinesterase (antiCHE) insecticides carbaryl (N-naphthyl-N-methylcarbamate) and dichlorvos (O,O-dimethyl-S-(2-dichlorovinyl) phosphorothioate), added at 0.5-50 uM to cultures of a mouse T lymphocyte line (CTL2), inhibited interleukin 2 (IL2) driven proliferation 10-60% without producing cell death. In view of a central role for esterase activity in IL2 signaling in lymphocytes and the high potencies of the antiCHE insecticides as esterase inhibitors, we propose that suppression of CTL2 proliferation was due to inhibition of a critical esterase(s). Carbaryl (CA) inhibits esterases via carbamylation of a serine hydroxyl in the catalytic site. The present study addresses the potential of CA to carbamylate macromolecules of CTL2 cells. CTL2 cells in exponential growth were resuspended in RPMI 1640 medium supplemented with fetal bovine serum and human recombiant IL2, then plated at 2.5-3.5 x 10^5 cells per well of a 24-well plate. Cells were incubated with 40 uM 14C-methyl CA [11 uCi/umol] for 4 hours, while control cells were exposed to 14C methyl CA for less than 2 minutes. The cold acetyl precipitate of the labelled cells contained 277 pmol of bound 14C-methyl CA per 10^5 cells. Label in the acetoB precipitate of the control cells was less than 0.5% of that in labelled cells. When cells were labelled under identical conditions with 14Naphthyl CA [11 uCi/umol], the cold acetyl precipitate contained 11 pmol of bound 14Naphthyl CA per 10^5 cells. Dichlorvos (DDVP) at 25 and 50 uM reduced binding of 14C-methyl CA 25% and 40%, respectively. These results suggest measurable carbamylation of acetoB precipitable targets common to CA and DDVP, and are consistent with the proposed mechanism of inhibition. U.S. Geologic Survey Supported.

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Effect of CPT-11, Camptothecin analog, on immune responses in mice. F Yamaguchi, H Hattori, K Tawara and S Takayama, Research Institute, Daichl Pharmaceutical Co., Ltd., Tokyo, JAPAN

CPT-11 was found to be one of the most potent camptothecin analogs in term of antitumor effects. Many antitumor drugs have immunosuppressive effects. Thus, we examined the effect of CPT-11 on the humoral and cellular immune responses in vitro and in vivo. In vitro experiments, the mitogenic response of murine splenocytes to LPS, PHA or Con A, IgG antibody production by PWM and mixed lymphocyte reaction were significantly suppressed in the presence of more than 10^{-9} M of CPT-11. Furthermore, treatment of the mice with CPT-11 after sensitization of IgM antibody response to sheep red blood cells (SRBC) and transplantation of graft vs host reaction (GVHR) resulted in suppression of these responses. However, no suppressive effects on IgM antibody response to SRBC and GVHR were observed in the mice by the pretreatment with CPT-11. According to these results, the immune responses were suppressed during the presence of CPT-11. Then, the mitogenic responses of splenocytes were measured at various time after treatment of CPT-11 in mice. The responses of splenocytes obtained from CPT-11-treated mice were higher than those obtained from control mice one day after the treatment. These findings suggest that immunosuppressive effects caused by CPT-11 are similar to antitumor drugs.
SPECIFICITY OF HOST RESISTANCE ASSAYS (HRAS) FOR IDENTIFYING IMMUNOMODULATORY COMPOUNDS. MJ Murray, PA Horn, PT Thomas, Procter & Gamble, Cincinnati, OH, IIT Research Institute, Chicago, IL.

HRAs are in vivo assays utilizing infectious or tumor challenges to demonstrate biological relevance of chemical immunomodulation. Cyclosporin (CsA), a semi-specific suppressant of cell-mediated immunity (CMI), was used to evaluate the specificity of Listeria monocytogenes (LM), and Streptococcus pyogenes (Strep) bacteria; or, Influenza A (flu) virus HRAs in identifying immunomodulatory compounds. Resistance to LM, Strep or flu is believed to be mediated primarily by CMI, natural immunity (NI) or humoral immunity (HI), respectively. The mixed leucocyte response (MLR), T-dependent antibody-plaque response (APR) and natural killer (NK) cell activity were quantitated as in vitro correlates of CMI, HI or NI. Mice were treated with CsA intraperitoneally for 14 days at doses from 1.0 to 100mg/kg/day. On day 15, spleen cells were used for the MLR, APR, and NK assays. For the HRAs, mice were challenged on day 9 of dosing or day 15 and monitored for mortality. Only host resistance to LM was decreased by CsA. Mortality ranged from 80% in controls to 75 and 100% in the 100 mg/kg groups challenged on day 15 or day 8, respectively. NK activity was unaffected by CsA treatment. Suppression (PC.05) of the MLR occurred at doses 50X lower than those suppressing the APR. The data indicate CSA can selectively suppress CMI and demonstrate specificity and utility of HRAs in identifying biologically relevant immunosuppressants.

STUDIES ON THE MECHANISM OF TOLERANCE DEVELOPMENT TO 2-BUTOXYETHANOL INDUCED HEMATOTOXICITY IN RATS. B.I. Ghannam, H.B. Matthews, and M. Sanchez, National Toxicology Program, NIHE, RTP, NC

Early work demonstrated that 2-butoxyethanol (BE) causes acute hemolytic anemia in rats. Treatment of rats with BE daily (125 mg/kg/day) for three consecutive days resulted in a time-dependent increase in the hemolysis of erythrocytes. However, when daily treatment continued beyond 3 days, the number of erythrocytes began to rebound and approached pretreatment levels within 10 days, suggesting development of tolerance to the hemolytic effect of BE. To investigate the mechanism of tolerance development, rats were treated with 125 mg BE/kg/day for 3 days and allowed to recover (with no treatment) for 7 days. Control rats were treated with water similarly. At the end of this recovery period, rats were treated with 125 or 250 mg BE/kg and the hematological profiles were assessed at 2, 8, and 24 hr. A significant decline in the sensitivity of BE-pretreated rats compared to vehicle-pretreated rats was observed. Furthermore, in vitro incubation of blood obtained from the recovered rats with the hematoxic metabolite of BE, 2-butoxyacetic acid (BAA), indicated that tolerance is due, at least in part, to a decline in the sensitivity of the newly formed erythrocytes. These data suggest that young erythrocytes (formed to replace hemolyzed erythrocytes during recovery) were less sensitive to the hemolytic effects of BAA than older erythrocytes. Therefore, continued exposure to BE would be expected to result in increased turnover of erythrocytes with resulting stress on the hematopoietic system.
Inhalation of asbestos results in a variety of pulmonary diseases including fibrosis and mesothelioma. In addition, asbestos inhalation is associated with changes in humoral and cell-mediated immunity. From a mechanistic and therapeutic point of view, it is important to know whether pathological changes that occur are accompanied and/or amplified by immunological factors. We have investigated the hypothesis that the immune system, and in particular T-lymphocytes, modulate asbestos-induced fibrosis. Normal C57BL/6J mice and T-cell deficient C57BL/6J-RU/RU mice (nude) were exposed (3 hrs/day for 3 days) to a level of chrysotile asbestos known to induce a fibrotic response. Animals were sacrificed at 2, 7, 28, and 56 days post exposure and bronchoalveolar lavage cells, lavage fluid and lung RNA were examined for inflammatory mediators or cytokine gene expression. Significant differences in the inflammatory response between the two strains of mice were observed. Specifically, normal mice treated with asbestos maintain an active macrophage recruitment as late as 8 weeks post exposure while nude mice lack this response. Nude mice exposed to asbestos demonstrate a significant difference in pulmonary neutrophil (PMN) kinetics when compared to normal animals. Specifically, T-cells appear to play a role in the initial influx of PMNs in addition to influencing their residence time in the alveolar space (present in the nude mice at 8 weeks, p<0.05). Despite this observation, T-cells do not appear to play a critical role in the development of fibrosis since typical asbestos fibrotic lesions are found around alveolar duct bifurcations in both strains of mice. These studies suggest that following asbestos exposure, T-cells play an important role in the inflammatory response but do not appear to contribute to asbestos induced fibrosis.

The beige mouse is an animal model of the Chediak-Higashi syndrome of humans characterized, among other effects, by defective neutrophil function. Neutrophils of beige mice have defective ability to release cytoplasmic granules, and these granules possess lower levels of elastase and cathepsin G. We used this animal model to examine the role of the neutrophil in silica-induced pulmonary fibrosis. Normal, heterozygous (C57BL/6J-b, "black") mice and beige (C57BL/6J-b, "beige") mice were exposed to silica (100 mg/m³, 6 hr/day, 3 days), and parameters of lung cytotoxicity and morphometric measurements of fibrosis were compared with values obtained from sham-exposed mice. Parameters of lung injury, including lavaged protein (indicative of alveolar epithelial permeability), lactate dehydrogenase (indicative of cellular death or toxicity), and N-acetyl glucosaminidase (indicative of lysosomal enzyme release) were not significantly different between black and beige mice immediately and one month after silica exposure. All values from exposed mice in both groups were significantly greater than the values from sham-exposed black and beige mice. The results indicate that defective neutrophil function has no effect on the early silica-induced increases in parameters of lung injury.

MDP-Lys(L18)-Induced Arthritis in Rats and Effects of Various Drugs on It. T. Sugawara, H. Kato, K. Furuhara and S. Takayama, Drug Safety Research Center, Research Institute, Dainichi Pharmaceutical Co., Ltd., Tokyo, Japan

MDP-Lys(L18), an analogue of muramyl dipeptide, increases the number of neutrophils in the blood and enhances resistance to bacterial infection in experimental animals and humans. When MDP-Lys(L18) was subcutaneously administered to rats, swelling of the tarsal joint was induced. In this study we examined the development of arthritis. The swelling appeared from Day 7 and continued during the course of treatment. However, it was easily reduced after the cessation of treatment. Histopathologically, synovocytes with vesicular cytoplasm were arranged in multilayer and neutrophils infiltrated in synovial membrane in the early stage after a single injection. This acute synovitis was gradually exacerbated until 24 hr later. We also examined effects of various drugs on the arthritis. Indomethacin and dexamethasone reduced the swelling of joint, suggesting that prostaglandins are included in the pathogenesis of the arthritis. An immunosuppressors, cyclophosphamide, reduced the swelling, but the other one, cyclosporin-A, enhanced. However, the two drugs completely inhibited an increase in IgG production induced by MDP-Lys(L18). From these results, it is speculated that development of MDP-Lys(L18)-induced arthritis is different from adjuvant-induced arthritis.

Formation of Benzo[a]Pyrene-7,8-Diol DNA Adducts by Stimulated Pulmonary Neutrophils. J. P. Petruska, G. Jakab, and M. A. Trosh, Johns Hopkins School of Hygiene and Public Health, Baltimore, MD.

Chronic respiratory tract infection can cause an increase in lung tumor incidence in rats (JNCI 49: 1107, 1977). The aim of our work was to determine if neutrophils could effect initiation of carcinogenesis via a myeloperoxidase (MPO)-dependent transformation of benzo(a)pyrene-7,8-diol (BP-7,8-diol), a pulmonary carcinogen. In this investigation, virus-free male DBA/2 mice were exposed by inhalation to the Gram negative bacteria Proteus mirabilis for 1 hour. For various timepoints post-exposure, bronchoalveolar lavage (BAL) was performed to determine total cellular MPO, total and differential cell counts, and superoxide (O₂⁻) release. Twelve hours after the exposure, cellular levels of MPO as well as percentage and total number of polymorphonuclear leukocytes (PMNs) peaked and began to decline subsequently thereafter. At this same timepoint, cells from BAL demonstrated increased production of O₂⁻ as measured by reduction of cytochrome c, after addition of phorbol myristate acetate (PMA) or opsonized zymosan. These cells also evoked biotransformation of BP-7,8-diol as evidenced by enhanced diol chemiluminescence, tetraol formation, and covalently bound adduct formation to exogenous DNA upon addition of a soluble or particulate stimulus, PMA and opsonized zymosan, respectively. Our results demonstrate an important role of PMNs, due to their content of MPO, in the initiation stage of carcinogenesis.

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Hemolytic anemia and methemoglobinemia induced by exposure to aniline is known to be mediated by its N-oxygenation metabolite, phenylhydroxylamine. The herbicide propanil (3,4-dichloroanilinoanilide), is an aniline derivative that is also thought to induce methemoglobinemia through the action of oxidized metabolites. In view of the hemolytic capacity of phenylhydroxylamine, studies were undertaken to determine the hemolytic potential of propanil, and, if possible, the role of metabolites in this hemotoxicity. The survival of previously administered $^{51}$Cr-labeled erythrocytes in rats was reduced in a dose-dependent manner by i.p. administration of propanil or its deacetylated metabolite, 3,4-dichloroaniline (ED50 for both ca. 1.8 mmol/kg). When labeled erythrocytes were exposed in vitro to propanil or 3,4-dichloroaniline and then administered to rats, no decrease in erythrocyte survival was observed, which indicated that these compounds were not direct-acting hemotoxins. In contrast, erythrocyte survival was markedly reduced by both i.p. administration or in vitro exposure to N-hydroxy-3,4-dichloroaniline. In addition, N-hydroxy-3,4-dichloroaniline was detected in the blood of propanil-treated rats in amounts sufficient to account for the hemotoxicity of the parent compound. These data suggest that N-hydroxy-3,4-dichloroaniline mediates propanil-induced hemolytic anemia, and that occupational exposure to propanil may result in an increased risk of hemolytic anemia. Supported by NIH Grant HL30038.


Acetaminophen has been shown to cause an infiltration and accumulation of macrophages into the centrilobular region of rat liver in the absence of necrosis (Leakin and Pilaro, Textol. Appl. Pharmacol., 66,204, 1986). These cells may play a role in producing necrosis. To test this hypothesis, we developed a model which examines the effect of non-necrogenic doses of acetaminophen on allyl alcohol hepatotoxicity. Twenty-four hour acetaminophen pretreatment increased the necrotic response to non-necrogenic doses of allyl alcohol. To determine if activated macrophages contributed to this potentiation, we used the effects two agents known to inhibit hepatic macrophage activity, dextran sulfate and gadolinium chloride. Dextran sulfate, a suppressant of macrophage phagocytosis (Sohami and Bradford, J. Reticuloendo. Soc. 16,75, 1974), administered with acetaminophen alleviated the ability of acetaminophen to potentiate the cytotoxicity of allyl alcohol. Gadolinium chloride, a selective selectively depletes Kupffer cells (Koudstaal et al., Cells Hep. Sin., in press), administered with acetaminophen decreased the potentiation of allyl alcohol. These two compounds suppress hepatic macrophage activity by different mechanisms. Both altered the ability of acetaminophen to potentiate the cytotoxicity of allyl alcohol. These data suggest that acetaminophen stimulated infiltration and activation of macrophages in the liver are required for the ability of acetaminophen to potentiate cytotoxicity. This data also supports the hypothesis that activated macrophages contribute to necrosis induced by some compounds.

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FALCARINOL. A NATURALLY OCCURRING INDUCER OF OXYGEN REACTIVE SPECIES. J. Alia, R. C. Boding, and F. Rodriguez. Environmental Toxicology Program, Department of Community and Environmental Medicine, University of California, Irvine, CA.

Polysacetylenes are common natural products in ingested vegetables, plant medicines, and spices such as parsnip, skirret, parsley, carrot, and celery. A major polysacetylenic constituent of celery (Apium graveolens) is the potent human contact allergen, Falcarinol, which has been implicated as a neurotoxic agent. Studies using 2,7'-dichlorofluorescin diacetate (DCFH-DA) were undertaken to explore the neurotoxicological properties of Falcarinol and its possible mechanism of action. DCFH-DA crosses cell membranes and is enzymatically hydrolyzed by intracellular esterases to non-fluorescent DCFH, which reactive oxygen species can rapidly oxidize to the highly fluorescent 2,7'-dichlorofluorescin. Tests in vivo of these species including superoxide anion, hydrogen peroxide, and hydroxyl radicals are believed to be initiators of oxidative cell damage. Crude liver, brain, and skin mitochondrial fractions, isolated from male Sprague-Dawley rats, were loaded with DCFH-DA and treated in vitro with various concentrations of Falcarinol. Falcarinol induced the formation of active oxygen species in liver, brain, and skin. This stimulation occurred in a dose-dependent manner. The potentiation of Falcarinol may be related to its constituent acetylenic and olefinic bonds, which are available for epoxide formation.

EFFECT OF TOLUENE AND ITS METABOLITES ON CEREBRAL OXYGEN RADICAL GENERATION. C. J. Mann, C. P. LeBel and S. C. Bondy. University of California, Irvine, Dept. of Community and Environmental Medicine, Irvine, CA.

Toluene has been implicated as a neurotoxicant and there is evidence that membrane associated phenomena may be integral to the mechanism of action of this organic solvent. The purpose of this study was to characterize the effect of toluene on oxygen radical formation and to identify what metabolites may contribute to the solvent's neurotoxicity. The rate of oxygen radical formation was measured using the molecular probe 2,7'-dichlorofluorescin diacetate. Exposure to toluene in vitro caused a dose dependent elevation of oxygen radical generation within crude synaptosomal fractions (P2) obtained from rat cerebral cortex. Pretreatment of the P2 fraction with the cytochrome P450 mixed function oxidase inhibitor SKF 525A, significantly inhibited toluene stimulated excess oxidative activity. Administration of toluene in vivo also enhanced cerebral oxygen radical generation and pretreatment with the mixed function oxidase inhibitor, metyrapone, blocked the oxygen radical-inducing properties of toluene. The results obtained from both the intact animal and the isolated system suggested that a metabolite of toluene may catalyze oxygen radical formation. In vitro studies showed that both benzyl alcohol and benzoic acid had free radical quenching properties, while benzaldehyde exhibited significant induction of oxygen radical generation. Benzaldehyde appears to be the metabolite responsible for toluene's acceleration of free radical production in the nervous system and may thus contribute to the overall neurotoxicity of toluene.
Oxovanadate Effects on Membrane Proteins and Proteolysis in Human Red Blood Cells. A M Mortensen and R F Novak. Institute of Chemical Toxicology, Wayne State University, Detroit, MI

Oxovanadium (NaVO₃) compounds have been implicated in plasma membrane-mediated stimulation of NADH oxidation, O₂⁻/H₂O₂ production and toxicity. NaVO₃ effects on membrane proteins and proteolysis in human red blood cells (RBCs) were examined using HPLC, SDS-PAGE and Western blot analysis. Incubations were performed for 6 hrs at 37°C with human RBCs (93% HCT) in isotonic buffer, pH 7.4, containing 10 mM glucose. HPLC analysis revealed an 52% and 100% decrease in the rate of lys and leu release, respectively, at 0.1 mM NaVO₃, and an 19% and 43% decrease in lys and leu release, respectively, at 1.0 mM NaVO₃ as compared to control. In contrast, 10 mM Na₂O₃ stimulated an 340% and 190% increase in the rate of lys and leu release, respectively, relative to control. SDS-PAGE analysis of isolated red cell ghosts revealed no significant difference from control for membrane proteins in the presence of 0.1 or 1.0 mM NaVO₃, whereas Na₂O₃ at 10 mM resulted in an attenuated intensity of band 2.1 and an 60 kDa protein and an increase in intensity of band 4.2. Western blot analysis using a sheep anti-human calpain Ab revealed a progressive increase in intensity of membrane-associated calpain with increasing NaVO₃ concentrations. These results suggest an inhibitory role for oxovanadate at low concentrations while higher concentrations damage proteins and stimulate proteolysis. Supported by NIH grant ES02521.

HEPATOTOXICITY OF BENZYL VILOGEN IN SPRAGUE-DAWLEY RAT LIVER SLICES. SJ Waters, TC Spaulding, K Bendel and AJ Gandolfi. Anaquest Inc., Murray Hill, NJ and Department of Pharmacology, University of Arizona, Tucson, AZ.

The toxicity of benzyl viologen (BV), a bi-pyrilid herbicide which produces hepatotoxicity via redox cycling, was examined in vitro using precision cut hepatic slices in dynamic organ culture. Slices viability (intracellular K⁺ content), and glutathione levels were well maintained in control slices during the 6 hour incubation, whereas BV-treated slices displayed decreased glutathione levels and a concentration-related [125, 250, 500 uM] decrease in viability (18, 51 and 64% respectively). Protein synthesis (¹⁴C-leucine incorporation) was inhibited by BV incubation at time points and concentrations where viability was not affected. Preincubation (1 hr) of hepatic slices with the inhibitor of glutathione reductase, [BCO₂], produced approximately a 2 fold potentiation of BV-induced effects on viability measurements and produced marked elevations in lipid peroxidation [malondialdehyde] measurements. End-points of hepatocellular injury consistent with oxidant stress may be examined in this in vitro system.

EFFECTS OF ANTIOXIDANTS ON CARBON TETRACHLORIDE- OR DIQUAT-INDUCED ETHANE EVOLUTION IN PRECISION-CUT RAT HEPATIC SLICES. T.H. Petry, G.H. Wolfgang, R.A. Jolly and W.J. Donarski. The Upjohn Company, Kalamazoo, MI.

Previous studies have demonstrated the abilities of novel antioxidants, U-74,006F and U-78,517G to inhibit chemically-induced lipid peroxidation (LP), as measured by thiobarbituric acid-reactive substances (TBARS) formation. Studies reported here evaluated ethane evolution as an alternative indicator of LP in slices. Slices were incubated for 30 min in the presence of U-74,006F (100 uM), U-78,517G (100uM) or the known antioxidant DPFD (1 uM) prior to challenge with either CCl₄ or diquat (DQ) at appropriate times, 2 ml headspace samples were removed and analyzed for ethane by gas chromatography. CCl₄ increased ethane evolution in a concentration-dependent manner; the order of efficacy for inhibiting ethane evolution was similar to that observed previously with TBARS formation, although the overall efficacy of the antioxidants vs ethane evolution was lower. DQ variably increased ethane evolution at a concentration of 3 mM. In experiments where DQ-dependent ethane evolution was observed, addition of the antioxidants completely inhibited the increase. In summary, ethane evolution is a viable alternative to, although less sensitive than, TBARS formation as an indicator of LP in precision-cut hepatic slices.

MARKED INTER-ANIMAL DIFFERENCES IN SUSCEPTIBILITY OF SPRAGUE-DAWLEY RATS TO DIQUAT-INDUCED OXIDATIVE STRESS. C. Madhu, Z Gregus and C D Klassen. Univ Kansas Med Ctr, Kansas City, KS

Biliary excretion of oxidized glutathione (GSSG) is used as an index of oxidative stress. We observed a marked inter-animal difference in susceptibility to diquat-induced oxidative stress. When diquat was administered to rats (120 μM/kg, M), a 60-fold increase in the biliary excretion of GSSG was observed in 40% of the rats (responders). In 60% of the animals, diquat did not increase the biliary excretion of GSSG (non-responders). This inter-animal variation is not due to a difference in the hepatobiliary transport of GSSG, as no inter-animal difference was observed after administration of another oxidative-stress inducing agent, t-butyl hydroperoxide (1.4 nmol/kg). We then examined the hepatobiliary disposition of diquat by HPLC. No differences in biliary bile concentration of diquat were noted between responders and non-responders. However, a marked difference was observed in the hepatic concentration of diquat in responders and non-responders. The responders exhibited a 4-fold higher hepatic diquat concentration than the non-responders (85 or 15 nmol/g, respectively). In conclusion, this study demonstrates that there is a marked inter-animal variation to the oxidative stress produced by diquat. This variation does appear to be due to differences in the plasma disappearance of diquat or the ability to excrete GSSG into bile, but rather due to a difference in the hepatic accumulation of diquat. (Supported by NIH Grant ES-03192)
THE EFFECT OF CARBON MONOXIDE ON MITOCHONDRIAL FUNCTION IN ISOLATED RAT HEPATOCYTES. Y. PARK and D. P. Jones, Department of Biochemistry, Emory University School of Medicine, Atlanta, GA.

Carbon monoxide (CO) induces tissue hypoxia due to binding to hemoglobin. However, CO also binds to cytochrome oxidase and inhibits mitochondrial function. In this study, we evaluated CO-induced hypoxic effects on mitochondrial membrane potential and ATP contents in isolated rat hepatocytes. We studied 4 different groups: Hepatocytes were incubated in Krebs-Henseleit buffer (pH 7.2) at 37°C saturated with 1% argon for 60 min, 2) 0.5% oxygen balanced with argon for 60 min, 3) argon for 30 min followed by 0.5% oxygen balanced with argon for 30 min, and 4) argon for 30 min followed by 0.5% oxygen balanced with carbon monoxide. ATP contents (nmol/million cells) in these groups were 1.0±0.70, 12.6±5.67, 9.1±1.67, and 8.5±2.39 respectively. Mitochondrial membrane potentials were 133±1.53, 156±1.33, 78±8.93, and 130±2.25 respectively. ATP content in group 4 treated with 0.5% oxygen balanced with CO showed no difference compared to that in group 3. However, mitochondrial membrane potential in group 4 was significantly different from that in group 3 and was no difference from those in group 1 and 2. These results show that the mitochondrial membrane potential falls more in the reoxygenation phase than during hypoxia and that CO protects against this loss in membrane potential. The underlying mechanism(s) of CO on preserving membrane potential might be due to metabolic suppression during hypoxia in mitochondria but exact mechanisms are still unknown. (Supported by the Office of Naval Research and NIH grant GM 36538)

α-TOCOPHEROL (VITAMIN E) METABOLISM IN CULTURED HEPATOCYTES TREATED WITH TERT-BUTYL HYDROPEROXIDE. P. A. Glasscott, E. Gilfor and J. L. Förber, Thomas Jefferson Univ., Phila., PA.

α-Tocopherol is a naturally occurring membrane-bound antioxidant that protects cells against the damage induced by free radicals. We have used tert-butyl hydroperoxide (TBHP) to study oxidative cell injury in cultured hepatocytes. Little is known about the involvement of α-tocopherol in the injury of cultured hepatocytes by TBHP. The concentration of α-tocopherol in cultured hepatocytes after 24 hr is 15% of the value in freshly isolated hepatocytes, despite the fact that Williams' E medium with fetal calf serum contains 18 nM tocopherol-phosphate ester and 40 nM α-tocopherol. Supplementation of cultures with 1 μM tocopherol-phosphate for 20 hours maintains a physiological concentration of α-tocopherol. After treatment with TBHP, cells supplemented with tocopherol-phosphate had decreased and delayed cell death and lipid peroxidation relative to unsupplemented cells. In supplemented cells, α-tocopherol decreased within 15 min after addition of TBHP, and this decline in tocopherol was greater with increasing concentrations of TBHP. These data demonstrate that α-tocopherol protects hepatocytes against injury by TBHP and the loss of α-tocopherol is associated with the peroxidation of lipids and the death of the cell. Thus, cultured hepatocytes can be used to study the metabolism of α-tocopherol during oxidative stress, if the cultures are supplemented with α-tocopherol ester to maintain physiological α-tocopherol concentrations.

COMPARISON OF ANTIOXIDANT EFFECTS ON CYSTEINE CONJUGATE, IODOACETAMIDE AND T-BUTYLHYDROPEROXIDE CYTOTOXICITY. J. L. Stevens and Q. Chen, W. Alton Jones Cell Science Center, Lake Placid, NY.

Our previous data indicate that the depletion of nonprotein thiols (NPSH) and lipid peroxidation (LPO) are involved in the cytotoxicity of S-(1,2-dichlorovinyl)-L-cysteine (DCVC). To determine if these are general events involved in chemical-induced cytotoxicity in LLC-PK1 cells, we compared the effect of antioxidants, an iron chelator and a thiol reducing agent on the cytotoxicity of DCVC to that of an alkylating agent, iodoacetamide (IDAM) and an organic peroxidant, t-butylhydroperoxide (TBHP). All three toxins caused LPO prior to cell death. Phenolic antioxidants DPPD, BHA, BHQ, PGA and BHT and the iron chelator deferoxamine inhibited both LPO and toxicity for all three toxins. The inhibition was not due to a decrease in covalent binding of DCVC and IDAM. The three toxins depleted cellular NPSH in the order IDAM>DCVC>TBHP. Antioxidants did not affect the loss of NPSH. The thiol reducing agent dithiothreitol (DTT) blocked the toxicity of all three toxins. Therefore, it appears that depletion of NPSH and LPO are common mechanisms for the cytotoxicity of DCVC, IDAM and TBHP in LLC-PK1 cells.

THE ROLE OF SINGLET OXYGEN IN GENOTOXIC MECHANISMS OF PHOTOACTIVE DYES. J. E. Schneider, J. Phillips, G. Liu and R. A. Floyd, Molecular Toxicology Research Program, Oklahoma City, OK.

Several photoactive dyes, some of which are experimental antitumor agents were compared for their relative efficiency for inactivation of the RNA bacteriophage, R17. Methylene blue (MB) was the most efficient of the 13 dyes tested, reducing the virus titer to less than 0.00001 percent of the initial titer, compared with inactivation to 0.02 percent by rose Bengal (RB) and to 0.6 percent by erythrosin B (EB). Singlet oxygen generation by these three dyes, examined by electron spin resonance spectroscopy (ESR) techniques, was in the order MB>RB>EB, corresponding to the order of their efficiency in killing R17 virus. Methylene blue plus light has been shown to mediate 8-hydroxyguanine formation in RNA and DNA. The 8-hydroxyguanine formation rates in RNA by RB yield 0.06 8-hydroxyguanine molecules per viral genome equivalent of HB under conditions that inactivated 99 percent of the virus. Thus, 8-hydroxyguanine formation is probably not the primary lethal event in viral inactivation by rose Bengal. Damage to the phage coat protein, crosslinking events, and RNA strand breaks remain as candidates for the primary lesion. Supported in part by NIH Grant No. CA42854. J.P. was a Fleming Scholar at OMRF during the summer of 1990.
3-AMINOBENZAMIDE, AN INHIBITOR OF POLY(ADP) RIBOSE POLYMERASE, ACTS AS A RADICAL SCAVENGER IN PROTECTING HEPATOCYTES FROM AN ACUTE OXIDATIVE STRESS. M.E. Kye, K. Yamamoto, and J.L. Farber. Toxicology Department, Rohm and Haas Company, Spring House, PA and Department of Pathology, Thomas Jefferson University, Philadelphia, PA.

The mechanism by which 3-aminobenzamide (3-AB), an inhibitor of poly(ADP) ribose polymerase, protects against the toxicity of hydrogen peroxide was studied in primary cultures of rat hepatocytes. Ten mM 3-AB inhibited by 50% the cytotoxicity of the hydrogen peroxide generated in the culture medium by glucose oxidase or as a consequence of the intracellular metabolism of menadione. Increasing the concentration of 3-AB afforded no further protection, and concentrations lower than 5 mM were ineffective. Hydroxyl radicals formed in the reaction of hydrogen peroxide and ferrous iron were scavenged by 3-AB at concentrations equivalent to those that prevented cell killing. By contrast, benzamide, an equipotent inhibitor of poly(ADP) ribose polymerase, protected only slightly from the killing of hepatocytes by menadione and not at all against that by glucose oxidase. Similarly, benzamide was considerably less effective than 3-AB in scavenging hydroxyl radicals. These data indicate that the protective action of 3-AB against the killing of cultured hepatocytes by an oxidative stress is more readily attributed to the scavenging of hydroxyl radicals that to the inhibition of poly(ADP) ribose polymerase.

DIFFERENTIAL EFFECTS OF THE PHOSPHODIESTERASE INHIBITOR ENOXIMONE ON PEROXISOMAL AND MITOCHONDRIAL \( \beta \)-OXIDATION OF FATTY ACIDS. J Youssefi, S Abdel-Akeem, C Franagakis and M Radclie. 1University of Missouri-Kansas City, MO and 2Department of Pharmacology, Glaxo Inc., NC.

Although oxidation of fatty acids occurs in both peroxisomes and mitochondria, \( \beta \)-oxidizing enzymes in these organelles have distinct differences in their specificity and sensitivity to inhibitors. In this study, the effects of the phosphodiesterase inhibitor enoximone on hepatic peroxisomal and mitochondrial \( \beta \)-oxidation were investigated. Cyanide-insensitive peroxisomal \( \beta \)-oxidation of palmitoyl CoA was inhibited progressively by increasing concentrations of enoximone. At 25 \( \mu \)M enoximone, peroxisomal \( \beta \)-oxidation was inhibited by about 20%, with complete inhibition attained at 250 \( \mu \)M enoximone. In contrast, mitochondrial \( \beta \)-oxidation of either palmitoyl CoA or palmitoyl-\( \beta \)-carnitine was not inhibited by up to 250 \( \mu \)M. However, mitochondrial \( \beta \)-oxidation of palmitic acid was inhibited by about 30% in the presence of 250 \( \mu \)M enoximone, whereas oxidation of the short-chain fatty acid hexanoate was not influenced by enoximone. These data show that enoximone selectively inhibits peroxisomal, but not mitochondrial \( \beta \)-oxidation of the CoA thiocesters of long-chain fatty acids. Data also suggest that enoximone is a selective inhibitor of the mitochondrial long-chain acyl-CoA ligase.

COMMERCIAL PREPARED XANTHINE OXIDASE IS CONTAMINATED WITH PROTEOLYTIC ACTIVITY THAT IS INHIBITED BY A NOVEL SERIES OF ANTIOXIDANT LAZAROIDS. K L Lineman and J Mark Breugler. Upjohn Research Laboratories, Central Nervous System Diseases Unit, The Upjohn Company, Kalamazoo, MI.

The 21-aminosteroid antioxidants U-74096F and U-74500A (lazaroids) have been shown previously to protect neurofilament proteins in crude rat brain homogenate from xanthine/xanthine oxidase (x/o)-induced damage, as well as afford partial protection against apparent free radical-mediated degradation of mitochondrial and dehydrogenase (GDPDH). Further investigation of this phenomena revealed that x/o was supplied by Sigma and used as a free radical generated was contaminated by proteolytic activity. The possibility that this protease(s) activity was responsible for the majority of protein degradation in systems using x/o was examined along with the possibility that the lazarois could inhibit this protease(s)-induced damage. Protease(s) activity was partially purified from Sigma's xanthine oxidase by column chromatography and examined for its ability to degrade GDPH. The isolated proteolytic activity contained no x/o activity, but effectively degraded GDPH during an incubation at 37°C. Despite the absence of x/o activity from the protease(s) preparation, a number of first and second generation lazarois at 100 \( \mu \)M were capable of partially protecting GDPH from degradation by this protease(s) activity with the percent inhibition ranging between 38-98%. These data suggest that certain lazarois may act as protease inhibitors in addition to their potent effects on lipid peroxidation.

DITHIOCARBAMATE HERBICIDES ARE POTENT HYDROXYL RADICAL SCAVENGERS. N J Keiner. Dept. Pathology, University of California, San Diego, CA.

Dialkylidithiocarbamates demonstrated hydroxyl radical scavenging ability when studied using either radiation or chemical hydroxyl radical generating systems. The dithiocarbamates demonstrated competitive inhibition with known hydroxyl radical scavengers and bimolecular K values from 5.9 x 10\(^9\) /M sec to 7.9 x 10\(^9\) /M sec were calculated. Production of carbon disulfide and diethylamine was detected in both types of hydroxyl radical generating systems. Formation of carbon disulfide was inhibited by hydroxyl radical scavengers. Together, these results suggest that dithiocarbamates react with a hydroxyl radical to form a thyl radical which undergoes umimolecular dissociation to yield carbon disulfide and a nitrogen-based radical.

Formation of this thyl radical may explain how dithiocarbamates are metabolized to form carbon disulfide and dialkylamines. Formation of the nitrogen-based radical may explain the teratogenic effects of dialkylidithiocarbamates at parts per billion concentrations. Supported by UC Toxic Substances Research Program.
A FLUORESCENT TECHNIQUE FOR MEASUREMENT OF INTRACELLULAR HYDROPEROXIDE CONCENTRATIONS IN LIVING CELLS. D W Bombick and D J Doolittle, R J Reynolds Tobacco Company, Cellular/ Molecular Biology Div., Winston-Salem, NC.

Hydroperoxides are reactive oxygen species which have been reported to cause cytotoxicity and genotoxicity. The objective of this study was to establish a technique for quantifying the concentration of hydroperoxides in a living mammalian cell. Intracellular hydroperoxides were measured by trapping 2,7-dichlorofluorescin (non-fluorescent) into CHO or WE rat liver epithelial cells by allowing cellular esterases to cleave the cell impermeable 2,7-dichlorofluorescin diacetate to the cell permeable 2,7-dichlorofluorescin. In the presence of hydroperoxide the non-fluorescent 2,7-dichlorofluorescin is converted to a stoichiometric manner to the fluorescent 2,7-dichlorofluorescin which can be quantified by a laser cytometer. Optimal concentration and time of incubation of 2,7-dichlorofluorescin diacetate were found to be 10 μM and 15 minutes, respectively. Intracellular hydroperoxide concentrations were determined after exposing cells to varying extracellular concentrations (0 to 880 μM) of hydrogen peroxide, t-butyl hydroperoxide, cumene hydroperoxide and benzoyl peroxide. Greater intracellular hydroperoxide concentrations were observed with organic hydroperoxide exposure compared to equivalent extracellular concentrations of hydrogen peroxide, with cumene hydroperoxide giving the greatest intracellular hydroperoxide concentrations. This method demonstrates a sensitive technique for assessing intracellular hydroperoxides in living cells.

REDOX STRESS IN DIQUAT-TREATED FISCHER-344 RATS AND THE BILARY EFFLUX OF IRON. G. V. Smith and S. L. Reddy. Dept. of Pediatrics, Baylor Col. of Medicine, Houston, TX

The availability of chemically reactive chelates of iron, which are usually low molecular weight, appears to be a critical determinant in the adverse biological effects of reactive oxygen species [Mol Pharm 32: 417]. Although the concentrations of these reactive iron chelates are normally kept very low in biological systems, diquat and similar compounds stimulate release of available iron from ferritin by ruddimentary metabolism. In vivo, diquat increases hepatic low molecular weight chelatable iron content in rats at 36 h [Tox Appl Pharm 93: 506], but the earlier events needed to be determined. We examined the effects of diquat on biliary efflux of iron in Fischer rats anesthetized with pentobarbital. Bile ducts were cannulated, samples collected, and iron was measured with ferrene-S in the presence of ascorbate [Clin Bioch 14:311]. Administration of 0.1 mmol/kg of diquat increased biliary iron concentrations from 6.0±1.2 (around 50 pmol Fe/min/100g body weight) to 213±2.8 μM at 80-100 min. No change was observed in saline-treated controls. The rise in iron efflux was not observed during the first 40 min, in contrast to the rapid responses in GSSG and in alkane expiration. Administration of a comparably hepatotoxic dose of acetaminophen (1.5 g/kg) caused a rapid and profound (75-85%) decrease in biliary iron. The acute changes in iron metabolism caused by these two hepatotoxins are thus quite different, but the biochemical bases of the two effects will require further study. (Supported by GM 44263 from NIH).

RICIN-INDUCED OXIDATIVE STRESS IN MICE. D F Muldoon and S J Stobbs. Creighton University Health Sciences Center, Omaha, NE.

Ricin is one of the most potent toxins known to man. Ricin exhibits hepatotoxicity, neurotoxicity, adrenal toxicity and central nervous system toxicity. This protein toxin is composed of two subunits, the B subunit being responsible for binding, while the A subunit carries the toxic activity. Ricin exhibits an RNA N-glycosidase activity which may be the specific mechanism involved in the inhibition of protein synthesis and production of toxicity. However, few studies have examined other biochemical alterations associated with ricin toxicity. Following the administration of 25 μg ricin/kg to mice i.p., the effects of ricin on hepatic lipid peroxidation and non-protein sulfhydryl content were determined. At 12, 24 and 48 hours after administration, hepatic non-protein sulfhydryl content decreased by 35, 50 and 35%, respectively, while lipid peroxidation increased by 2.1, 6.5- and 3.3-fold, respectively. Ricin had comparatively little effect on protein bound sulfhydryl content and total sulfhydryl content. In addition, time-dependent decreases in spleen, liver, and intestinal weights relative to body weight were also observed. The results indicate that an oxidative stress is produced by ricin which is maximal at 24 hours post-treatment.

TIME-COURSE OF LIPID PEROXIDATION IN LIVER OF MALE AND FEMALE RATS TREATED WITH HEXACHLOROCYCLOPENTADIENE (HCB). S Baud and M Charbonneau. Dép. de Mèd. Trav. & Hyg., Mil., Université de Montréal, Québec, Canada.

Free radical formation may be related to HCB-induced porphyria in female rat liver. Lipid peroxidation, assessed by malondialdehyde (MDA) and conjugated dienes (CD) formation, was measured in mitochondria isolated from male and female rat livers. Animals were given corn oil or HCB (100 mg/kg, p.o., 10 ml/kg in corn oil) for five consecutive days. Rats were killed at day 6, 10, 20, 30, 40, 46, 52 and 65. In all groups, CD levels were high at day 6 and then decreased, while MDA formation kept increasing during the same period of time. MDA and CD levels in HCB-treated male rats were not significantly different from controls. In HCB-treated female rats, the decrease in CD levels was more marked and accompanied by a higher rate of MDA formation compared to controls; MDA level peaked at day 40. Ethoxyresorufin-O-deethylase (EROD)/aminopyrine-N-demethylease ratio increased and peaked at day 20 in HCB-treated female rats, shortly before the elevation of MDA levels. Results suggest that corn oil induces lipid peroxidation in rat liver. HCB exacerbates this process in female rats only, where EROD induction may accelerate the rate of CD transformation into lipid peroxides, leading to a greater MDA release from membrane (Supported by NSERC and FRSQ).
BIOCHEMICAL CHANGES IN MOUSE LUNG AND BRAIN AFTER A SINGLE SUBCUTANEOUS INJECTION OF A SULFUR MUSTARD. J M Blakemore, S T Omeye, and D W Kope, Jr. Letterman Army Institute of Research, San Francisco, CA and School of Public Health, University of California, Los Angeles, CA.

Sulfur mustard (HD), a vesicant and chemical warfare agent, is not believed to affect tissues distant from the original site of administration. Current treatments for HD injury only address the localized blistering effects. We previously reported that subcutaneous (sc) administration of the monofunctional HD analog, butyl 2-chloroethyl sulfide (BCS), caused significant biochemical changes in the lungs, brains and kidneys of athymic nude mice. In this study, we examined whether sc administration of a bifunctional HD analog, 4-chlorobutyl 2-chloroethyl sulfide (CBCS), would induce similar changes. We injected Swiss-Webster mice with a sublethal dose (3 μg/mouse) of CBCS and examined lung and brain tissue after 1, 24 and 48 hrs. We found significant alterations in superoxide dismutase, catalase, glucose-6-phosphate dehydrogenase, and glutathione S-transferase activities, and total glutathione content in both organs predominately after 24 hrs. The changes ranged from 20% to 60%. These changes were generally consistent with free radical-mediated effects. We therefore concluded that sc administration of the bifunctional HD analog CBCS causes biochemical changes in organs or tissues far removed from the site of administration. These observations may contribute to a better understanding of the mechanisms underlying sulfur mustard injury and may also offer new insights for treatment of and protection from these compounds.

EFFECTS OF IN VITRO ALUMINUM ON NAPDH-INDEPENDENT AND -DEPENDENT MOUSE HEPATIC MICROSONAL LIPID PEROXIDATION (LP). B Fulton and E H Jeffery, University of Illinois, Urbana, IL.

The effect of physiological levels of aluminum (Al:25-400 μM) on in vitro NADPH-independent, xanthine/xanthine oxidase (X/XO)-dependent, and NADPH-dependent iron-initiated LP was investigated using female Swiss-Webster mouse hepatic microsomes. Microsomes were incubated with buffer (final concentration 0.06 M Tris or HEPES, pH 7.4), ferrous sulfate, aluminum sulfate or chloride, and a G6P-containing NADPH-generating system as indicated for 5 or 10 minutes at 37°C. The thioalbuminic acid assay was used to measure malondialdehyde formation and H2O2 was determined by the formation of HCHO from the oxidation of 100 mM methanol by catalase (300 U); blanks contained 0.5 mM ascorbate. In the absence of NADPH, Al (400 μM) stimulated iron-dependent LP 1500%. Using X/XO to generate O2, but in the absence of NADPH, Al (400 μM) stimulated iron-dependent LP 160%. In the presence of NADPH, Al inhibited iron-dependent LP significantly (range 16-87%) when Al was present at equivalent or greater molar concentrations than iron. Microsomal NADPH-dependent H2O2 formation was inhibited 10% by 400 μM Al. All differences sig. at P < 0.05. These data confirm the work by B. Fulton et al. (J Biol Chem 962:196-200, 1988) that Al stimulates NADPH-independent LP. However, NADPH-dependent LP, which is approximately 10-fold greater than NADPH-independent LP, appears to be inhibited by Al at a point prior to the production of O2. (BF supported by a 1990 IUUC-COM Hazel I. Craig Fellowship.)

LIPID PEROXIDATION IN RATS SUBACUTELY EXPOSED TO A LOW CONCENTRATION OF AEROSOLIZED CADMIUM. D Marcha, A Ricard, R Vincent, B Trottier, H V Traa and G Chevalier. Université du Quebec, Montreal, Canada.

Lipid peroxidation (LPO) was evaluated at 1, 3, 10, 15, and 30 days post-exposure in the lungs, liver, and kidneys of rats exposed to cadmium chloride (50 μg Cd/ml; CDIC: 0.3 μm; Vf = 1.3) during 4 h/day for 14 days. Results demonstrate that LPO was significantly increased in lung tissue 10 h after the first exposure (1.3% P < 0.01) while activities of the antioxidant defense components, namely GPX, GR and SOD were not affected. Activity of G6PDH was significantly increased at day 1. Subsequent exposures had no effect on lung LPO but produced significant changes in GR and G6PDH, while GPX and SOD remained unchanged. These parameters were not altered in liver and kidneys. These results demonstrate that LPO is an early response of the lung tissue and that it may serve as an early indicator of the intensity of exposure. Alterations of the antioxidant defense system are not responsible for the stimulation of the LPO reaction.

INHIBITION OF IRON-ASCORBATE STIMULATED LIPID PEROXIDATION IN RAT LIVER MICROSONES BY PURINE AND PYRIMIDINE TRIPHOSPHATES. J. Palamanda and LP. Keffer, Division of Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX.

Both iron-ascorbate and iron-NADPH stimulated lipid peroxidation in rat hepatic microsomes can be inhibited by the addition of glutathione. The effect of glutathione is mediated by a heat- and trypsin labile factor, and is believed to require vitamin E. The effect of purine and pyrimidine triphosphates on microsomal lipid peroxidation has not previously been tested. Lipid peroxidation was induced by the addition of 400 μM ADP, 10 μM ferric chloride, and either 450 μM ascorbic acid or 400 μM NADPH (final concentrations). Rats liver microsomes (5mg/ml) were incubated at 37°C and sealed under 95% O2/5%CO2. One ml of the incubation mixture was removed for the measurement of thiobarbituric acid reactive substances (TBARS) at specific time points. The effect of exogenously added purine (ATP & GTP) and pyrimidine (UTP & CTP) triphosphates were evaluated on TBARS formation in both the iron-ascorbate and the iron-NADPH stimulated systems. The addition of all four triphosphates at 400 μM inhibited TBARS formation in the iron-ascorbate microsomal peroxidation system by 40% when compared with the control system without added triphosphate. The ability of ATP and GTP to inhibit TBARS formation in the iron-ascorbate stimulated system was lost upon heating the microsomes prior to initiating lipid peroxidation. None of the triphosphates inhibited TBARS formation in the iron-NADPH stimulated system. These data suggest that the inhibiting effects of nucleotide triphosphates in the iron-ascorbate system are not due to an iron chelating action. The exact mechanism underlying this inhibition remains to be determined although it appears to involve a microsomal heat labile factor which may interact with ascorbate. (This work was supported by NIH grant HL40695.)
Lipoxygenase activity in rat brain cytosol was studied using linoleic acid (LA) as a substrate. O₂ uptake was measured using Clark electrode while the formation or utilization of LA-OOH was monitored spectrophotometrically at 234 nm. The LA addition to native brain cytosol exhibited a biphasic response. Initially a rapid depletion of preformed LA-OOH was observed with concomitant appearance of a peak at 280-285 nm, indicating formation of oxo-compounds. This phase was followed by LA dioxygenation. In the dialysed cytosol, the dioxygenase activity was stimulated by nanomolar concentrations of H₂O₂ and micromolar concentrations of LA-OOH, and was inhibited by serotonin and catecholamines (IC₅₀ 25-43 μM). A significant peroxidase activity was also observed towards xenobiotic substrates viz. guaicol, benzidine, tetramethylphenylenediamine, thio benzensulfonate and aldrin in the presence of LA-LA-OOH. Supported in part by Grants from The Council for Tobacco Research USA, Inc. and S.C. Johnson and Son, Inc.

Neuronal cell bodies, except in dorsal root ganglia (DRG), contain no appreciable glutathione (GSH). Since sensory neuropathies involving the DRG are the most common expression of neurotoxicity, it is important to determine colocalization of related phase I and II enzymes. Paraflin sections (6μm) of DRG and spinal cord from adult female rats perfused with 4% paraformaldehyde were rehydrated and incubated with polyclonal antibodies (Abs) to glutathione-S-transferases (GST) with reactivity towards class alpha (α), one specific for subunit 1, mu (μ), cytochrome P450 (IE1 and IAI) and gamma-glutamyl transpeptidase (GGT). Sections were developed by using an ABC-peroxidase DAB complex. No immunoreactivity was observed with either the α-class or subunit I GST Abs in any of the nervous tissues examined. Neither anterior horn nor DRG neurons appeared to exhibit immunoreactivity with any of the GGT or GST Abs. Strong staining of DRG satellite, spinal cord epineurial cells and white matter of both cord and DRG was observed with GGT Ab. The satellite cells of the DRG stained intensely for μ-class GSTs and both P450 isozymes, whereas the anterior horn cells displayed moderate staining for P450 IAI and IIE1. Spinal epineurial cells appeared to contain high levels of both P450 isozymes and μ-class GSTs. Differences in the location of these phase I and II enzymes may provide a mechanistic basis for the selective vulnerability of sensory and motor neuron sub-types to xenobiotics. [Supported by NS23325 (HEL), GM42620 (RFN) and ES04976 (KRR)].

Earlier studies from this laboratory demonstrated that lipooxygenase catalyzes oxidation of number of xenobiotics. The present study reports for the first time the desulfitation and dearylation reactions catalyzed by highly purified soybean lipooxygenase. A K Naidu A K Naidu and A P Kukarni, Toxicology Program, College of Public Health, University of South Florida, Tampa, FL.

Male Sprague Dawley rats were pair-fed isocalorically a Lieber-DeCarli liquid diet with 38% of total caloric intake substituted by ethanol. The control rats received dextrose-maltose instead of ethanol. The rats were continued pair feeding on this diet for 18 months. Three of the 5 rats fed ethanol were given, i.p., vitamin E for 2 days at a dose of 400 mg/kg/day. These rats were placed in ethanol-collection chambers and ethanol exhaled was collected on cotton-ethyl alcohol filters, heat desorbed and measured by gas chromatography. The rats were then killed and diene conjugates and fluorescent lipids in their liver samples were determined. The results showed that ethanol-fed rats exhaled remarkably high levels of ethane compared to the control-fed rats. Vitamin E treatment reduced ethane exhaled to ethanol to intermediate levels indicating that Vitamin E inhibits ongoing free radical attack on lipids. Diene conjugates and lipid fluorescence were also increased with ethanol consumption but without vitamin E showing an effect suggesting that these materials are formed or removed slowly and a short administration of vitamin E did not affect their levels. [Supported by NCI grant No. 51088].

Vitamin E inhibits lipid peroxidation induced by chronic ethanol consumption. D.L. Muhl and C.D. Ekelson, Departments of Surgical Biology and Pharmacology and Toxicology, University of Arizona, Tucson, AZ. Sponsor: J.G. Sipes.

The health risk assessment of a chemical or a mixture of chemicals should represent all the available integrated scientific evidence on the plausible toxicities of chemicals of interest. Available empirical and mechanistic information suggests that mixtures may contain components that enhance or diminish the toxicity of other components. EPA-Cin (1988) suggested the development of an interaction scheme for mixtures, analogous to the IARC type of classification. Presented will be a preliminary approach to express the weight of evidence for the interactions in binary mixtures based on information from human and animal bioassay data, studies on pharmacokinetics, metabolism and Structure Activity Relationships. Also included in this approach are factors such as the relevance of route, duration and sequence of exposure, toxicological significance of interactions or the quality of in vivo and in vitro data. The utility of this approach to estimate and predict interaction factors for binary mixtures will be discussed. Such an approach, when fully developed, will help risk assessors decide consistently the weight to be given to each piece of information from interactions in the overall determination of potential hazards of chemical mixtures.


TCE, 1,1-DCE and chromium were found as drinking water contaminants in an area of Tucson, AZ which had an excessive number of children born with cardiac malformations. Both TCE and DCE were found to cause specific cardiac teratogenicity in the chick and rat. In order to study possible interactions between the halogenated hydrocarbons and chromium in vivo, rats were injected SC with 1µCi Na2 51CrO4/g body weight. Some animals received 50 µL "neat" TCE or DCE SC daily. Rats were sacrificed at 24,48 or 72 h and 51Cr in major organs quantitated by gamma counting. The highest labeling ratios were seen in kidney, liver and spleen in both male and female rats. Treatment with both TCE and DCE significantly decreased 51Cr in a number of organs, including the heart. The possibility of interactions between TCE or DCE and chromosome at the site of injection was ruled out by obtaining UV spectra of neat solvents and solvents over chromosome. Chromium is known for its toxicity/mutagenicity but is also thought to be essential for many functions. The ability of TCE and/or DCE to affect the distribution and metabolism of chromosome may play a role in the observed cardiac teratogenic effects of these compounds. (Supported by AZ Dis. Con. Res. Com. #82-9279, Am. Heart Assoc. (AZ Affiliate) #G22888 and NIEHS Superfund #1-P42ES04940-01).

EFFECTS OF PRETREATMENT OF MONOCHLORACETATE ON VINYLDENE CHLORIDE HEPATOTOXICITY. J B Wijeweara, H E Davis and W O Berndt, Dept. of Pharmacology & Toxicology, W. Va. Univ. Health Sci. Ctr. Morgantown, WV and Univ. of Nebraska Medical Ctr., Omaha, NE.

Vinyldene chloride (VDC) is found in ground water as a contaminant in considerable concentrations. VDC is both hepatotoxic and nephrotoxic in experimental animals. Chlorination of drinking water for disinfection produces mono-chloroacetic acid (MCA). Previous experiments in the lab showed that MCA caused significant depilation of glutathione. Reactive VDC metabolites are detoxified by conjugation with glutathione. Therefore it is hypothesized that MCA would increase VDC hepatotoxicity. Male Sprague Dawley rats (275-330g) were pretreated with 188mg/kg MCA + increasing doses of VDC (0,100,200,300,500mg/kg in 4ml peanut oil), and NaCl + increasing doses of VDC. The control rats received NaCl + peanut oil. Rats were anesthetized with ether after 24 hours and blood samples were drawn from the abdominal aorta for the determination of GPT. Pretreatment with MCA caused significant elevation of GPT. Statistical analysis showed that MCA caused a significant leftward shift of the dose response curve for VDC (mean values for the two curves, NaCl=4+37.1, MCA+VDC=826.5). These results show that MCA caused a significant potentiation of VDC hepatotoxicity. (Supported by AF Contract F49620-86-C-0096).

MONOCHLORACETATE PRETREATMENT INCREASES CHLOROFORM TOXICITY BUT NOT BINDING. H E Davis and W O Berndt, Dept. of Pharmacology & Toxicology, W. Va. Univ. Health Sci. Ctr. Morgantown, WV and Univ. of Nebraska Medical Ctr., Omaha, NE.

Monochloroacetate (MCA) and chloroform (CHCl3) are products of chlorination disinfection of drinking water. Previous studies have shown that MCA greatly increases the toxicity of CHCl3. The present studies were performed to determine if MCA pretreatment increases bioactivation of CHCl3. Male and female Sprague-Dawley rats were gavaged with neutralized MCA (188 or 94 mg/kg) or saline (equimolar for Na+) and 1 h later injected with 14CHCl3 (0.75 ml/kg CHCl3 or peanut oil vehicle. They were killed 2, 6 or 24 hr later. Samples of liver and kidney were processed for total label and label bound to tissue lipid and protein. Binding of label to kidney was not different in MCA and saline groups. In males, 6 hr after 14CHCl3, binding was increased in the MCA group for liver protein (26±6 vs. 8±1) and lipid (47±15 vs 10±3), however there was also more 14CHCl3 present in the tissue (protein bound to total 0.425±0.104 for MCA and 0.299±0.051 for NaCl). In females, the increase of binding to protein in the MCA group (52±14 vs 15±2) exceeded that of label (bound to total 0.67±0.203 in MCA and 0.285±0.006 in NaCl). These results indicate that increased formation of reactive metabolites increased toxicity and alterations in the distribution of 14CHCl3 contribute to the increased toxicity. (Supported by AF Contract F49620-86-C-0096).
A study was conducted to characterize alterations in the PK of CCl₄ and TCE upon co-administration. Male Sprague-Dawley rats (300-400 g) were prepared with indwelling jugular vein and carotid artery cannulas 18 hr before dosing. TCE and CCl₄ were administered iv in PEG 400, either as a single bolus or as a constant infusion in the presence of a constant infusion of the other chemical. Seven different regimens were employed with total doses of 17 mg/kg for CCl₄ and 66 mg/kg for TCE. Noncompartmental analysis of the blood concentration-time data yielded estimates for total systemic clearance (CL) and volume of distribution at steady-state (Vss). Comparison of PK parameters obtained following single bolus and constant infusions of CCl₄ or TCE revealed significant changes in CLr for CCl₄ and CCl₄ and Vss for TCE. CL of both compounds was reduced following bolus administration, which is consistent with metabolic saturation. CCl₄'s CLr and Vss, determined following a single bolus or a constant infusion, were not significantly changed in the presence of steady-state TCE infusion. TCE's CLr and Vss, following administration either as a single iv bolus dose or as a constant iv infusion, decreased in the presence of a constant CCl₄ infusion. Reductions in TCE's CLr ranged from 25% to 30%, whereas the Vss decreased 40% to 50%. It is apparent that CCl₄ has a greater inhibitory effect than TCE, which can be attributable to both competitive and suicide metabolic inhibition by CCl₄. Reduction of TCE's Vss by CCl₄ suggests CCl₄ interferes with tissue uptake of TCE, although how this occurs is unknown. (Supported by U.S. EPA CR-816528)

**EFFECTS OF MIXTURES OF TRICHLOROETHYLENE (TCE), DI(2-ETHYLHEXYL)PHOSPHATE (DEHP), AND HEPTACHLOR (HEPT) IN AN IN VIVO DEVELOPMENTAL TOXICITY SCREEN: A PILOT STUDY.**

M G Narotsky's, B T Hamby's, V M Chinchilli's, and B J Kallcol's. NIESI-Environmental Sciences' and US EPA, RTP, NC, and Medical College of Virginia, Richmond, VA.

In a preliminary investigation of the developmental effects of chemicals in mixture, three compounds were combined using two doses of each agent (a 2x2x2 design). F-344 rats were gavaged on gestation days (GD) 6-15 with solutions of TCE (32 or 320 mg/kg/d), DEHP (78 or 780 mg/kg/d), and HEPT (0.8 or 8 mg/kg/d) in corn oil. Each high dose was selected to produce minimal maternal toxicity when administered alone. The dams were allowed to deliver and their litters were examined on days 1 and 6. Implants were counted to determine prenatal loss. Maternal weight gain on GD 6-8 showed main effects of all three chemicals, but no significant interactions. Gestational weight gains exclusive of live-litter weights revealed TCE and HEPT main effects as well as a TCE-HEPT interaction. Developmentally, full-litter resorptions occurred in 20-75% of the dams in groups receiving a high dose of at least two of the compounds. Prenatal losses indicated a marginal TCE-HEPT interaction. On day 1, main effects of TCE and DEHP were present for pup weight, while by day 6, only TCE exerted a main effect. Dose responses and synergistic effects of these toxicants are currently being pursued using additional doses in a 5x5x5 design.
Polychlorotrifluoroethylene (PCTFE) is a mixture of tetrafluoroethylene and hexafluoropropylene. PCTFE is used as a hydraulic fluid in advanced Department of Defense weapon systems. PCTFE inhalation studies have demonstrated peroxisomal proliferation in the livers of chronically exposed rats. Inhalation studies have also associated dose-related body weight loss, increased organ weights and abnormal hepatic enzyme activities with exposure to PCTFE. The carboxylic acids of PCTFE oligomers have been associated with hepatotoxicity in rats. This is manifested by increased liver weights and the proliferation of hepatic peroxisomes. The same type of liver injury has been observed in perfluorodecanoic acid exposure studies. These data suggest that a carboxylic acid metabolite of PCTFE may be a toxic moiety, with liver being a target organ. However, the presence of carboxylic acid metabolites of PCTFE has not been confirmed in biological samples. This investigation has used combined GC/MS to verify the presence of carboxylic acid metabolites of PCTFE in the liver of rats exposed to PCTFE. (Supported by DoD Contract No. F33615-86-O-5032).

Increased MEOH use as a transportation fuel would result in greater potential for inhalation exposure. We examined potential of CC14 hepatotoxicity by exposure to inhaled MEOH as a function of MEOH concentration. Adult, male F-344 rats were exposed to 0, 1000, 2500, 5000 or 10,000 ppm MEOH by inhalation, 6 hr/day, for 1 or 3 days and gavaged 24 hr later with 0 or 0.075 ml CC14/kg. Hepatotoxicity was assessed 24 hr later. Inhaled MEOH alone was not overtly hepatotoxic; exposure to CC14 alone resulted in mild/minimal centrilobular degeneration and necrosis and a ~2-3 fold increase in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) relative to control. 10,000 ppm MEOH strongly enhanced CC14 hepatotoxicity (moderate/marked centrilobular degeneration/necrosis, ~100-fold increase in AST and ALT relative to CC14 alone). 5000 ppm MEOH was above, 2500 ppm MEOH was close to, and 1000 ppm MEOH was below the potentiation threshold concentration. The hepatic response to CC14 was qualitatively similar after 1 and 3 days of MEOH exposure. In conclusion, acute exposure to inhaled MEOH resulted in a concentration-dependent increase in CC14 hepatotoxicity. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

Because groundwater is an important source of drinking water, we examined the hepatic effects of a mixture of 25 chemicals (19 organics and 6 inorganics) frequently found in groundwater and the mixture's effect on CC14 hepatotoxicity. In a pilot experiment, adult male F344 rats were exposed to deionized water or the 25-chemical mixture via drinking water for 14 days then challenged with 0 or 0.075 ml CC14/kg by gavage. The 25-chemical mixture alone was not overtly hepatotoxic but apparently enhanced CC14 hepatotoxicity (increases in severity of histopathologic changes and in serum alanine (ALT) and aspartate (AST) relative to CC14 alone). The 25-chemical mixture resulted in decreased feed and water consumption. In a confirmatory experiment, rats were exposed to deionized water or the 25-chemical mixture for 14 days then challenged with CC14 (0, 0.0375, 0.05, 0.075 and 0.15 ml/kg). Water and feed restricted controls were included. Exposure to the 25-chemical mixture again resulted in apparent enhancement of CC14 hepatotoxicity. However, water and feed restriction appeared to account for a significant portion of this observed enhancement of CC14 hepatotoxicity. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy).

The hepatotoxicity and lethality of CCl4 were studied in 2, 20, 35, 45, and 60 days old rats with or without dietary chlordane (CD) preexposure for 15 days. A low dose of CCl4 (100µl/kg, ip) did not cause any significant hepatotoxicity or death in rats fed normal diet at any age. CD+CCl4 combination also failed to induce any hepatotoxicity and mortality in 2 and 20 days old rats. This combination caused increased hepatotoxicity in 35- and 60 days old rats, as indicated by elevation of serum ALT, but without any mortality. However, CD+CCl4 treatment resulted in greater hepatotoxicity in 45 and 60 days old rats with mortality of 16 and 90%, respectively. The CD precipitated in hepatic tissue of 2 or 20 days old rats through dietary exposure of their dams to 30 ppm CD for 15 days was comparable to that in the hepatic tissue of rats exposed to 10 ppm CD diet for 15 days. Preexposure to CD led to increased cyt p-450 content in all age groups. Hepatic microsomal cytochrome p-450 contents increased with age, but they were not significantly different among rats older than 35 days, regardless of CD treatment. Hepatocellular proliferation decreased rapidly up to 20 days and more slowly thereafter, as indicated by 3H-thymidine incorporation in hepatic nuclear DNA. Age-related differences in CCl4 bioactivation and ongoing hepatocellular proliferation may both impart resiliency to CD-potentiation of CCl4 toxicity in developing rats. (Supported by AFOSR-88-0099).

We previously reported (Toxicologist, 1990, 10(1) and Toxicol., Appl. Pharmacol., 1990, 106) that the polychlorinated biphenyl (PCB) congeners that human primates follow exposure to Aroclor 1016 are nonplanar and ortho-substituted and reduce brain concentrations of dopamine (DA).

These same congeners also cause dose-dependent decreases in PC12 cellular DA content. PC12 cells synthesize, store, release and metabolize DA. We now extend these studies to include additional PCB congeners and structurally similar polychlorinated biphenyls (PCBs). Cells were exposed to either PCBs or PBBs for 6 hr. Changes in cellular concentrations of DA were determined by HPLC with EC detection. Results are expressed as the IC₅₀ for reductions in cellular DA. The most active PCBs had IC₅₀≤100 μM and were di-ortho substituted. Two of the most active PCBs were 2,2' (IC₅₀=65 μM) and 2,6 (IC₅₀=106 μM). Complete ortho substitution reduced activity (2,6,2',6' had an IC₅₀=1000 μM). PCBs with chlorine substitutions in the meta or para position also showed low potency. The most planar, dioxin-like PCBs (3,4,3',4' and 2,3,5,3',4') had IC₅₀<100 μM. PCB congeners have IC₅₀ similar to their PCB homologs with 2,2' PBB having an IC₅₀ of 60 μM and 2,6 PBB having an IC₅₀ of 95 μM. Meta-substituted PBBs also had lower potencies. These results confirm our earlier observations and demonstrate that a non-planar conformation produced by ortho-substitutions of the biphenyl rings with Cl or Br allows these molecules to interact at a specific site on the dopaminergic neuron. Supported by grants from USEPA (818380) and NIH (ES03884).


Groups of 18 female Macaca mulatta monkeys, whose average estimated age at study initiation was 11±4 years, self-ingested capsules containing daily doses of 0.5, 20, 40 or 60 μg of Aroclor 1254/kg bw for 2 years at which time 93% of the treated monkeys had attained a qualitative pharmacokinetic steady state. They were mated to untreated males while continuing to receive a daily dose of Aroclor 1254 during mating, gestation and the first 6 weeks of nursing. All infants were nursed for 22 weeks and then separated. The infants received no further PCB. Monitored parameters included daily health status; feed and water consumption; menstrual status (daily); body weight (weekly); hematology; serum chemistry; detailed clinical evaluation; PCB analysis of blood, adipose tissue and feces; TG levels (monthly); evaluation of estrogen and progesterone levels during one menstrual cycle just prior to breeding and immunological testing prior to and post breeding. Major treatment findings included: Reproduction - a dose dependent effect upon reproductive parameters; Immunology - several treatment related effects; PCB analysis - fecal analyses indicated greater than a 90% retention of the administered dose; Clinicals - changes in nail beds with subsequent nail loss, inflammation and distalization of tarsal glands; Hematology - decreases in reticulocytes, mean platelet volume, hemoglobin, red blood cells; Serum biochemistry - decreases in cholesterol and total bilirubin; Infants - little effect on growth and development.


Polychlorinated biphenyls (PCBs), are resistant to metabolism in most animal species. The dog has the unique ability to metabolize and eliminate certain PCB congeners, as a result of the activity of the cytochrome P-450 isozyme PBD-2. An expressible cDNA coding for PBD-2 has been introduced into the genome of tobacco plants. PBD-2 cDNA and a screenable marker gene coding for neomycin phosphotransferase were introduced into tobacco leaf disks using a binary Agrobacterium tumefaciens vector system. Southern and Western blot analyses have confirmed chromosomal integration of the cDNA and expression of the PBD-2 polypeptide. Differential centrifugation and Western blot analyses have shown the PBD-2 protein to be associated with a membrane fraction in transgenic tobacco leaf homogenates. Our goal is to develop transgenic plants in which the PBD-2 protein metabolizes PCBs, thus providing a novel method for bioremediation of PCB-contaminated soils.
The freshwater planarian, *D. dorotocephala*, is being developed as a model to test environmental samples for tumorigenesis. The induction of p-nitrophenetole (pNPEt) Q-dealkylase by Aroclor 1254 was investigated at doses known to enhance cadmium tumorigenesis. Planarians were pretreated in Aroclor 1254-coated beakers and, at designated times, were transferred to beakers containing 50 uM pNPEt, exposed for 24-46 hours and p-nitrophenol (pNP-OH) determined in hydrolyzed medium. Exposure to 5-25 uM Aroclor 1254 results in minor (≤20%) changes in activity after 1 week and moderate enhancement of activity at 2 weeks. Higher doses (50-100 uM) cause induction at one week and a return toward or below control values by 2 weeks of continuous exposure. This return toward control values can be reversed by feeding after 2 weeks exposure and before introducing the planaria into the pNPEt. After more than 2 weeks of exposure, pNP-OH production is also suppressed in unfed planaria receiving lower doses. The planarians accumulate Aroclor in a dose-dependent manner and the dose-induction relationship parallels the 11-day dosecarcinogen concentration relationship. This information warrants further studies.

**Effects of Selected PCBs and PBBs on Retinoid Levels in Various Rat Tissues.**

Male Sprague-Dawley rats (about 40g) were fed a vitamin A-adequate (4000 IU/kg) diet for 30 days before being given a single IP injection of one of 7 polyhalogenated biphenyls (150 μmol/kg) in corn oil (10 ml/kg) or vehicle alone. Rats served as controls. From 3 weeks later, except for 3,3',4,4',5,5'-HBB, all PCBs and PBBs studied significantly decreased serum retinol levels (p<0.05). The levels of hepatic retinol were decreased by 2,2',3,3',5,5'-HCB, 2',3,3',4,5-PCB and 3,3',4,4',5-PCB while those of hepatic retinyl palmitate were decreased by 2',3,3',5,5'-HCB, 2',3,3',4,5-PCB and 3,3',4,4',5-TCB, 3',4',4'-TBB and 3,3',4,4',5,5'-HBB. All halogenated biphenyls, except 3,3',4,4',5-PCB, which caused a decrease in hepatic retinyl palmitate, also caused an increase in renal retinyl palmitate. The acutely toxic (non-ortho substituted) congeners had more pronounced effects on hepatic, renal and serum retinoids while other biphenyls only decreased serum retinol levels. These data indicate that the structure-activity relationships for regulating hepatic retinoids differ from those for serum retinol, implying the involvement of multiple mechanisms.
Organic dyes are used by the military in colored-smoke marking grenades. The potential inhalation exposure of munitions workers to such dyes, particularly as mixtures, necessitates the assessment of their pulmonary toxicity. A red dye mixture (RDM) consisting of two red dyes, 1,4-diamino-2-methoxy-anthraquinone (Disperse Red 11) and 1-(2-methoxyphenylazo)-2-naphthol (Sudan Red), at a ratio of 9.4:90.6 was delivered as a dry aerosol (MMAD 2.4 μm, ef=2.3) to male F-344 rats (SPF; 90 days at the start). Groups of 10 rats were evaluated for lung dysfunction immediately after 4 or 13 wk of exposure (6h/d; 5d/wk at 0, 30, 100, or 300 mg/m³) or after a period (2 or 4 wk, respectively) in filtered air. Compliance (Cr,g), peak flow during forced expiration (PKFLOW), and body weight (BW) decreased, while end-expiratory volume increased, after 4 wk of exposure. These effects did not resolve after 2 wk in filtered air. After 13 wk RDM, significant effects were found for Cr,g, PKFLOW, Ny washout, total lung capacity, vital capacity, and BW; most of these effects also did not resolve. These data suggest the development of a subtle but significant lung restriction disorder. The lung dysfunction will be correlated with tissue structure/composition. The 300 mg/m³ RDM exposure group showed the most marked effects, although similar trends were seen at the lower concentrations. (This abstract does not necessarily reflect Army or EPA policy)

TOXIC INTERACTIONS OF BINARY MIXTURES OF NO₂ & CO, NOₓ, NO, & HCN. NO₂, O₂, AND NO₂ & CO₂. B C Levin, M Pasbo, and M Navarro. National Institute of Standards and Technology (NIST), Gaithersburg, MD.

A model to predict the toxic interactions of the major gases produced in fires is being developed at NIST and includes six gases - CO, NO₂, HCN, reduced O₂, HCl, and HBr. The current objective is to add NO₃ to the model. The toxicity of individual gases, CO, NO₂, reduced O₂, HCN, and NO₃ in air as well as various two, three and four gas combinations have been examined in Fischer 344 male rats exposed for 30 min and observed for 14 days. LC₅₀'s for HCl and HBr were obtained from the literature. Deaths from NO₃ in air occurred only in the post-exposure (PE) period and its LC₅₀ is 200 ppm (PE). Carbon dioxide has synergistic toxicological effects when combined with any of the other gases tested at NIST. The LC₅₀ for NO₂ in the presence of 5% CO₂ is 90 ppm (PE). CO produces only within-exposure deaths (WE) and its 30 min LC₅₀ is 6600 ppm. In the presence of 200 ppm of NO₂, the WE toxicity of CO increased. At the new LC₅₀ of CO, the PE toxicity of NO₂ also increased. Deaths from HCN occur primarily during or within 24 hours following exposure and its LC₅₀'s are 200 ppm (WE) and 150 ppm (PE). An antagonistic effect is observed with NO₂ and HCN; in the presence of 200 ppm of NO₂, 2.3 to 2.5 times the HCN LC₅₀ is needed to produce one death within-exposure. Deaths from reduced O₂ occur primarily within exposure and its 30 min LC₅₀ is 5.4%. In the presence of 200 ppm of NO₂, the WE LC₅₀ of O₂ and its toxicity increased. At this new LC₅₀ of O₂, the PE toxicity of NO₂ increased. Thus, NO₂, a PE toxic gas, increases the toxicity of the WE toxic gases (except HCN) and vice-versa.

FURTHER EVALUATION OF PULMONARY EFFECTS OF AEROSOLIZED MACHINING FLUIDS IN MICE AND GUINEA PIGS. K Detwiler and M Schaper. University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA.

The sensory and pulmonary irritating properties of 10 aerosolized machining fluids were recently evaluated in mice and for each fluid, the concentration capable of evoking a 50% decrease in respiratory rate (RD50) was obtained. Four of these fluids were studied here. Groups of mice were exposed to each fluid at its RD50 on Days 1, 2, 3, 4, 5 and 14 for 3 hours/day. The same relative decrease in respiratory rate was evoked each day, thus, there was no evidence of a cumulative effect. Groups of guinea pigs were also exposed to each fluid at its RD50 on Days 1, 2, 3, 4 and 5 for 30 minutes/day. Similar exposures were conducted on Days 19, 33, 47, 61, and 75. No pulmonary effects were observed on Days 1-5 or on Day 19. However, on Days 33-75, bronchoconstriction occurred in 2/4 animals that were exposed to a semi-synthetic fluid. No response occurred in the other exposed animals. These data suggest that the semi-synthetic fluid may contain a sensitizing ingredient. This protocol may be useful for screening other machining fluids for sensitization potential. Supported by UAW-GM.


Two refinery waste water sludges, API Separator Bottom Sludge (API Sludge) and Dissolved Air Float at (DAF) Float, were evaluated for sub-chronic (SC) and developmental toxicity. In the API Sludge studies, the material was applied to the clipped backs of rats at dose levels of 0, 500 and 2000 mg/kg; dose sites were occluded. Pregnant rats were exposed to the sludge on gestation days (GD) 0-20; in the SC study, male rats were exposed for 90 days, 5 days/wk. In the DAF Float studies, the test material was applied to non-occluded sites at dose levels of 0, 60, 250, and 1000 mg/kg to male and female rats for 90 days, 5 days/wk. Pregnant rats were exposed to DAF Float at 0, 125, and 500 mg/kg on GD 0-20 or to 1000 mg/kg on GD 0-15. In the SC studies, API Sludge did not produce overt signs of toxicity; DAF Float produced skin irritation, decreased body wt, altered hematology, altered organ wt and reduced sperm count. Both sludges produced maternal and developmental toxicity. Adverse fetal effects included an increase in intrauterine death, decreased body wt, and reduced skeletal ossification. The systemic and developmental effects may be related to levels of polynuclear aromatic compounds derived from petroleum. Refinery streams containing polynuclear aromatic hydrocarbons (PAH) and PAH containing nitrogen are known to cause SC and developmental toxicity.

Exxon conducted a chemical characterization of thermal process emissions on several polyethylene resins using a variety of extrusion processes. The air emissions were complex and varied with polymer composition and processing conditions. About 200 chemicals were identified from the resins representing at least 10 classes of chemicals. Nonaromatic hydrocarbon vapors (alkanes/alkenes) were generally present in the largest amounts in the emissions. The highest concentrations were found in the C$_6$-C$_{12}$ fraction. Non-volatile aerosols were also present in major amounts and appear to consist of higher alkanes (>C$_{12}$). With the possible exception of paraffin fumes, none of the identified chemicals exceeded their respective ACRI TLV's. Overexposure to the emissions can lead to irritation of the eyes and respiratory tract and these effects may be due to paraffin fumes and/or aldehydes and acids. Based on a review of exposure and toxicity data of emission components, there is, at present, no conclusive evidence that these emissions will produce irreversible lesions in man.


Reactive oxygen intermediates, in particular hydrogen peroxide (H$_2$O$_2$), have been implicated as mediators of skin inflammation and tumorigenesis. Both ultraviolet light B (UVB) and the phorbol ester, TPA, modulate HP and tumor formation in mouse skin. In the present studies we compared production of HP in epidermal cells from dorsal skins of CD-1 mice treated with UVB or TPA. Isolated epidermal cells were analyzed using flow cytometry and the HP specific dye dichlorofluorescin diacetate. Both UVB (60 mJ/cm$^2$) and TPA (17 nmol) were potent inducers of epidermal HP. However, the kinetics of HP formation in response to the two inducers was distinct. UVB induced a 50% increase in HP production over controls that peaked within 4 hr and then decreased. In contrast, TPA-induced HP production increased to 50% over controls within 4 hr and to 100% over controls within 24 hr. These data suggest that the actions of UVB and TPA in the skin are distinct. (ES03647)


Results from other laboratories (Cancer Res. 46:5096, 1986; Carcinogenesis 10:1769, 1989) have indicated significant biochemical differences in the responsiveness of peritoneal macrophages (MPs) elicited from phorbol ester-sensitive (SENCAR) and relatively resistant (C57Bl/6) mice to 12-O-tetracanoylphorbol-13-acetate (TPA) in vitro. Recent studies in this laboratory have indicated that MPs elicited from B6C3F1 mice, another mouse strain resistant to skin tumor promotion by TPA, also did not generate superoxide (quantitated spectrophotometrically at 550 nm as a reduction in ferricytochrome-c) when stimulated by 200 nM TPA in vitro. Alternatively, TPA-stimulated SENCAR MPs demonstrated a time and dose-dependent release of superoxide anion (4-7 nmoles/10$^6$ cells) which was significantly inhibited (50%) by preincubation with H-7 (40 uM), a specific protein kinase C (PKC) inhibitor. A similar strain-dependent induction of superoxide was observed when MPs were stimulated with thapsiargin (THAP), a tumor promoter which has been shown to act independently of PKC. THAP-stimulated SENCAR MPs released significant superoxide (2-3 nmoles/10$^6$ cells) which was not inhibitable by H-7 whereas MPs from B6C3F1 mice demonstrated negligible stimulation. Preincubation of SENCAR MPs with dibutylcyclohexylphosphoramide (100 uM), an inhibitor of phospholipase A$_2$, completely suppressed the superoxide stimulated by TPA or THAP. Therefore, arachidonic acid metabolite production may be a common biochemical pathway by which phorbol and non-phorbol-type tumor promoters activate inflammatory cells in SENCAR mice (Supported by ACS grant IN-17).

MOUSE STRAIN DIFFERENCES AFFECT ENHANCED SUSCEPTIBILITY TO RESPIRATORY INFECTION FOLLOWING EXPOSURE TO OXIDANT GASES. MT Gilmour, P Park, and RAK Silverdale. Center for Environmental Medicine UNC, Chapel Hill, NC, and Health Effects Research Laboratory, US EPA, RTP.

Reports that C57/B6 mice are more susceptible than C3H/HEJ mice to O$_3$ induced inflammatory responses prompted us to investigate strain differences in enhanced susceptibility to respiratory infection. Mice were exposed to O$_3$ (0.4 or 0.8ppm), NO$_2$ (2 or 5ppm), phosgene (0.025 or 0.05ppm), or air for 3hrs prior to receiving a sublethal infection of Streptococcus zoeoepidemicus. In contrast to the inflammatory responses, the C3H mice were more susceptible to infection after being exposed to the gases, than the C57 mice. Alveolar macrophages (AM) from the nonexposed C3H mice had a lower baseline phagocytic activity than the C57 controls, and both strains demonstrated similar decrements in function following O$_3$ exposure. The streptococci proliferated in the lungs of O$_3$ exposed mice, and displayed a mucoid colony appearance indicative of capsule. These studies demonstrate inherent differences in susceptibility to infection between C3H and C57 mice following exposure to oxidant gases which, in the case of O$_3$, correspond to AM phagocytic activity. The reason for the exacerbation and possible development of virulence factors in the bacteria isolated from O$_3$ exposed animals is not known but may be linked to the observed phagocytic defect. This abstract does not necessarily reflect EPA policy.
THE EFFECTS OF HYDROGEN PEROXIDE AND OZONE ON THE YEAST, SACCHAROMYCES CEREVISIAE. B L N Blau and C S McLaughlin. Dept. of Community and Environmental Medicine, Univ. of California, Irvine, CA. Sponsor: D B Menzel.

Reactive oxygen species (ROS), which are produced from incomplete reduction of oxygen during metabolism or by many xenobiotics, are potentially harmful to cells by interacting with biomolecules. ROS are known to induce the synthesis of a set of specific proteins in prokaryotes. This oxidative stress response may occur in the yeast S. cerevisiae. Yeast serves as a useful model for the study of toxic agents in eukaryotic cells. In this study we analyzed the effects of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), a ROS, and ozone, which is known to decompose to ROS, on the S. cerevisiae strain W303a. The sensitivity of yeast to these compounds was measured by cell survival. The survival of yeast exposed to H\textsubscript{2}O\textsubscript{2} concentrations of 0.5 to 100 mM, for up to two hours, was shown to decrease with increasing H\textsubscript{2}O\textsubscript{2} concentrations. To assure that cell death was due to H\textsubscript{2}O\textsubscript{2}, and not another ROS, we performed inhibition studies employing the antioxidant enzymes, superoxide dismutase and catalase, and the iron chelator, desferal. Our results suggest that superoxide anions and hydroxyl radicals were not involved in cell death. Cells growing in media were exposed to ozone entrained in air. Ozone concentrations greater than 30 ppm decreased cell survival. Changes in yeast protein composition due to the effects of ROS were examined by two-dimensional gel electrophoresis and will be described.

E-BUTYLLHYDROPEROXIDE METABOLISM IN HEPATOCYTES IS PRIMARILY DEPENDENT UPON NADH AND NOT UPON NADPH. D P Jones, D P Kowalski and T Y Aw. Dept. of Biochem. and Winship Cancer Ctr., Emory Univ., Atlanta, GA

In studies of the effects of lactate accumulation during anoxia on the susceptibility of hepatocytes to post-anoxic oxidative stress, we found that lactate protects against oxidative injury due to \textsuperscript{\textsuperscript{-}}\textsuperscript{BuOOH}. Measurement of \textsuperscript{\textsuperscript{-}}\textsuperscript{BuOOH} metabolism showed that added lactate stimulated peroxide elimination. Under these conditions, pyruvate accumulation occurred at 50-80% of the rate of peroxide loss. Measurement of NADH and NADPH concentrations as a function of added lactate in the presence of \textsuperscript{\textsuperscript{-}}\textsuperscript{BuOOH} showed that both pools are decreased by peroxides, but added lactate only increased NADH. Measurement of the rate of NADPH supply for reduction of GSGL shows that the rate (8 mmol/10\textsuperscript{6} cells per min) is only about 20-25% of the rate of peroxide elimination (35-40 mmol/10\textsuperscript{6} cells per min). Thus, NADPH is not supplied at a rate fast enough to account for peroxide elimination and, under these conditions, most of the metabolism occurs by NADH-dependent mechanisms. These results suggest that GSH peroxidase may not be the quantitatively most important pathway for \textsuperscript{\textsuperscript{-}}\textsuperscript{BuOOH} elimination, and that lactate or other NAD\textsuperscript{+}-linked donors may be useful for protection against oxidative injury. Supported by NIH grant GM-36538.

POSSIBLE ROLE OF ACTIVE OXYGEN IN MANIFESTATION OF SELENITE CYTOTOXICITY IN RAT HEPATOCYTES. Y Seko, J Kitahara and N Imura. School of Pharmaceutical Sciences, Kitasato Univ., Minato-ku, Tokyo, Japan.

The possibility of active oxygen generation in the presence of selenite and sulfhydryl compounds and its role in the cytotoxic action of selenite were investigated. The emission of chemiluminescence (CL) and a decrease in dissolved oxygen were observed on the addition of glutathione (GSH) to the solution of selenite and luminol. The CL emission was inhibited to less than 10% by superoxide dismutase (SOD). Further, the CL emitted on exposing an oxygen-free H\textsubscript{2}Se solution to the air was also depressed by SOD. These results suggest that superoxide anion (O\textsuperscript{2-}) was generated by the reaction of oxygen molecules with H\textsubscript{2}Se, which was produced by reduction of selenite with GSH.

Decrease in the GSH content and increase in the O\textsubscript{2} consumption rate were observed after the addition of selenite to rat hepatocyte suspension. Subsequently, the amount of thiobarbituric acid-reactive substances and the activity of lactate dehydrogenase were gradually increased in the supernatant of hepatocyte suspensions. A desferrioxamine-Mn complex, a SOD mimic, reduced the selenite-induced cytotoxicity much more than desferrioxamine itself. These results indicate that O\textsuperscript{2-}, generated in rat hepatocytes exposed to selenite, may participate in the manifestation of selenite cytotoxicity.

INTRACELLULAR DISTRIBUTION OF PROTEIN AND NON-PROTEIN THIOLS IN SINGLE CULTURED HEPATOCYTES BY MULTIPARAMETER DIGITIZED VIDEO MICROSCOPY (MDVM). A-L Nieminen, B Herman and JI Lemasters. Department of Cell Biology & Anatomy, University of North Carolina, Chapel Hill, NC.

Cellular glutathione plays an important role in protecting cells against damage from oxidant chemicals. The fluorescent probes monochlorobimane (mBCl) and monobromobimane (mBBr) have been used previously to measure intracellular glutathione and protein thiols by flow cytometry (J Biol Chem 263, 14107). Here, we use bimane probes to determine the intracellular distribution of thiols in single hepatocytes. Overnight cultured rat hepatocytes were stained with 200 \textmu M mBCl or 500 \textmu M mBBr. Fluorescence was imaged at 380 nm excitation and 470 nm emission using MDVM. Intracellular distribution of fluorescence was determined by opening intracellular compartments with increasing strengths of detergent. mBCl fluorescence which identifies non-protein thiols (predominantly glutathione) was 92% localized in cytosol. mBBr fluorescence released by 10 \textmu M digitonin corresponded principally to glutathione, whereas the remainder corresponded to protein thiols. Protein and non-protein thiols were measured during chemical hypoxia with 2.5 mM KCN and 0.5 mM lodoacetate and during oxidative stress with 50 \textmu M HgCl\textsubscript{2}. HgCl\textsubscript{2} decreased protein thiols by 70% and glutathione by 95% in 90 seconds. In chemical hypoxia, glutathione was 90% depleted after 20 min but protein thiols declined more slowly. In conclusion, mBCl and mBBr are useful probes to measure protein and non-protein thiols simultaneously in single cells during injury.
MITOCHONDRIAL AND GLYCOLYTIC DYSFUNCTION IN HEPATOCYTES EXPOSED TO t-BUTYL HYDROPEROXIDE: CONTRIBUTION OF A MITOCHONDRIAL PERMEABILITY TRANSITION TO LETHAL INJURY. R Imberti, A-L Nieminen, B Herman and JJ Lemasters. Department of Cell Biology & Anatomy, University of North Carolina, Chapel Hill, NC.

In isolated mitochondria, t-butyl hydroperoxide (t-BuOOH) promotes a permeability transition characterized by increased permeability to small ions, swelling and loss of membrane potential. Cyclosporin A (CyA) and trifluoperazine (TFZ) inhibit this permeability transition. Here, we investigate the role of the mitochondrial permeability transition in lethal injury from t-BuOOH. Hepatocytes from fasted rats were isolated by collagenase perfusion, and cell viability was assessed by propidium iodide fluorescence. Mitochondrial membrane potential was evaluated by rhodamine 123 (Rh 123) retention. Glycolysis was determined from lactate plus pyruvate production. t-BuOOH caused dose and time-dependent cell killing. Fructose, a substrate for glycolytic ATP formation, protected at lower (<50 μM) but not at higher concentrations of t-BuOOH. At 100-300 μM, t-BuOOH, oligomycin (10 μg/ml) conferred protection upon fructose. CyA (0.5 μM) plus TFZ (5 μM) could replace oligomycin in conferring protection. In single cultured hepatocytes, t-BuOOH caused abrupt leakage of Rh 123 from mitochondria after about 30 min which preceded cell death. In hepatocyte suspensions, CyA plus TFZ in the presence of fructose prevented mitochondrial depolarization. At 1 mM, t-BuOOH inhibited glycolysis. In conclusion, our findings support the hypothesis that inhibition of mitochondrial function is the common final pathway leading to cell death after exposure to t-BuOOH.


Lucigenin-derived chemiluminescence measures the production of superoxide anion (O₂⁻) by the cell, either extracellular O₂⁻ produced by NADPH oxidase or intracellular O₂⁻ produced by the reduction of molecular oxygen by the mitochondrial electron transport chain. Rat alveolar macrophages (AMs) have a ten fold greater resting lucigenin CL when compared to rat peritoneal macrophages or human monocytes due to increased mitochondria-derived lucigenin CL. Although the addition of 30 ng/mL TPA (12-O-tetradecanoyl phorbol-13-acetate) to AMs increases the production of O₂⁻ by NADPH oxidase the net result is a decrease in lucigenin CL resulting from inhibition of mitochondria-derived CL. Since protein kinase C (PKC) has been shown to be the cellular receptor through which TPA mediates its effects, it was postulated that activation of PKC inhibits mitochondria-derived CL. Studies performed with 50 μM H-7 (a PKC inhibitor) support this hypothesis by blocking the inhibition of mitochondria-derived CL by TPA, while its negative analogue HA-1004 has no effect on the system. These results demonstrate that mitochondrial respiration may be modulated by PKC. Moreover, this observation suggests a novel mechanism whereby xenobiotics which modulate PKC may affect cellular function. Supported by ES03760, ES07141, ACS SIG-3.


Patulin, a fungal metabolite, causes lipid peroxidation in cultured renal cells. Patulin-induced lipid peroxidation, blebbing, calcium uptake, and LDB release can be prevented by antioxidants. However, cell death is not prevented by antioxidants. Thus, the cause of cell death is due to factors which are not a result of lipid peroxidation. The purpose of this study was to determine those patulin-induced events which might explain patulin’s cytotoxicity. We have found that patulin causes an increased rate of **Rb⁺ efflux and reduced intracellular potassium content at times long before any changes in intracellular sodium. **Rb⁺ uptake or lipid peroxidation are observed. The increased potassium conductance can not be prevented by antioxidants. Therefore we looked to see if patulin was acting by oxidizing protein bound sulphydryls. What we discovered was that patulin had little effect on protein bound sulphydryls but readily depleted non-protein sulphydryls. The depletion of non-protein sulphydryls was not prevented by antioxidants and occurred concurrent to significant increases in potassium conductance. We suspect that patulin-induced depletion of non-protein sulphydryls and increased potassium conductance are mechanistically linked phenomena.

DEMONSTRATION OF CELL-SPECIFIC TMJURY USING RAT LUNG SLICES. K Brendel, MS Stefaniak, RD Spall, AJ Gandolfi. Dept. of Pharm/Tox., University of Arizona, Tucson, AZ

The pneumotoxicants bleomycin (2-200 mU/ml) or amiodarone (25-100 μg/ml) were incubated with agar-filled, precision-cut rat lung slices in a dynamic organ culture system for 6-48 hr. Direct cytotoxicity, as measured by 3Cr release, and protein synthesis was minimal with either drug. Bleomycin at 100 or 200 μM/ml induced a selective increase in phosphatidylcholine (surfactant) synthesis by 24 hr continuous exposure. These changes were paralleled by a pronounced Type II cell hypertrophy, the surfactant-producing cells of the lung; identity of the Type II cell was confirmed by histochemical demonstration of alkaline phosphatase activity. Amiodarone failed to produce marked changes in phospholipid synthesis, but elicited histologic evidence of macrophage phospholipidosis at 100 μg/ml. Demonstration of macrophage B-galactosidase activity indicated a state of activation. These experiments demonstrate application of the agar-filled lung slice model to the study of cell-specific injury in vitro. [NIH GM 28290] and a Ciba-Geigy Graduate Student Fellowship]
TOXICITY OF REDOX CYCLING COMPOUNDS IN RAT LUNG SLICES. MS Stefaniak, AJ Gandolfi, K Brendel, Dept. of Pharm/Tox., University of Arizona, Tucson, AZ

Agar-filled precision cut rat lung slices were incubated in a dynamic roller culture system with the redox cycling compounds nitrofurantoin (NF) or paraglut (PQ) (10^{-3}-10^{-5}M) for 6-48 hrs. Cytotoxicity (³⁸Cr release) was concentration dependent. Toxicity was enhanced under conditions of high (95%) O₂ tension, and attenuated by prior addition of catalase (1100 U/ml). Lipid peroxidation as measured by the disappearance of polyunsaturated fatty acid, was observed with 10^{-3}M NF or PQ by 12 hr. Total nonprotein sulfhydryl levels declined only after 24 hr, while enhanced oxidation of 1-³⁸C-glucose, but not 6-³⁸C-glucose to ³⁸CO₂ by both compounds at 2 hr indicates an early, selective stimulation of the pentose phosphate pathway. Stimulation by PQ was much greater than by NF; this blunted response may relate to the ability of NF to directly inhibit glutathione reductase. These experiments demonstrate application of the agar-filled lung slice model to mechanistic investigations in toxicology.

[NHGM 38290, T-32-ES07091, and a Ciba-Geigy Graduate Student Fellowship]
854 INHALED LEAD OXIDE (PbO) INDUCES AIRWAY HYPERRESPONSIVENESS MEASURED IN VITRO. H.A. El-Fawal, P.B. Schlesinger and I.T. Zeilieff, Institute of Environmental Medicine, NYU Medical Center, Tuxedo, NY.

Lead is a major airborne pollutant in industrial environments which poses a serious health threat. Little is known about the effects of inhaled lead on airway hyperresponsiveness (smooth muscle responses to pharmacological mediators). We examined tracheal (TR) and bronchial rings (BR) obtained from rabbits exposed by inhalation to PbO (30 µg/cm²) for 4 d (3 hfd) and sacrificed 24 h (124) and 72 h (172) after the final exposure. The rings were maintained at 37°C in Krebs-Henseleit buffer, at resting tensions of 1 and 0.5 g, and the responses to acetylcholine (ACh) and histamine (H) assessed. Air exposed rabbits served as controls. TR at 124 and 172 were 100x and 1000x more sensitive to ACh, respectively, than controls. BR at 124 and 172 were 100x more sensitive to ACh compared to controls. No change was observed in contractile tension in response to ACh. TR from control rabbits relaxed in response to H. However, at 124, TR from rabbits exposed to PbO contracted at higher concentrations of H; at 172, responses were characterized by contraction at low concentrations followed by relaxation, and then contraction at high concentrations. BR contracted in response to H in all groups, with 124 and 172 being 100 and 10,000x more sensitive than control, respectively. Magnitude of contraction was 3 and 2x greater than control at 2x10⁻² M H. Histological analysis of serial sections of TR and BR showed an intact epithelium. This study suggests that PbO induces airway hyperresponsiveness independent of epithelial desquamation; such altered airway responses may contribute to lung disease.

855 INTRACELLULAR pH OF TYPE II ALVEOLAR LUNG CELLS EXPOSED IN VITRO TO ACID AEROSOL CONDITIONS.

B Ziegler, R Vandagriff, R Rasmussen and DB Menzel, Dept. of Community and Environmental Medicine, Univ. of California, Irvine, CA

Intracellular pH (pHi) has been measured quantitatively in cultured human (A549) and rat (ARL) lung cells using a fluorescent pH indicator (BCECF) examined with a quantitative fluorescence confocal microscope (Meridian 570). pH was calibrated by treating cells with 10 µM nigericin in high K⁺ (130mM) medium to facilitate H⁺ exchange between the medium and the cells. Confocal images revealed that the resting pH varied over the range of 7.010 ± 0.449 (nucleus) to 7.892±0.128 (cytosole), depending on the region of the cell examined. The average intracellular pH was 7.418±0.049. The regional differences in pHi were complex, but reproducible representing local pH associated with subcellular organelles. On treatment with medium in the pH range (pH 6.7) estimated to occur on inhalation of acid aerosols, the pH fell rapidly within 2 min by 0.2 pH throughout the cell and slowly began to recover towards the resting pH. Since the value of pHi regulates many intracellular functions, these data suggest that total acidity may be a fundamental and important toxic property of acid air pollutants. Supported by a contract from the California Air Resources Board.

856 CYTOTOXICITY OF REFRACTORY CERAMIC FIBERS TO CHINESE HAMSTER OVARY CELLS IN CULTURE. G McCoy, M Newman, W Bunn, and T Hesterberg, Manville Technical Center, Littleton, CO.

Chronic animal inhalation studies are in progress to assess the toxicity/oncogenicity of refractory ceramic fibers (RCF) in rodents. There is a need to develop short term models to screen fibers for their toxicologic potential to reduce testing time and cost. In the present study, the toxic effects of four RCFs were determined using Chinese hamster ovarian (CHO) cells grown in culture. These RCFs were the same size selected fibers (approx. 1 µm X 22 µm) that were used in animal inhalation studies, providing a direct correlation of findings in the two systems. CHO cells were treated with RCF (1 to 30 µg/cm²) 24 hr after seeding into 60 mm culture dishes. Inhibition of cell proliferation and colony formation were determined at day 4 and day 6, respectively after treatment of the cells. Crocidolite asbestos (CD) was used as the positive control. A concentration dependent inhibition of cell proliferation and colony formation was observed after treatment with RCF. The LC-50 for cell proliferation and the different RCFs ranged from 1000 to 3000 µg/cm². The LC-50 for CD was 5 µg/cm². The RCFs induced similar inhibitions of colony formation. Work has begun to assess the genotoxic potential of these fibers using micronucleus induction. A 30% incidence of micronuclei was observed at 48 hr after treatment with 5 µg/cm² CD, and a 35% incidence with 2 µm mycotoxin C. A number of other fibers that are also being studied in chronic animal inhalation studies will be tested using this in vitro system. These data will help to validate this in vitro model, and could then be used in a battery of short term toxicity tests for screening man-made fibers.

857 AN EVALUATION OF THE COMPARATIVE METABOLISM AND KINETICS OF 1-NUITRO[14C]PYRINE BY RABBIT, RAT AND HAMSTER TRACHEAL EPITHELIAL CELLS: KINETIC ANALYSIS. LC King, E Hodgson and J Levens, U.S. Environmental Protection Agency, Research Triangle Park, NC; and North Carolina State University, Raleigh, NC.

The metabolism of 1-nitro[14C]pyrene by isolated rabbit, rat and hamster tracheal cells has been investigated in order to determine the steady-state kinetic parameters K and V for the reaction, and kinetic constants for the reaction, and kinetic constants for the reaction. Results suggest a similar enzymic affinity for L-NP, rabbit tracheal cells, however, have a faster rate of L-NP metabolism than either hamster or rat tracheal cells. This abstract does not necessarily reflect EPA policy.
The epithelial cells of the gastrointestinal tract have the capacity to engage in biotransformation of ingested chemicals. The presence and inducibility of cytochrome P450 (P450) isozymes in the epithelial cells of the colon and small intestine of male Sprague-Dawley rats was examined by Western analysis using polyclonal antibodies raised against rat liver enzymes. The appearance of P450IA1 was observed in both tissues after oral administration of β-naphthoflavone (BNF) (40 mg/kg/3 days). The appearance of this P450 isoform was concurrent with increases (up to 150-fold) in P450-related 0-deethylation of 7-ethoxyccomarin and 7-ethoxyresorufin.

Following administration of phenobarbital (PB), P450IIIB was identified immunochemically in the small intestine; however, this isozyme could not be detected in colon. These data suggest that intestinal epithelial cells respond to BNF and PB in a manner similar to the liver, whereas colonic epithelial cells may have a greater capacity to respond to P450IA1-type inducers. Evidence exists that differences in P450 isozyme composition can affect the ultimate metabolic fate of ingested chemicals, including carcinogens, and thus a role for colonic P450-dependent monooxygenase activity in the biogenesis of cancer in this tumor-susceptible tissue is suggested.

To assess the biotransformational capability of ocular tissues in the rabbit, representative phase I and phase II enzymes were assayed in five tissues from the eye, and in the liver, kidney and intestine. Within the eye, the iris/ciliary body exhibited the highest glutathione S-transferase activity, whereas the cornea possessed the highest specific activities for N-acetylation, sulfoxide and UDP-glucuronyltransferase activity. Cornea, iris/ciliary body, choroid and retina exhibited significant activities of N-acetyltransferase, 2-naphthol sulfoconjugation, and 1-chloro-2,4-dinitrophenol conjugation with glutathione. UDP-glucuronosyltransferase activity was relatively independent on tested substrates and tissues. When compared to liver, kidney or intestine, N-acetyltransferase activity in the iris/ciliary body nearly matched the rate measured in kidney, glutathione S-transferase activity in cornea and iris/ciliary body was nearly 70 and 89% of the rate in intestine, and corneal sulfoconjugation activity was greater than that in kidneys. Little phase I enzyme activity was measured in ocular tissues as compared to liver. These data suggest that biotransformation pathways are present in the eye, and particularly in ocular tissues having adequate blood supply or interfacing with the external environment.

1-Aminobenzotriazole (ABT) is a suicide substrate inhibitor of both hepatic and pulmonary cytochromes P450. The present studies were conducted to more fully characterize the effects of ABT on hepatic and renal metabolism. Hepatic and renal microsomes and cytosol were prepared from male Sprague-Dawley rats following ABT pretreatment (0-100 mg/kg ip) for various times. ABT produced comparable maximal losses of both renal and hepatic P450 within 2 hr; less of P450 in both tissues persisted for at least 32 hr. ABT-induced destruction of P450 was dose-dependent. Maximal inhibition of about 75% of total hepatic P450 occurred at dosages of ABT equal to or greater than 10 mg/kg. Maximal inhibition of 84% of total renal P450 occurred at dosages of ABT equal to or greater than 50 mg/kg. In addition, in both liver and kidney, only P450 content and P450-dependent activities were significantly decreased. Cytochrome b5, NADPH-cytochrome c reductase, glutathione S-transferase, glucuronide transferase, and reduced glutathione content were unaltered. These data suggest that ABT is a selective and effective inhibitor of both hepatic and renal P450. ABT may be a useful tool to probe the potential role of P450 in the bioactivation of certain compounds.
862 INDUCTION OF RAT CYTOCHROME P450IE1 BY INHALED PYRydINE VAPOR. A R Dahl, J A Hotchiss, S G Kim*, and R Novak*. Inhalation Toxicology Research Institute, Albuquerque, NM. *Institute of Chemical Toxicology, Wayne State University, Detroit, MI.

Cytochrome P450IE1 is induced by solvents, including pyridine, trichloroethylene, isopropanol, ethanol, benzene, carbon tetrachloride, and diethyl ether. Experiments were conducted to examine whether inhalation exposure to pyridine vapor resulted in increased expression of P450IE1 in hepatic and extrahepatic tissues. Rats were exposed to 5 ppm pyridine, 6 h/day for 4 days or to 440 ppm pyridine, 6 h/day for 1 or 4 days. Control rats were exposed to filtered air for 1 or 4 days. Liver and kidney tissues were removed from the air- and pyridine-exposed rats and expression of P450IE1 was examined in tissue sections using immunohistochemistry and in microsomal suspensions using Western blot analysis. Rats exposed to 5 ppm pyridine for 4 days or to 440 ppm pyridine for either 1 or 4 days had significantly elevated levels of immunoreactive P450IE1. Immunohistochemical analysis of liver sections showed that inhalation of pyridine resulted in elevated expression of P450IE1 in hepatocytes surrounding terminal hepatic veins. The results of this study indicate that inhalation of pyridine vapors at levels equal to or greater than the current TLV of 5 ppm results in induction of P450IE1 in rat hepatic and renal tissues. (Research supported by NIH Grant ES05656 and U.S. DOE/OHER under Contract No. DE-AC04-76-EV01013.)

863 METABOLISM OF ACRYLONITRILE (ACN) AND 2-CYANOETHYLENE OXIDE (CEO) BY RODENT BRAIN ENZYMES. G L Keeders and R Batra. CII, Research Triangle Park, NC.

ACN causes brain tumors in rats, but has not been tested in other species. The metabolism of ACN and its DNA-reactive epoxide CEO by microsomes and cytosols from the brains of male F-344 rats and B6C3F1 mice was studied to determine the capacity of this target organ to activate or detoxicate ACN. ACN was oxidized to CEO by microsomes from rat and mouse brain with a Vmax of 3 and 165 pmol/min/mg and Km of 12 and 102 mM, respectively. These rates are 200 and 17 times lower than the respective rates in liver microsomes. Inhibition studies indicated that cytochrome P450 was the catalytic enzyme. The very low affinities and low rates of oxidation suggest that the brain does not significantly activate ACN. The rate of conjugation of ACN and CEO (5 mM) with glutathiones (GSH; 2 mM) at pH 7.3 (5 and 3 nmol/min) increased only 1.6-fold after addition of brain cytosols, suggesting that the chemical reaction with GSH is more important than the enzyme-catalyzed reaction. The rate of hydrolysis of CEO at pH 7.3 (0.7 nmol/min) was not enhanced by addition of brain microsomes or cytosols. These results suggest that the brain can detoxicate CEO by GSH conjugation but not by enhancing its hydrolysis.

864 ISOYME-SPECIFIC BIOACTIVATION OF 3-METHYLLINDOLE BY PULMONARY CYTOCHROMES P450. D R Bushka and G B Marcus, Dept. Pharmacology & Toxicology, Purdue Univ., W. Lafayette, IN.

Ruminants are most susceptible to the selective pneumotoxic 3-methylindole (3MI). Rat and bovine pulmonary P450 isozymes metabolize 3MI to a reactive imine methide. Bioactivation of 3MI by pulmonary P450 was measured by trapping this intermediate with glutathione (GSH). 3MI-GSH adducts formed in vitro using 3H-3MI were quantitated by reverse-phase HPLC coupled with radioimmune and fluorometric detection. Pulmonary microsomal incubations were conducted in the absence and presence of isozyme-selective P450 inhibitors. Adduct formation by rat pulmonary microsomes (29 pmol P450/mg) was 1.4 ± 1.3 pmol/min/mg protein. 100 μM 1-phenylimidazole, (PI; selective for P450IIB isozymes), and 100 μM α-naphthoflavone (αNF; selective for P450I A isozymes), decreased adduct formation 57% and 21%, respectively. Adduct formation by bovine pulmonary microsomes (228 pmol P450/mg) was much greater, 659 ± 83 pmol/min/mg, and was inhibited 94%, and 83%, by the same concentrations of PI and αNF, respectively. 3MI bioactivation in rat lungs appears to be catalyzed largely by P450IIB-like isozymes, and in bovine lungs by both P450IIB- and P450I A-like isozymes. Species differences in 3MI bioactivation and pneumotoxicity thus appear to arise from both qualitative and quantitative differences in P450 expression. (Supported by a NIA Research Starter Grant)

865 IDENTIFICATION OF 3-METHYLLINDOLE METABOLITES FORMED BY ISOLATED CLARA CELLS. W K Nichols, P H Zimmerman, M O Covington, M L Appleton, G L Skiles, and G S Yost. Dept. Pharmacol. and Tox., Univ. of Utah, Salt Lake City, UT.

Several metabolites of the selective pneumotoxic, 3-methylindole (3MI), have been identified from the urine of mice, rats, and goats. Cytochrome P-450-mediated oxidation of 3MI is important in the formation of toxic intermediates involved in covalent binding to pulmonary proteins of several mammalian species. Clara cells from rabbits were incubated under the same conditions shown in earlier studies to produce cytotoxicity. Ethyl acetate extracts of sonicated Clara cells were examined using HPLC and purified standards of known metabolites. Rabbit Clara cells produced several metabolites of 3MI found in the urine of other species. The metabolites identified included 3-methylindoxil, 3-hydroxy-3-methylindole, and an apparent thioether adduct of 3MI. These in vitro techniques provided important information about the identification of the metabolites formed by isolated lung cells and the formation of toxic intermediates within these specific cells. GSY is a recipient of a Research Career Development Award from the NIH (HL02119) (supported in part by USPHS grant HL13645)
INDUCTION OF FLAVIN-CONTAINING MONOOXYGENASE (FMO) IN RABBIT LUNG DURING GESTATION. MY Lee⁴, DM Stroesser⁵, RN Hines¹,², and DE Williams¹,². Dept. of ¹Food Sci. and ²Toxicology program, Oregon State Univ., Corvallis, OR, and ³Dept. of Pharmaco., Wayne State Univ. Sch. Med., Detroit, MI.

FMO is not induced by xenobiotics but is regulated by endogenous factors like hormones. During the 31 day gestational period of rabbit, the level of progesterone linearly increases until parturition. It has been shown that FMO is induced during late gestation in rabbit. Rabbit lung tissues were collected throughout gestation (0, 10, 15, 20, 25, 31, 31 day, 1 day and 1 week postpartum). Microsomes and total RNA were prepared from each tissue. ¹⁴C-dimethylamine N-oxidase increased with pregnancy and was highest in the later period of gestation and reduced after parturition. Immunoprecipitation by western blots was consistent with FMO activity; protein levels were highest on the 31st day of gestation. A rabbit lung FMO C-terminus antisense probe was used to quantitate FMO RNA by northern blotting and showed that mRNA levels were also highest on the 31st day of gestation. This suggests that the levels and activity of FMO are regulated by endocrine factors and controlled by gene expression. Supported by NIH HL 38650.

METABOLISM OF THE PNEUMOTOXIN, 3-METHYLLINDOLE, BY CDNA-EXPRESSED CYTOCHROME P-450 ISOFORMS. J R Thornton-Manning, WRusuflidikarn and G S Yost. Dept. of Pharm. and Toxicol, Univ. of Utah, Salt Lake City, UT.

3-Methylindole (3MI) is a microbial degradation product of tryptophan, which is produced in the large intestine and in the rumen, is selectively pneumotoxic to several animal species after systemic circulation. Studies in our laboratory have indicated that the toxicity results after cytochrome P-450 (P-450)-mediated bioactivation. It is possible that the species susceptibility of 3MI pneumotoxicity is the result of differences in P-450 isozymes. In this study, cell lysates from HepG2 cells containing vacuolar expression vectors subconed with individual P-450 cDNAs were used to measure 3MI metabolism. Lysates containing P-450 isozymes IIA3 and IIF1 had a 3MI turnover rate of 0.772 and 0.693 nmol/mg protein/min. Cytochrome P-450 1V1B1 was also capable of metabolizing 3MI, however to a lesser extent than IIA3 and IIF1. The IIB7 isozyme metabolized 3MI to a negligible extent. These data indicate that IIA3, IIF1 and/or IVB1 may play a role in 3MI-mediated pneumotoxicity. Goats are highly susceptible to 3MI toxicity and therefore probably express the P-450 isoform(s) responsible for 3MI bioactivation. Initial results from northern blots produced from goat lung mRNA indicate the presence of a transcript that hybridizes to a cDNA probe encoding P-450 1V1B1. Human lung tissue has been shown to express 1V1B1 mRNA, suggesting that 3MI may be pneumotoxic to humans. Supported by Grant HL13645 from the U.S. Public Health Service, National Institutes of Health. G.S.Y. is a U.S. Public Health Service Research Career Development Awardee (HL02119).

PULMONARY ACTIVATION AND TOXICITY OF METHYL-CYCLOPENTADIENYL MANGANESE TRICARBONYL (MMT) AND CYCLOPENTADIENYL MANGANESE TRICARBONYL (CMT). R J Clay and J A Morris. Toxicology Program, School of Pharmacy, University of Connecticut, Storrs, CT.

MMT and CMT are organomanganese compounds which have been used as fuel additives. Previous studies in our laboratory have shown that these compounds are selectively toxic to the lungs following sc administration. Current studies have focused on the role of cytochrome P450 monooxygenase (P450) in the bioactivation of MMT and CMT to toxic metabolites in pulmonary tissues. MMT and CMT are metabolized by lung and liver microsomes in vitro. Micrometabolism was NADPH-dependent and inhibited by both piperonyl butoxide and mepirapone, suggesting MMT and CMT are metabolized via P450 in both tissues. Phenobarbital pretreatment increased hepatic metabolism of MMT and CMT 2-3 fold without significantly effecting pulmonary metabolism. Phenobarbital pretreatment protected animals from both MMT- and CMT-induced pulmonary toxicity (as assessed by bronchoalveolar lavage parameters). These results suggest MMT and CMT are metabolized in pulmonary tissues via P450 to a toxic metabolite(s). The liver does not appear to be involved in the generation of the pneumotoxic metabolites of MMT and CMT.
PEROXIDASE CATALYZED XENOBIOTIC OXIDATION IN HUMAN TERM PLACENTA. P Joseph and A P Kulkami. Toxicology Program, College of Public Health, University of South Florida, Tampa, FL.

Hydrogen peroxide dependent oxidation of xenobiotics in human term placental membranes (nuclei, mitochondria and microsome) was investigated. Guaiacol was employed as a model compound and its oxidation was found to be dependent on the concentration of enzyme protein, \( \text{H}_2\text{O}_2 \) and substrate.

Several other classical substrates for peroxidases viz. pyrogallol, thioribamidate, tetramethyl benzidine, tetramethyl-p-phenylenediamine, p-phenylenediamine, o-dianisidine, benzidine and azinobis were also found to undergo oxidation. The xenobiotic oxidizing capacity of the membranes was found to be retained by \( \text{CaCl}_2 \) (0.5M) extract as well as by the partially purified enzyme obtained by affinity (conconavalin A) chromatography. These reactions were inhibited by NaNO\(_2\), KCN and BHA. Since the partially purified enzyme preparation used were free of catalase, hemoglobin and cytochrome P-450, the observed oxidation of xenobiotics appears to be catalysed by the peroxidase, a constitutive protein present in placental membranes.

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STUDIES ON THE MECHANISM OF CADMIUM NEPHTROTOXICITY. C Dorian, V H Gattone Li and C D Klaassen, Univ Kansas Med Ctr, Kansas City, KS.

Chronic, but not acute exposure to Cd produces renal damage. However, a single injection of Cd-metallothionein (CdMT) produces renal injury. The nephrotoxicity induced by chronic exposure to Cd may be due to CdMT synthesized by the liver, released into the circulation and taken up by the kidney. In the present study, this hypothesis was tested. CdMT at dosages as low as 0.2 mg Cd/kg produced proteinuria, glucosuria and injury to S1 and S2 segments of the proximal tubules within 24 hrs. These changes are similar to those produced by chronic exposure to Cd. Following administration of \(^{109}\text{CdCl}_2\) only 5% of the dose was in the kidney, while as much as 90% of CdMT accumulated in the kidney. The renal uptake of Cd after \(^{109}\text{CdCl}_2\) was very rapid, as maximal concentrations of \(^{109}\text{Cd}\) were observed 30 min after injection. Within the kidney, \(^{109}\text{Cd}\) distributed almost entirely to the cortex. In the cortex, the highest concentration of Cd was observed in the S1 and S2 segments of the proximal tubules, the site of Cd nephrotoxicity. The concentration of Cd in the kidneys remained relatively constant for at least a week after CdMT administration. Uptake of \(^{35S}\text{CdMT}\) was also quantitated, and a similar rapid uptake by the kidney was observed, but \(^{35S}\text{-MT}\) rapidly disappeared from the kidneys. These data support the hypothesis that chronic Cd nephrotoxicity might be due to relatively specific uptake by the S1 and S2 segments of CdMT which is rapidly degraded to release the Cd that produces nephrotoxicity. (Supported by NIH Grant ES-01142 and a Merck Fellowship)

THE UPTAKE AND ACCUMULATION OF CADMIUM IN RAT RENAL CORTICAL EPITHELIAL CELLS. M Yoshida, Z A Shaikh and S A Jordan, Department of Pharmacology and Toxicology, University of Rhode Island, Kingston, RI.

Although the renal effects of Cd are well known, little information exists regarding the mechanisms of its uptake into renal cells. Primary cultures of rat renal cortical epithelial cells were used to study the uptake and accumulation of 3 \( \mu \text{M} \) Cd at 4 and 37°C, in the presence or absence of Ca, Zn, Cu, Fe, Ni or La. The uptake of Cd was rapid during the first 5 min at both 4 and 37°C but plateaued within 15 min at 4°C. After 30 min, the level of Cd in the cells at 37°C was 2.5 times that observed at 4°C. The initial rate of uptake of Cd was the same in the presence or absence of 1.2 \( \mu \text{M} \) Ca but after 30 min the cells incubated in the presence of Ca accumulated 1.5 times less Cd than in the absence of Ca. Similarly, 30 \( \mu \text{M} \) Zn, Cu, and Ni decreased the accumulation of Cd by 36, 31 and 92%, respectively. In contrast, La and Fe increased its accumulation at 37°C by 184 and 118%, respectively. All metal-induced changes in Cd accumulation were concentration-dependent with the exception of Ni. These results suggest that renal cells, like hepatocytes, take up Cd by passive as well as temperature-sensitive facilitated diffusion. Moreover, Ca, Zn and Cu lower Cd uptake while La and Fe enhance its uptake by yet unknown mechanisms. (Supported by PHS grant ES03187)
Potassium dichromate (K₂Cr₂O₇) and cadmium chloride (CdCl₂) are nephrotoxic metals which primarily affect renal proximal convoluted tubules (PCT) in vivo. Here we examine the dose response and differential targeting of these metals in purified PCT segments. PCT were isolated from rabbit renal cortical cortices by collagenase digestion followed by purification on a discontinuous Percoll gradient. Tubules were resuspended (1 mg/ml) and preincubated for 45 min at 37 °C in Krebs buffer prior to a 45 min metal exposure at various doses. Toxicity was assessed by measuring the cellular release of lactate dehydrogenase (LDH), which serves as a marker for plasma membrane integrity, control LDH release values averaged 14 ± 3%. Toxic responses to dichromate and cadmium were dose dependent with LDH release values of 17, 35 and 42% for 0.01, 0.1 and 1.0 mM doses of each metal, respectively. Since cadmium readily conjugates with cysteine (Cys) or metallothionein (MT), we investigated the differential effects of Cd-Cys or Cd-MT conjugates (0.1 mM) in PCT segments. Differential responses to these cadmium analogs were observed with a toxicity rank order of Cd-MT > CdCl₂ > Cd-Cys > Control, with LDH release values of 74, 41, 24 and 14%, respectively. These results suggest that targeting of cadmium, and possibly other, metals to PCT segments may be manipulated by conjugation with either Cys or MT. (Supported by PHS grants ES-05468 and DK-28616).

Hepatic glutathione (GSH) and renal γ-glutamyl-transpeptidase (γ-GTP) have recently been reported to play key roles in renal uptake of mercury compounds. In the present study, the mechanisms of renal transport of three of these heavy metals: Ag, Bi, and Cd, were investigated. Pretreatment with 1,2-dichloro-4-nitrobenzene (DCNB), a specific depletor of hepatic GSH, reduced the renal uptake of Ag and Bi which preferentially accumulate in the liver and kidney, respectively. Their renal accumulation were markedly increased by coadministration of GSH. Inhibition of renal γ-GTP by pretreating with acivicin reduced the renal uptake but enhanced urinary excretion of Ag and Bi. Renal uptake of Cd remained unchanged on pretreatment with either DCNB or acivicin. Preadministration of probenecid, an inhibitor of peritubular organic acid transport, did not affect the renal uptake of Ag, Bi, and Cd. These results suggest that both Ag and Bi are transported to the kidney as a complex with GSH released from the liver and incorporated into the kidney by a γ-GTP dependent system as in the case of Hg, while Cd appears to accumulate in the mouse kidney through an alternative mechanism.
MERCURY-ENHANCED PRODUCTION OF REACTIVE OXIDANTS (H$_2$O$_2$ AND O$_2^-$) BY KIDNEY MITOCHONDRIA. B O Lund, D M Miller and J S Woods. Dept. of Environmental Health, University of Washington, Seattle, WA.

Mercury causes oxidative tissue damage, possibly by promoting formation of reactive oxidizing species. We studied the effects of Hg(II) in vitro on the production of superoxide (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) in rat kidney mitochondria (mito). In mito supplemented with a respiratory chain substrate and electron transport inhibitor (succinate/antimycin A or malate-glutamate/rotenone), Hg(II) caused a linear increase in H$_2$O$_2$ production at conc. up to 10 uM. At 6 uM Hg(II), H$_2$O$_2$ production was increased to 500% and 300% of control rates at the ubiquinone-cyt. b and NADH-dehydrogenase regions, respectively. At low conc. of Hg(II) (<2 uM) an increased formation of O$_2^-$ was observed. However, at higher Hg(II) conc. a O$_2^-$-scavenging effect, probably leading to formation of H$_2$O$_2$, resulted in a decreased detection of O$_2^-$. In mito supplemented with substrate, antimycin A increased the rate of lipid peroxidation (LP) from 22 to 48 nmoles malondialdehyde (MDA)/min, whereas rotenone caused a 20% increase. Addition of 3-6 uM Hg(II) further increased the rate of LP to 55-65 and 80-90 nmoles MDA/min in antimycin A and rotenone-inhibited mito, respectively. These results demonstrate that Hg(II) enhances the production of reactive oxygen species by kidney mitochondria under conditions of impaired electron transport. The increased production of reactive oxidants may contribute to oxidative tissue damage caused by mercury compounds. Supported by ES03628 & ES04696. BL was partly supported by the Swedish Institute.

REDOX ACTIVITIES OF MERCURY-THIOL COMPLEXES. IMPLICATIONS FOR MERCURY POISONING AND TOXICITY. D M Miller and J S Woods. Dept. of Environ. Health, Univ. of Washington, Seattle, WA.

Mercury causes biochemical alterations to kidney proximal tubule cells, including increased porphyrin excretion (porphyria). This porphyric action is due to inhibition of renal porphyrin metabolism as well as to Hg-promoted oxidation of reduced porphyrins (porphyrinogens) in the presence of glutathione (GSH) and H$_2$O$_2$ (Woods et al. Mol Pharmacol 38:253,1990). To further elucidate the mechanism of porphyrinogen oxidation by mercury, we compared the ability of Hg(II) to bound GSH, or of Hg(II) by GSSG, to catalyze uroporphyrinogen (urogen) oxidation in vitro. Both systems oxidized urogen in the presence of H$_2$O$_2$; however, Hg(II)/GSSG was 40-fold more effective than Hg(II)/GSH on a molar basis. The Hg(II)/GSSG/H$_2$O$_2$ system was unaffected by the removal of oxygen, whereas the Hg(II)/GSH/H$_2$O$_2$ system was stimulated by about 40%. Urogen oxidation occurred at a similar rate if t-butyli or cumene hydroperoxide were substituted for H$_2$O$_2$ in either system. GSH could be replaced by several thiols, all of which catalyzed urogen oxidation in the presence of Hg(II) and H$_2$O$_2$ except 2,3-dimercapto-1-propanesulfonic acid. Finally, either Hg(II)/GSH or Hg(II)/GSSG catalyzed the decomposition of H$_2$O$_2$, albeit slowly. These findings suggest that mercury-thiol complexes possess oxidase activity in biological systems, which promotes oxidation of reduced porphyrins and possibly other biomolecules. At least 2 oxidizing species may be formed by Hg-thiol complexes in the presence of H$_2$O$_2$, possibly including a GS radical. Supported by ES03628 and ES04696.

NICKEL-INDUCED OXIDATION OF RENAL DNA GUANINE RESIDUES IN VIVO AND IN VITRO. K S Kasprzak, M Misra, R E Rodriguez, and S L North. Laboratory of Comparative Carcinogenesis, NCI-FORDC, Frederick, MD.

Effect of nickel on the contents of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in renal DNA was studied in male mice of four strains having different susceptibility to nickel-induced lipid peroxidation (Misra et al., Proc. Am. Assoc. Cancer Res. 31, 146, 1990) and in cultured NRK-52 and NIH 3T3 cells. 8-OHdG was determined by means of HPLC with electrochemical detector. During the 48-hr period following a single i.p. injection of 170 &mu;mol nickel(II)acetate (NiAcet)/kg B.W., the 8-OHdG contents increased by 150% over the control in BALB/c mice and tended to increase (by a maximum of 13%) in C57BL mice, but remained practically unchanged in C3H and B6C3F1 mice. In the cultured cells, the response by 8-OHdG to NiAcet included changes varying from 0.20 - 30%, vs. the control values, depending on NiAcet concentration (0.001 - 2.5 mM) and treatment time (up to 24 hr). Under the same conditions, lipid peroxidation in the cells was generally lower than in kidneys, NIH 3T3 cells being more responsive than NRK-52 cells. Thus, in mice the magnitude of 8-OHdG increase by NiAcet was greatest in kidneys of BALB/c mice, i.e., mice which were also most susceptible to NiAcet-induced lipid peroxidation. No such concurrence was observed in the cultured cells.

INSOLUBLE PARTICLE RETENTION IN THE CONDUCTING AIRWAYS OF DOGS. J W Spoo, M B Snipes, R A Guilmette, B A Muggenburg and M D Hoover. Inhalation Toxicology Research Institute, Albuquerque, NM. Sponsor: J M Benson

Current models for clearance of insoluble particles from the conducting airways assume that clearance is complete within 24 hours of deposition. However, recent studies indicated that the clearance of inhaled insoluble particles from conducting airways is biphasic, with a long-term component having a retention half-time of 10 to 20 days in humans. We studied the retention patterns of insoluble particles in conducting airways of 8 Beagle dogs. Monodisperse 3 &mu;m diameter polystyrene latex microspheres radiolabeled with either 59-Fe or 85-Sr in 20 &mu;L of saline were deposited through a 1-mm diameter microspray nozzle introduced through a flexible fiberoptic bronchoscope. Thoracic counts were made for 42 days post-exposure using a NaI radiation detection system. After 2 days, less than 1% of the particles originally deposited in the trachea was retained. Particles that remained after 5 days did not clear perceptibly during the 42-day study. Six weeks after particle deposition, 7 - 9% of the original amount deposited in both the 15- and 8-mm id airways was retained, and 20% retention occurred in the 4-mm airway. We conclude that there is biphasic clearance for particles that deposit in the conducting airways of dogs, which has important implications for respiratory tract dosimetry. (Research supported by U.S. DOE/OGER under Contract No. DE-AC04-76EV01013.)

We hypothesized that a high rate of delivery of particles to the lung would overwhelm the normal clearance mechanisms, resulting in excess accumulation of particles in the lung and an increase in the inflammatory response to the particles. We exposed F344/N rats over a 12-wk period to the same concentration times time product of carbon black particles (CB), but at three different exposure rates: 0.5 mg/m³, 16 hr/day, 7 days/wk; 13 mg/m³, 6 hr/day, 5 days/wk; or 98 mg/m³, 4 hr/day, 1 day/wk. Lung burdens of CB were measured after 3, 6, 9 and 12 weeks of exposure and at 1, 2, 3, 4, and 6 months after the exposure ended. The inflammatory response was quantitated by analysis of bronchoalveolar lavage fluid after 6 and 12 weeks of exposure and at 1, 3, and 6 months after exposure. The histopathology of the lung was evaluated at the end of the exposure and at 6 months after the exposure. On the basis of all the evaluations, there were no differences in the response of the lung to the delivery of the CB at the three rates. The acquired lung burden at the end of the exposure (all groups) was 4.8 ± 0.17 (x ± SE, n=18) mg/lung. There was a mild inflammatory response to the particles as indicated by an influx of neutrophils and a 2-3 fold increase in protein and in lactate dehydrogenase activity in the BALF. The histopathology was consistent with a mild focal alveolitis, localized primarily in the centriacinar region of the lung. We conclude that, under the conditions of the study, there was no apparent effect of the rate of delivery of the particles on the response of the lung. (Research sponsored by U.S. DOE/OHER under Contract No. DE-AC04-76EV01013.)


Male F344/N rats were exposed to 0.01, 0.0, 0.25, and 1.0 mg CdO/m³ for 6 hours/day, 5 days/week, for 13 weeks. The MMAD of the CdO aerosol ranged from 0.3 to 1.6 µm, with SDIs from 1.6 to 2.0. Lung burdens of CdO and Cd concentrations in blood and kidneys were determined on study days 3, 9, 30, and 93. Lung burdens increased with exposure concentration, but the lung burden accumulation rate decreased as exposure concentrations increased. This was attributed to differences in deposition as exposure concentrations increased, presumably resulting from respiratory changes in the exposed animals. Lung burdens were 29, 52, and 86 µg CdO/lung after 93 days of exposure to 0.1, 0.25, and 1.0 mg CdO/m³, respectively. Steady-state lung burdens were estimated from measured lung burdens and the clearance rate of CdO from the literature. Cadmium concentrations in blood were very low, which was most likely a result of rapid clearance from the blood to other tissues. Kidney concentrations were 3, 6, and 15 µg Cd/g after 93 days of exposure to 0.1, 0.25, and 1.0 mg CdO/m³, respectively. The total mass of Cd in the kidneys represented a significant fraction of the accumulated lung burden of CdO. Cadmium concentrations in the kidneys were linearly proportional to lung burden. Projected accumulation of Cd in the kidneys of rats exposed to a maximum concentration of 0.1 mg CdO/m³ indicates that kidney Cd concentrations should be well below the human toxic threshold limit of 180 µg Cd/g (literature value) at the end of a 2-year chronic study. Together with pathology findings, steady-state lung burdens suggest a maximum chronic exposure concentration of 0.1 mg CdO/m³ would reduce the probability of premature death of the study animals.

LUNG CLEARANCE OF INHALED GALLIUM ARSENIDE (GaAs). B J Greenspan, J A Dill, T J Mast, B J Chou, K H Stoney, R Morrissey*, and J Roycroft*. Battelle, Pacific Northwest Laboratories, Richland, WA and *NIEHS/NIEH Research Triangle Park, NC.

Ga and As were measured in the lungs, lymph nodes (LALN), and whole blood of rats exposed by inhalation to GaAs to determine the deposition and clearance kinetics of the material. Male F344/N rats were exposed to 0.1, 1, 10, 37, and 75 mg GaAs/m³ for 6 h/d, 5 d/wk for 13 wk. The aerosol MMAD was 1.2 µm with SD values between 1.5 and 2.4. Lung burdens measured on days 23, 45, and 93 showed Ga and As present in nearly equimolar amounts suggesting that the material was cleared as intact particles. The lung burden accumulation rate decreased as exposure concentration increased. A group of male Sprague-Dawley (SD) rats was exposed to 10, 37, and 75 mg GaAs/m³ for 6 h/d for 12 consecutive days. Analyses of Ga and As were performed on lungs, LALN, and whole blood samples from these animals up to 6 wk post-exposure. Ga concentrations in the blood were <1.0 µg/g, but As continued to accumulate, presumably bound to erythrocytes. Ga and As were found in nearly equimolar amounts in the LALN, further suggesting clearance from the lungs as intact particles. The clearance of both Ga and As from the lungs could be described by simple first-order kinetics with an average clearance half-time of 17 days. This is consistent with reported values for insoluble particles in rats. A third group of pregnant female SD rats were exposed concurrently with the male rats. Arsenic levels in blood samples from these animals were still increasing at 20 days of gestation, while Ga was consistently <1.0 µg/g. Our results differ from other published studies showing preferential clearance of As over Ga in animals exposed by intratracheal administration.

REDUCED LUNG CLEARANCE INDUCED BY LOW LUNG BURDENS OF BERYLLIUM METAL IN RATS. G L Finch, P J Haley, M D Hoover, A F Eidson and R G Cuddihy. Inhalation Toxicology Research Institute, Albuquerque, NM.

As people may accidentally be exposed by inhalation to beryllium metal, we investigated the acute effects resulting from the deposition of beryllium metal in the respiratory tract. Rats were exposed by inhalation to beryllium to result in lung burdens ranging from 1.5 to 50 µg. The rats were co-exposed to inert particles of 85Sr-labeled fused aluminosilicate particles (FAP) to permit quantitation of clearance. After exposure, the rats were periodically counted for whole-body 85Sr radioactivity. Selected animals were sacrificed at 6, 16, 40, and 90 days after exposure for quantitation of beryllium content in the lungs. As determined by whole-body radioactivity counting up to 60 days after exposure, rats not exposed to beryllium cleared the 85Sr-FAP tracer with a clearance halftime of 38 days. In contrast, clearance half-lives of 64, 112, 145, and 232 days were observed for rats that received beryllium lung burdens of 1.5, 2, 10, and 50 µg, respectively. The difference in clearance compared to controls was not significantly different at the lowest level of beryllium. We concluded that beryllium metal can induce lung clearance reductions in rats at low levels of beryllium deposited in lung. (Research supported by the U.S. Department of Energy under Contract No. DE-AC04-76EV01013.)
886 UTILIZATION OF INTERSPECIES SCALING TECHNIQUES TO SUPPORT DOSE SELECTION IN THE DEVELOPMENT OF A MONOCLONAL ANTIBODY GN 1445. D. Maneval, D. Thomas, J. Mordenti, and J. Green, Dept. of Safety Evaluation, Genentech, Inc., South San Francisco, CA.

GN 1445 is a murine monoclonal antibody that is being developed as a potential therapeutic agent in breast and ovarian cancer in which the HER-2/neu gene is amplified. Patients in which this gene is overexpressed have been associated with extremely poor prognosis for survival. The preclinical safety evaluation profile for GN 1445 was determined by performing a series of single dose pharmacokinetic, tissue distribution and acute toxicity studies in which the doses employed were established by employing interspecies scaling techniques and selecting dosing multiples based on the concept of pharmacokinetic equivalence. This information was then utilized to support the clinical dosing regimen and subsequent single dose escalations. Multiple dose clinical protocols were supported by the concurrent initiation of limited repeat-dose toxicity studies. In selected cases, this approach can lead to decreased development time and more rapid initiation of Phase I studies. This poster will describe the pharmacokinetic data obtained from this integrated development approach, and advantages and disadvantages will be discussed.

887 TOXICOKINETIC ANALYSIS OF CADMIUM IN EARTHWORMS UTILIZING ARTIFICIAL SOIL AND FILTER PAPER AS EXPOSURE MEDIA. M E Honeycutt, A Dalu, L N Ace, and B L Roberts, School of Pharmacy, Northeast Louisiana University, Monroe, LA.

The toxicokinetics of cadmium in the earthworm Eisenia fetida were investigated utilizing two exposure media commonly used in earthworm research, artificial soil and filter paper. Exposed earthworms were sampled at various time intervals to determine the uptake and depuration of Cd via the two exposure media. Kinetic constants were estimated and used along with the shapes of the curves obtained to compare exposure media. Cadmium appears to follow two compartment uptake and elimination with both exposure media. The shapes of the curves obtained are almost superimposable, the only difference being the time axes, which are measured in hours for filter paper, and in days for artificial soil. Body burdens obtained in 48 hours with filter paper are similar to those obtained in 14 days with artificial soil. These data lend support to the use of filter paper as an exposure medium comparable to artificial soil which can be used to facilitate exposure for a more rapid assessment of biological effects.

888 DOSIMETRY-TOXICITY RELATIONSHIPS IN 2-METHOXYETHANOL TERAUTOGENESIS. D O Clarke, E Maudlin and F Welsch, CIT, Research Triangle Park, NC.

The pharmacokinetics and teratogenic outcome of bolus sc injection versus continuous sc infusion of 2-methoxyethanol (2-ME) were compared in CD-1 mice on gestation day 11. Our cumulative data indicate that it is the total embryonic exposure (area under the concentration-time curve; AUC, mmol/h/l) to the primary metabolite 2-methoxyacetic acid (2-MAA), rather than the peak 2-MAA concentration (PC, mmol/l), that best correlates with embryotoxicity (% fetuses with digit malformations). In addition, a good correlation exists between the 2-MAA AUC in maternal plasma (MP), embryo (E) and extra-embryonic fluid (EF), which demonstrates that the AUC in MP is a reasonable estimator of toxicity. We have further investigated the importance of AUC versus PC in 2-ME teratogenesis by using bolus and infusion dosing regimens in combination to effect an increase in the 2-MAA AUC without an increase in PC. Mice received either (a) 175 mg 2-ME/kg sc alone or (b) 175 mg 2-ME/kg sc at 0 h and 27 mg 2-ME/kg/h during 2.5 to 10.5 h from a sc implanted osmotic pump. The 2-ME PC was the same (2.7 MP, 4.0 E, 5.4 EF at 2 h) but the AUC increased from (a) 28 MP, 42 E, 56 EF to (b) 41 MP, 54 E, 78 EF. This resulted in an increase in abnormal fetuses from 29% to 74%, supporting our hypothesis that total exposure to 2-MAA is the important determinant of 2-ME teratogenesis. These data, integrated with a physiologically-based mathematical description of 2-ME disposition, which we are developing, will provide a more realistic means of estimating potential developmental toxicity in other species, including humans.

889 INHIBITION AND INDUCTION OF METABOLISM OF ETHYL CARBAMATE (EC) IN MICE; PHENOBARBITAL (PB) TREATMENT EFFECTS. R A Kemper, E Kuratsuki, H H Hurst and V J Waddell, Dept. of Pharmacology and Toxicology, University of Louisville, KY.

Ethanol (EtOH) and acetone (Ace) previously have been shown to act as acute inhibitors, and with pretreatment as inducers, of metabolic clearance (Cl) of EC from mice. Acute and pretreatment effects of PB on Cl of a standard EC dose (11.1 mg/kg) were determined from areas under the curve (AUC) of EC blood levels after EC/NS assay. Treatment mean Cl values (= dose/AUC ± SE ml/hr/kg) of EC were: pooled controls, 865 ± 26; PB (80 mg/kg ip) given 1 hr before EC, Cl = 1369 ± 107; PB (80 mg/kg/day x 4 days) 24 hr prior to EC, Cl = 1410 ± 163; PB (80 mg/kg/day x 4 days) 48 hr prior to EC, Cl = 1989 ± 230. Comparisons (p<0.01) indicate that PB, like EtOH and Ace, effectively induces Cl of EC. [EtOH (10% in drinking water 48-12 hr prior to EC), Cl = 1225 ± 23; with Ace (2 g/kg ip, 48 & 24 hr prior to EC), Cl = 2623 ± 123.] Maximal PB induction was observed 48 hr after treatment, consistent with the known induction interval for P450IIB isozymes. Unlike EtOH and Ace, concurrent PB treatment does not inhibit Cl of EC. [Concurrent EtOH (2 g/kg po), Cl = 195 ± 13; concurrent Ace (50 mg/kg ip), Cl = 185 ± 3.] The enhancement of EC Cl following acute PB requires further explanation. These findings fit an hypothesis that microsomal EC metabolism is mediated by P450IIB and P450IIE1 subfamilies.

Toxicokinetics of p-chloro-α,α,α-trifluorotoluene (CTFT) after administration as an aqueous α-cyclodextrin molecular encapsulated suspension (α-CD vehicle) or in corn oil solution (corn oil vehicle) were compared. Groups of male F344 rats were administered CTFT intragastrically with α-CD vehicle or corn oil vehicle at 10, 50 and 400 mg/kg. Another group of male F344 rats were administered CTFT intravenously using a 10% Tween 80 aqueous solution. Blood samples were taken from a jugular cannula up to 50 hrs after dosing and the CTFT concentrations in whole blood were determined by gas chromatography. Evaluation of the data revealed that the elimination of CTFT from the systemic circulation was not affected by the vehicle used. However absorption of CTFT from the α-CD vehicle was found to be much faster than from the corn oil vehicle. The average t1/2 of the absorption phase from the α-CD vehicle and the corn oil vehicle were 7 and 150 min, respectively. Despite the differences in the rate of absorption, no statistical difference was observed in the calculated area under blood concentration versus time curves (AUC) obtained from rats dosed with either vehicle. Dose proportionality for CTFT was estimated up to 400 mg/kg for both vehicles. Bioavailability of CTFT was shown to be complete for both α-CD and corn oil vehicles.

DOSE-RENAL EFFECT RELATIONSHIP DURING SHORT-TERM EXPOSURE OF RATS TO URANIUM (U). M W Hammelstein and E J O'Flaherty, Dept. Environ. Health, Univ. of Cincinnati, OH.

Prolonged retention of bone-seeking elements leads to concerns over potential health effects of chronic exposure. As part of a project to develop an anatomically and physiologically based model for the kinetics and dynamics of U in the rat, a study was undertaken to establish dose-effect and tissue-to-blood concentration relationships. Twenty-four male Long-Evans rats were divided equally between two dose rate groups. Four rats served as controls. Uranyl nitrate was administered intraperitoneally by osmotic pump. Treated rats were killed after 5, 7.9, 11.8, or 14.8 days of continuous exposure, immediately following overnight timed urine collection. Control rats were killed after 14.8 days. Concentrations of U in tissues were measured by alpha pulse height analysis. Concentrations in the kidney, tibia, and urine increased with dose rate and duration of exposure. Urinary excretion of U was proportional to the concentration in the kidney. The concentration of U in blood approached the detection limit of the analytical method, but a kidney-to-blood partition coefficient of 480 was estimated, which compares reasonably well with published values. Renal toxicity, measured as protein-to-creatinine ratio in the urine, was directly dependent on the concentration of U in the kidney, irrespective of dose rate or duration of exposure. (Supported by DOE EG&G Mound Applied Technologies, Miamisburg, OH).

LACTATIONAL TRANSFER OF HEXACHLOROBENZENE D H Taylor, E S Goldey and J W Fiske, Department of Zoology, Miami University, Oxford, OH. and Toxic Hazards Division, AAMRL, WPAFB, OH.

The maternal transfer of hexachlorobenzene (HCB) to fetuses and suckling pups was assessed using a prebreeding dosing design. Two weeks prior to breeding, virgin Sprague-Dawley rats were given a total oral dose of HCB in corn oil of 10 or 100 mg/kg body weight over four days. Concentrations of HCB were determined in the tissues of dams and their offspring for each of days 9, 15 and 20 of gestation, and on postnatal days 4, 7, 10, and 14. Throughout gestation, the HCB tissue concentrations for the 10 and 100 mg HCB/kg body weight groups differed by 10 fold. The maternal body burden of HCB was quickly depleted by lactational transfer of the HCB to the suckling pups as reflected by HCB concentrations in the milk and pups. However, across treatment groups, only a 2-3 fold difference existed between tissue concentrations of HCB in both dams and pups during lactation. The HCB concentrations in the blood from rats in the 10 and 100 mg HCB/kg body weight groups were similar during lactation as were the milk concentrations. Our data suggest that HCB stored in tissues equilibrates with the lipoprotein rich blood during lactation, resulting in the pups receiving similar doses of HCB for the maternal 10 and 100 mg HCB/kg body weight dose groups.

COMPARISON OF SINGLE AND MULTIPLE ORAL BOLUS DOSAGE REGIMENS ON PHARMACOKINETICS OF TRICHLOROETHYLENE IN THE RAT. S Muralidhara, RO Manning, K Lee, V Srivastana, IV Bruecker and JM Gallo, Dept. Pharmacol. & Toxicol. and Dept. Pharmaceutics, College of Pharmacy, University of Georgia, Athens, GA.

A variety of volatile organic compounds (VOCs) have been identified as contaminants of drinking water. Exposure to a chemical via drinking water during the course of a day may result in a very different kinetic profile (and toxic response) than that following a single bolus exposure. In an effort to better understand the kinetics of VOCs administered in very small divided doses, the following studies were conducted. Unanesthetized male Sprague-Dawley rats (N=6-8) were administered 0.8 or 75 mg trichloroethylene (TCE)/kg bw in either a single oral bolus or in a multiple dose regimen. The multiple dose regimen consisted of 5 separate doses of 20% of the total dose administered at 90-min intervals. Blood samples were collected from an indwelling carotid artery cannula for several hours during and after the exposures to delineate the kinetic profile of TCE in the blood. When TCE was administered in divided doses, the area under the blood concentration-time curve and maximum concentration in the blood were markedly lower than after a single oral bolus. This relationship held true for both the low dose of 8 mg/kg and the high dose of 75 mg/kg. Terminal elimination half-life was not altered dramatically by either dosage regimen or dose level. The kinetic profiles indicated that TCE reaches and remains at steady-state during the repetitive oral exposures, and that metabolic saturation does not occur, even after administration of the 75 mg/kg in 5 divided doses. These findings support the hypothesis that oral dosage regimen can substantially affect the pharmacokinetics of VOCs. (Supported by U.S. EPA CR-816283 and U.S. Air Force AFOSR 88-0277)
ALERTED HEPATOBILIARY (HB) DISPOSITION OF ACETAMINOPHEN GLUCURONIDE (AG) IN THE ISOLATED PERFUSED RAT LIVER (IPL) AFTER ACUTE AND IN VIVO PHENOBARBITAL (PB) ADMINISTRATION. S D Studenberg and K L R Brouwer, Curr. in Tox. & Sch. Pharm., UNC, Chapel Hill, NC. Sponsor: D Holbrook.

The influence of PB on the HB disposition of acetaminophen (APAP), AG, and APAP sulfate (AS) was examined in the recirculating IPL. PB was administered as a 5 μmol bolus to the IPL (acute) or in vivo (75 mg/kg/day i.p. x 5 d), followed by a 48 hr washout, prior to addition of an APAP (66 μmol) bolus to the IAC. Acute PB decreased significantly the rate constant for bile excretion of AG (Kb-A) (0.002 ± 0.001 vs. 0.014 ± 0.006 min⁻¹). PB in vivo PB induced significantly the AG formation rate constant (0.016 ± 0.003 vs. 0.005 ± 0.001 min⁻¹). The sinusoidal egress rate constant for AG after in vivo PB was increased significantly (0.074 ± 0.006 vs. 0.013 ± 0.001 min⁻¹), while Kb-AG was decreased significantly (0.002 ± 0.001 vs. 0.013 ± 0.001 min⁻¹). Kp-AG and biliary recovery of AG were decreased by the same extent by PB. Hydroxyphenobarbital (PBH) was not detectable after either treatment; PB was detectable only after acute administration. PBH glucuronide (PBH-G) was present in perfusate and bile after both acute and in vivo PB. These data support the hypothesis that PBH-G inhibits AG excretion into bile. Supported by Training Grant 5-T32-ES07126 and a PMAF Research Starter Grant.

NON-LINEAR TOXICOKINETIC BEHAVIOR OF TCDD-LIKE HALOGENATED POLYCYCLIC AROMATIC HYDROCARBONS (HPAH) IN VARIOUS SPECIES. G Carrier and J Brodeur. Département de Médecine du Travail et Hygiène du Milieu, Université de Montréal, Montréal, Québec, Canada.

Meta-analysis of literature on TCDD-like HPAH in rats, monkeys and humans shows differences in toxicokinetics between substances and between species. Differences are related to the dose and the affinity of HPAH for liver binding sites. The hepatic concentration appears to be the best indicator of toxic effects for all 3 species, similar concentrations in the liver producing virtually similar degrees of response. Relationship between absorbed dose and concentrations in various organs is non-linear. This can be explained as follows: 1) below hepatic saturation, contribution of liver load to total body burden increases as body burden increases, tending toward second order kinetics; 2) at saturation, body burden elimination follows zero order kinetics; 3) as dose increases, the intestinally absorbed fraction decreases. A toxicokinetic model was developed which allows a better understanding of the dose-response relationships in a given species and facilitates interspecies comparison as it pertains to risk assessment of health effects in humans.


The disposition of 14C-labeled MDEA was evaluated in male and female Fischer 344 rats. Initially, 50 and 500 mg/kg IV doses were given only to male rats to characterize systemic pharmacokinetics over 0-48 hr. After IV doses, plasma radioactivity disappeared bieplexonally and was excreted almost exclusively in urine (50-68% in 48 hr) while retention of 16-24% of the 14C dose in the carcass was also noted. Large terminal t½ values of 38 and 70 hr resulted for the 500 and 50 mg/kg doses, respectively, which did not indicate linear PK after IV dosing. An elimination t½ value of 27-39 hr was estimated from the PK analysis of urine data after these IV doses. For cutaneous dosing, only the 500 mg/kg dose was applied (both sexes) and the PK (evaluated over 0-72 hr) showed similar disposition in males and females. MDEA in continuous 72 hr contact readily penetrated skin, with 41-49% of the dose absorbed. In separate experiments with both sexes, a water wash-off step at 5 hr after dose application reduced the penetration to 17-21% over 72 hr. Cutaneous penetration appeared to be zero-order, since plasma 14C increased slowly to apparent steady-state levels, even after the 5 hr wash step, and the penetration process was not complete up to 72 hr after MDEA application. After cutaneous dosing, most of the 14C recovered in urine was unmetabolized MDEA and carcasses retained at least half of the absorbed dose. Thus, MDEA given by either route results in long plasma elimination half-lives accompanied by retention of 14C in the carcass and urinary excretion of mostly unmetabolized MDEA.

PHARMACOKINETICS OF COCAINE AND LIDOCAINE IN COMBINATION IN RATS. A M Kadry, X Ming, and M S Abdel-Rahman, Pharmacology/Toxicology Dept., New Jersey Medical School, Newark, NJ.

Cocaine is often adulterated with lidocaine as a way of reducing the purity of street cocaine while retaining the drug's anticipated effects. (H) cocaine and 14C-lidocaine were administered separately or in combination to male rats. Blood samples were collected as a function of time and assayed for total cocaine or lidocaine activity. The presence of lidocaine increased cocaine X and β phases of elimination half lives 68 and 7-fold, respectively. The tissue distribution study revealed that lidocaine increased the concentration of cocaine 38, 14, 18 and 17-fold in liver, heart, spleen and kidney, respectively, 72 hr after the administration. Lidocaine decreased cocaine excretion in the same time period and significantly increased fecal excretion of cocaine 3-fold compared to cocaine alone. The data suggest that the combination of cocaine and lidocaine may increase the toxicological effect of cocaine.
Both diltiazem and hydrochlorothiazide are effective hypotensive agents when administered as sole therapy. Due to different antihypertensive mechanisms, an additive effect was observed when administered in combination. The purpose of this study was to examine the effect of administered dose on the pharmacokinetics of a series of combination doses of DTZ/HCT in beagle dogs. Two male and two female dogs received 10/2, 20/4, 40/8, 50/10, 60/12 or 70/14 mg/kg/day of DTZ/HCT for 14 days. Plasma levels of HCT, DTZ & DTZ active metabolites desacetyl (DAD) and N-demethyl (DMDTZ) were analyzed by an HPLC method. No apparent sex difference in pharmacokinetic parameters between the male and female was found. The formation of DAD/DMDTZ metabolites were not affected by the combination doses. Mean plasma levels of metabolites relative to DTZ, were 136% for DMDTZ and 13% for DAD. The elimination half-lives of DTZ (1.8 hr), DMDTZ (2.1 hr), DAD (2.3 hr) and HCT (2.9 hr) were also constant throughout the dose range. The peak concentration and extent of absorption of DTZ and HCT were reasonably linear up to the 50/10 mg/kg/day dose, but declined at the 60/12 and 70/14 mg/kg/day dose. Incomplete absorption and accelerated renal clearance of DTZ and/or HCT at higher doses may cause the non-linearity.

The kinetics of absorption, distribution and elimination of the carbonic anhydrase inhibitor, acetazolamide(AZ) have been studied in the Rhesus Monkey. Animals were given an i.v. dose of 2 or 10mg/kg of AZ and blood and a 24-hr urine collected for assay of AZ by HPLC. About one month later the same animals were administered a single oral dose of 10 or 50mg/kg of AZ in 0.5% methyl cellulose. AZ was measured in the plasma up to 8 hours after oral and i.v. dosing. The plasma half-life of AZ ranged from 0.6 to 1.3 hours. In contrast, AZ was detected in the erythrocytes for 7 days post-administration with a half-life that ranged from 22-40 hours. Approximately 78% and 67% of the dose was excreted in the 24-hr urine after i.v. and oral dosing, respectively. Bioavailability after oral dosing was over 95%.

This study was conducted to determine whether the rate of infusion would effect the toxicity. One group received the dose (16 mg/kg) over a 5 minute period and the second group over a 90 minute period. Toxicity in this study was determined by the mortality, clinical signs of toxicity, body weight, food consumption, hematology and serum chemistry changes over a 21 day period. Gross and microscopic examinations of the kidney and bone marrow were performed. The results showed that a short infusion rate (5 minute) and a long infusion rate (90 minute) produced a similar toxicity profile.

Microscopic findings included moderate to severe hypocellularity of the bone marrow in all animals which died or were sacrificed moribund in both treated groups. No microscopic evidence of renal damage was seen in any of the early death animals in either group. Very slight tubular regenerative changes were seen in scheduled sacrifice animals from both treated groups. In conclusion, there was no evidence that the toxicity was altered by changing the infusion rate. This suggests that the AUC rather than the C_max is the critical toxicokinetic parameter in determining the toxicity of Carboplatin.

Previous work has associated brain lesions with convulsions in non-human primates intoxicated with phosphonoformic acid methyl methylphosphonofluoridate (soman) and given a standard therapeutic regimen of atropine and pralidoxime chloride. Administration of the sedative diazepam has been shown to be variably effective in ameliorating convulsions. In the present work, nine male rhesus monkeys were administered diazepam IM at three dose levels (70, 110, and 220 mg/kg) in a triple-crossover study design. Blood samples were collected for 24 hr after each dose, and total (free plus bound) plasma diazepam concentrations were fitted to a single-compartment, open pharmacokinetic model. A wash-out and recovery period of approximately one month was used between doses. This work demonstrates a high degree of inter-animal variability in peak plasma diazepam concentration and other pharmacokinetic variables at each dose level and may explain why diazepam varies in efficacy as an anticonvulsant following soman intoxication. [Supported by USAMRIC DAMD17-89-C-9050.]
Inhalation vs. Systemic or Topical Administration of Some Natural Toxins: Poor Route-to-Route Extrapolations. D.A. Creasia, R. W. Wannemacher, Jr., M. K. Rippy and J. D. Thurman, U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD.

We compared the acute toxicity of some natural toxins after either inhalation exposure or intravenous, dermal, or ocular administration and found little route-to-route similarity. T-2 toxin, a non-protein mycotoxin, induces dermal necrosis when applied to the skin, but not pulmonary necrosis when inhaled, and is 30 times more toxic by inhalation than by intravenous administration. Saxitoxin, a marine neurotoxin, is 10 times more toxic when inhaled than by intravenous administration; but there is no histological evidence of damage to pulmonary tissue and is non-toxic dermally. Ricin, a lectin derived from the castor bean, is not a dermal or ocular irritant, but produces severe, diffuse necrosis to respiratory tract tissue after a single inhalation exposure, but not when intravenously administered. Ricin is also more toxic by inhalation than by intravenous administration. Microcystin, a hepatotoxop cyclic polypeptide derived from aquatic cyanobacteria, induces no dermal toxicity and produces similar toxicity when either inhaled or intravenously administered. Thus, except for microcystin, each toxin causes a toxic effect unpredicable by extrapolation of data from other routes of administration.


Several chemicals proposed for regulation under the new Clean Air Act are carcinogenic following oral exposure, but inhalation data are lacking. The feasibility of route-to-route extrapolation (RRE) of oral-based potency estimates must be determined to develop inhalation risk assessments for these chemicals. RRE was evaluated based on key pharmacokinetic parameters, target organ and first pass effects, carcinogenic mechanism, and completeness of the data base. This indicated that carcinogenic potencies by the oral and inhalation routes are expected to be similar for both aniline and 1,4-dioxane. RRE for isophorone and benzyl chloride is more difficult because the oral studies were by gavage, which involves much faster compound delivery than inhalation dosing. For benzyl chloride, inhalation may be more potent due to hepatic first pass metabolism; this can be confirmed by pharmacokinetic modeling with existing data. For isophorone, elimination data needs to be developed to allow RRE based upon the gavage study. We conclude that the uncertainty surrounding RRE for aniline, 1,4-dioxane and benzyl chloride is within reason, but that RRE for isophorone involves considerably greater uncertainty.

Risk of Cancer Due to Airborne Vinyl Chloride (VC). S.V. Dawson and G.V. Alexeoff. California Department of Health Services, Berkeley, CA.

Three sets of laboratory rodent bioassays and one human occupational study provide cancer incidence data appropriate for a quantitative risk assessment of VC. Based on the saturation kinetics that produce the active metabolite of VC, as characterized by the Michaelis-Menten equation, the analysis used a metabolic exposure reflecting the tissue concentrations. The saturation constant (Km) was 336 ppm for rodents and 150 ppm for humans. A two-stage model obtained good maximum-likelihood fits of the rat and mouse incidence data on liver angiosarcoma and lung and breast cancer. Extrapolating to humans by scaling for body surface area yielded 95% upper confidence limits (UCLs) for lifetime unit risk from $3.7 \times 10^{-5}$ to $20 \times 10^{-5}$ ppb$^{-1}$. Using a single-stage model in which historical exposure data were converted to metabolic exposures, a most useful occupational study (Mawellener 1976) yielded UCLs on lifetime unit risk of $2.5 \times 10^{-5}$ ppb$^{-1}$ for liver cancer and $4.5 \times 10^{-5}$ ppb$^{-1}$ for all tumors. When adjusted for female-to-male differences by using the ratio of the UCLs on unit risk obtained for the rat, the UCL on lifetime unit risk for all tumors in the occupational study was $1.4 \times 10^{-5}$ ppb$^{-1}$, which is near the top of the range extrapolated from rodents.
COMMUNITY EXPOSURE GUIDELINE (CEG) FOR VINYL CHLORIDE MONOMER. D.A. Edwards, A.E. Chin and B.L. Lockhart. Exxon Biomedical Sciences, E. Millstone, NJ and Esso Chemical Canada, Toronto, Canada. Sponsor: R.A. Scala

Vinyl chloride (VC) is a colorless gas used primarily for the manufacture of polyvinyl chloride. Exposure to VC is known to result in both acute (hepatotoxicity, CNS effects [high doses]), and chronic (liver angiosarcomas) toxicities. A Community Exposure Guideline (CEG) of 80 μg/m³ was derived for VC using the method of Lewis, Lynch, and Nikiforov. The CEG represents an estimate of a safe lifetime community exposure level which would protect nearly all exposed individuals including children, the aged, pregnant women and hypersensitive individuals. The derivation method is predicated on adjustments of experimentally determined "no-effect" (or minimum effect*) levels from laboratory animal studies, taking into account scientific considerations such as species differences, sensitivity of the exposed population, the potency of the toxic agent and the quality of the database. A comparison of the CEG with other ambient air standards for VC was also performed.


Frequently there are two or more data sets available on which quantitative risk estimates for carcinogenicity could be based. Combining these data may in some cases result in improved estimates. A statistical method for evaluating the hypothesis that two or more sets of data are compatible with a common dose-response model would aid the biologist in making the determination whether to combine or not combine data sets. A statistical method based on the likelihood ratio principle is presented for comparing two or more data sets to a common linearized multistage model. This test with several examples and the results of a Monte Carlo study of the power of the test are presented. The theoretical relationship between the proposed test and the methods commonly used to estimate parameters and confidence limits for the linearized multistage model are discussed.


Propylene oxide (PO) is an important industrial chemical used in the synthesis of glycols, glycol ethers, resins, and surfactants. It has been identified as both genotoxic and carcinogenic in animals. Based on a review of available data, it was concluded that data from garage studies in rodents is inappropriate for quantitative cancer risk assessment and that data from long term inhalation studies should be used.

PO is also a direct acting DNA alkylating agent which yields a uniform alkylation pattern in various tissues. The rate of detoxification of PO does not vary widely in various mammalian species, including man. For these reasons, the surface area extrapolation model for estimation of human equivalent dose may not be appropriate and previously derived cancer potency factors should be revised downwards. Based on the incidence of hemangiomas and hemangiosarcomas in male mice, a 95% upper limit of 4.6E-04 (mg/kg/day)-1 is obtained.

AN ANALYSIS OF DOSE-RESPONSE INDUCTION OF DNA ADDUCTS BY AFLATOXIN B1 IN LIVER: IMPLICATIONS TO CANCER RISK ASSESSMENT. W.N. Choy Reproductive and Cancer Hazard Assessment Section, California Department of Health Services, Berkeley, CA.

A cancer risk assessment of aflatoxin B1 (AFB1) has been conducted for the California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65). Thirty-seven animal bioassays were evaluated. The rat is the most sensitive species and liver is the most sensitive site. The Wogan et al. (1976) low dose rat bioassay (life-time equivalent dose: 0 to 5.5 μg/kg-day) was selected for the estimation of animal cancer potency. To examine if pharmacokinetic dose corrections are required, published dose-response studies on AFB1-DNA adduct induction in rat liver were reviewed. An analysis of three low dose ingestion studies (Lutz, 1986; Wild et al. 1986 and Garner et al. 1988) revealed that the dose-response curve of DNA adduct formation is linear over a wide dose range (1 ng/kg to 0.1 mg/kg). A similar linear dose-response was also observed in an injection study (10 ng/kg to 1 ug/kg) (Appleton et al. 1982). This finding indicates that at the doses used in the Wogan (1974) study, the applied dose is linearly proportional to the target tissue dose and thus pharmacokinetic dose adjustments are not necessary. Also, the lack of threshold in both dose-response curves supports the use of the linearized "multistage" polynomial model for AFB1 risk assessment.
The regulation of neurotoxicants has usually been based upon setting reference doses by dividing a no observed adverse effect level by uncertainty factors that theoretically account for interspecies and intraspecies extrapolation of experimental results in animals to humans. Recently, we have proposed a four-step alternative procedure which provides quantitative estimates of risk as a function of dose. The first step is to establish a mathematical relationship between a biological effect or biomarker and the dose of chemical administered. To enhance the certainty of this procedure we considered the pharmacokinetics, the uptake kinetics into the target cell(s) and/or membrane interactions, and the presumed receptor site(s) interaction of the chemical or metabolite. Because these theoretical factors each contain a saturable step due to definitive amounts of required enzyme, reuptake or receptor site(s), a nonlinear, saturable dose-response curve would be predicted. When data generated from rats administered methylendioxymethamphetamine (MDMA) were plotted as biological effect (decreases in hippocampal serotonin concentrations) versus dose, a saturation curve best described the observed relationship. The use of dose-response data may enhance the certainty of quantitative risk assessment.

Dioctane (DK) was selected as an impurity warranting a health-based residue limit because it is considered a probable carcinogen. The FDA procedure for setting health-based residue limits for organic volatile impurities in chronically administered pharmaceuticals was applied to DK. Data pertaining to chemical and physical properties, use and occurrence, bio-disposition, toxicology and effects in people were collected and evaluated. A weight-of-evidence test suggested that both a safety factor approach and use of statistical models were appropriate for DK. Acceptable daily intake (ADI) values calculated for oral, parenteral and inhalation routes and encompassing different toxicological endpoints ranged from 13 to 67 mg/day. Based upon a consideration of all of the data for DK and the ADI values derived from the data, a health-based residue limit of 10 mg/day is considered to be appropriate as a lifetime average for chronically administered pharmaceuticals, i.e., pharmaceuticals intended for continuous use for a year or more in a lifetime.
NON-TRANSPANTABLE HUMAN TISSUES IN RESEARCH. *D C Cook, J Paparo and B Bardsley. International Institute for the Advancement of Medicine (IIAM), Phoenix, AZ. and Exton, PA. Sponsor: K Bredel.

Thousands of chemicals will be examined this year for their potential toxicities. Because of species variation, data extrapolation from experimental animals to humans is often problematic. Human in vitro biotransformation technology is useful in determining the metabolism and cellular effects of potential toxic environmental/industrial chemicals or other drugs in man without exposing humans or laboratory animals to the actual compound. However, the logistics of human tissue acquisition and distribution are complex; few investigators are able to gain access to human tissues without considerable effort. IIAM has developed a unique approach in the acquisition of non-transplantable organs and tissues from the United Network for Organ Sharing (UNOS) and numerous organ & tissue banks and subsequent distribution to biomedical research centers nationwide. From our experience, current preservation solutions allow most tissues and organs to remain viable up to 56 hrs. This allows for adequate time for the transportation of tissue to research laboratories. Efforts are underway to evaluate new solutions which will augment tissue viability beyond 2 days.


To assist in the preliminary evaluation of compounds of toxicological and environmental interest to the U.S. Environmental Protection Agency (USEPA), a scoring system was devised as a collaborative effort between the USEPA and the Oak Ridge National Laboratory. The scoring system combines objective guidelines with professional judgement to evaluate chemicals and consists of 11 separate scoring parameters, 6 of which pertain directly to toxicity, e.g. developmental toxicity and oncogenicity. The remaining parameters are related to environmental fate and occupational, consumer, and environmental exposure. The scoring system was designed to rapidly score chemicals in a minimal amount of time with readily available information. It is used by the Office of Toxic Substances of the USEPA as a tool to help set priorities in conjunction with other criteria. It is particularly useful in performing preliminary evaluations involving large chemical classes, such as petroleum distillates.


Evaluation of toxicological data is an important task for toxicologists. There are only few rules and guidelines for how it should be done. Knowledge and acquired experience are the main implicit means of support. There is no description of how the toxicologist has performed the evaluation. Which data has he considered, in which way and what is the bases for his conclusions? It has been shown that there is a great variation among toxicologists with regard to how they evaluate basic concepts in toxicology (T Malmfors, N Kautz and P Slovic, Intuitive toxicology - how toxicologists judge toxicological data, SOT 1990). In order to explore the feasibility to use knowledge-based techniques, the carcinogenicity evaluation in the NTP reports was used as a model. 208 studies were used to build an inductive expert system and 20 studies were then evaluated with the system in order to test its performance. The outcome shows a good correspondance between the computer expert system and the expert panel. Furthermore, the analysis gives further insight into the evaluation process of carcinogenicity studies.


Regulatory oversight of biotechnology products represents a crucial hurdle for the commercialization of many new crop varieties possessing an impressive array of beneficial traits. Such oversight will require innovative and flexible approaches. The standard methods of chemical risk assessment are in many cases insufficient for the evaluation of the potential risks posed by genetically engineered food products. The informational nature of newly introduced DNA, as well as the catalytic properties of enzymes that might be encoded by such DNA, make considerations of their concentrations and/or doses simplistic approximations of their potential for biological effects. A novel approach has been developed that incorporates aspects of traditional risk assessment, including hazard identification, exposure assessment and risk characterization, while also addressing specific concerns unique to genetically engineered products. Risk assessments are conducted for both the introduced genes and the corresponding gene products, in addition to an environmental assessment. A cascaded probability analysis is also included for estimating the risk of interspecies transfer of the engineered genes.
AN OVERVIEW OF THE TOXICOLGY OF METHYL METHACRYLATE. R A Young, M L Daugherty, S S Talmage. Biomedical and Environmental Information Analysis, Health and Safety Research Division, Oak Ridge National Laboratory, Oak Ridge, TN; D McKean, U.S. Environmental Protection Agency, Cincinnati, OH.

Methyl methacrylate (MMA) is used for the production of acrylic polymers that have industrial and medical applications. The primary route of exposure is by inhalation, although oral, dermal and iatrogenic exposures are also of concern. MMA is readily absorbed following inhalation or oral exposure and has a large volume of distribution. Toxic effects in humans exposed to 100 mg MMA/m^3 include nasal passage irritation and neuropsychiatric effects (fatigue, memory loss, irritability). Allergic dermatitis is well documented for dental and medical personnel. Acute toxicity studies in laboratory species indicate very high oral LD_{50} and LC_{50} values. Inflammation and necrosis of the nasal cavity have been demonstrated in laboratory species following chronic and subchronic inhalation exposure, although subchronic exposure to very high concentrations (>2800 mg/m^3) increased mortality in rats and mice. Chronic inhalation exposure studies provided no-observed-adverse effect levels (NOAEL) in the 400 to 500 mg/m^3 range depending on species, and lowest-observed-adverse effect levels (LOAEL) in excess of 1000 mg/m^3. Subchronic exposure of dogs to 1473 ppm MMA in the diet, and chronic exposure of rats to 68 ppm MMA in drinking water failed to produce significant signs of toxicity. Operated by Maritett Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400.

GLUTATHIONE S-TRANSFERASE DEFICIENCY IS ASSOCIATED WITH HIGHER LYMPHOCYTE SCE LEVELS IN HEAVY SMOKERS. G van Poppel, P van Bladeren, P J Kok. NWO Toxicology and Nutrition Institute, Zeist, Netherlands.

Persons deficient in glutathione-S-transferase μ isozyme have been reported to be at higher risk for smoking-induced lung cancer. Smoking induced cyto-genetic damage includes increased levels of sister chromatid exchanges (SCE) in smoker's lymphocytes. We therefore studied the association between transferase-μ deficiency and SCE's in 157 smokers who volunteered for an intervention study on β-carotene and cyto-genetic damage. All smokers (>15 cigarettes/day) were healthy males without exposure to chemicals or drugs known to induce SCE's. 73 smokers were μ-deficient. Plasma cotinine levels and SCE's showed a weak significant correlation (R=0.17, p=0.03). SCE levels were higher in μ-deficient smokers compared to non-deficient smokers (5.24 vs. 4.96 SCE/lymphocyte; p=0.07) This μ-related difference in SCE's was more pronounced in smokers having high (> median of 351 ng/ml) plasma cotinine levels (5.50 vs. 4.94; p=0.007). In smokers having low cotinine levels, μ-related differences were absent (4.95 vs. 4.97; p=0.91).

Our results indicate that GSH-transferase μ deficiency is a marker of cigarette smoke induced chromosome damage, which may be especially important at high exposures. This observation suggests that increased genetic damage in GSH transferase μ deficient subjects is a mechanism explaining the epidemiological association between μ-deficiency and lung cancer.

Supported by the Dutch Prevention Fund.


Extrapolation modeling has been used to derive factors to adjust inhalation exposure concentrations for dosimetric differences between experimental animal species and humans. The dosimetric adjustments differ depending on whether the inhalant agent is a particle or gas and on whether the observed toxicity is in the respiratory tract or peripheral to the respiratory tract. These dosimetric adjustments are used in the interim methodology developed by the U.S. EPA to calculate human equivalent concentrations (HEC) to No-Observed-Adverse-Effect-Levels (NOAELs) or Lowest-Observed-Adverse-Effect-Levels (LOAELs) in experimental animal studies. The HEC values are then used to operationally derive inhalation reference concentration (RIR) risk estimates by application of uncertainty factors (UF). Since this methodology was based on the oral reference dose (ROD) paradigm, usually a 3-fold UF for extrapolation from experimental animal data was applied. Recently, a 3-fold UF has been suggested for use with dosimetric adjustments. Calculation of HEC values for selected particles and gases with differing physicochemical properties is performed from experimental species with appropriate data to illustrate the application of the dosimetric adjustments. Analysis of the variability across HEC's extrapolated from various species for different chemicals provides an index of the validity of the dosimetric adjustments and provides insight on the appropriate magnitude for the uncertainty factor applied with experimental animal data. The impact of the dosimetric adjustments to interspecies extrapolation used in risk assessment is discussed. (This is an abstract of a proposed presentation and does not necessarily reflect U.S. EPA policy).


A "carcinogen" classification system is suggested for classifying chemicals according to the potential that each substance poses a human cancer hazard. The system uses a weight of the evidence evaluation and is a logical extension of the systems developed by the International Agency for Research on Cancer, the U.S. Environmental Protection Agency, and the American National Standard for Hazardous Industrial Chemicals. The system evaluates human studies, animal bioassays, correlative evidence from bioassays, metabolic information, mechanistic studies and all other information relevant to predicting potential carcinogenic hazard to humans. Six categories and two additional designations are suggested: (Category 1) "Known Human Carcinogen"; (Category 2) "Carcinogenic Activity in Animals, Probable Human Carcinogen"; (Category 3) "Possible Human Carcinogen"; (Category 4) "Carcinogenic Activity in Animals, Probably Not a Human Cancer Hazard"; (Category 5) "Carcinogenic Activity in Animals, Considered Not a Human Cancer Hazard"; (Category 6) "Evidence of Noncarcinogenicity"; (Designation "EH") 'Equivalent Evidence for Carcinogenic Activity"; and (Designation "I") "Evidence Inadequate for Classification."
Silica (silicon dioxide) exists in amorphous and crystalline forms. Although OSHA has established a PEL of 0.1 mg/m³ TWA for the quartz form of crystalline silica in respirable dust based on the risk of developing silicosis, it may be inappropriate to use threshold values for silica. This is because IARC has concluded that there is sufficient evidence to show that crystalline silica causes cancer in experimental animals and limited evidence for the carcinogenicity of crystalline silica to humans. The carcinogenicity of crystalline silica was assessed to provide health-based standards to risk managers at hazardous waste sites in California. Three crystalline silica inhalation studies in rats show that inhalation of high concentrations of aerosol dusts of quartz causes malignant lung tumors in rats. Using the linearized multistage model and the method of Allen et al. (1987), the U.S. Environmental Protection Agency estimated an incremental lifetime risk of $10^{-4}$ for human cancer induced by crystalline silica was estimated to be $9 \times 10^{-7}$ mg/kg/day ($q_{(a)} = 1.11$ (mg/kg/day)$^{-1}$), based on a response of combined benign and malignant lung tumors in rats. The inhalation dose associated with one extra cancer in a million persons exposed is 0.002 µg/m³ which is considerably lower than the PEL of 0.1 mg/m³.

CNS, the Catalog of Neurotoxic Substances, is a database system designed to assist in relating chemical substances to reported neurotoxic symptoms or effects. CAS (Chemical Abstract Service) numbers and RTECS (Registry of Toxic Effects of Chemical Substances) numbers are listed whenever possible. This system enables users to rapidly obtain output reports, the contents of which depend on selections made from multiple-choice menus. They may request searches for information about substances by entering names, CAS or RTECS numbers, effects, or uses. Partial word text strings may be entered. Up to three different requests may be combined with "and" or "or" evaluation per search. Users may obtain either a full list of effects reported in referenced sources and uses for specific chemical substances, or more compact lists of chemicals, effects or uses. Reports requested are displayed on the video screen, as a printed copy or as an ASCII text file on disk. The initial contents of the database is the information retailer on a personal computer and were described previously (Toxicologist 1987;1:237). The database system occupies 1.4 megabytes of disk storage. The names, effects and uses of these chemicals are in separate databases related through the use of an arbitrarily assigned neurotoxic number. Utilities are provided to insert and edit information about additional chemicals, their effects, and their uses in the existing files. The programs and menu selection software are provided in source code format so later modification by the user is possible.

Carcinogen risk assessments conducted by the U.S. EPA have most frequently been based on results of a bioassay from a single sex/strain/species of animal. Use of more of the available data may result in a higher level of confidence in the risk estimate. Several biological factors should be considered before combining data sets from different animal sexes, strains, species or tumor sites. The relevance of the animal models, study design and execution, dose selection and route of administration are study quality factors which influence whether studies should be combined. The decision to combine data sets is also based on what is known of the mechanism of action of the agent; i.e., whether the agent is thought to be an initiator or to act at later stages, its pharmacokinetics, any species/sex specificity of the effect, and considerations regarding tumor site specificity. The use of these factors in the decision to combine or not combine data sets is discussed.

The EPA has recently compiled a listing of site of application (skin)-specific gross and histopathology criteria for selection of a Maximum Tolerated Dose (MTD) in rodent dermal bioassays based on preliminary 13-week subchronic studies. We evaluated the proposed EPA histologic criteria by comparing the MTDs selected by three different pathologists based on their evaluation of skin slides from three 13-week mouse skin painting studies by the National Toxicology Program. Key diagnoses on which the pathologists disagreed were: (1) crust versus epidermal hyperkeratosis and parakeratosis; (2) microcircular versus degeneration/necrosis; and (3) identification of adnexal hyperplasia. These differences in diagnosis led to large differences in the determination of an MTD. Post-grading discussions of problem diagnoses among the pathologists led to better concurrence in selection of MTDs upon re-evaluation of the slides. We anticipate that this work will lead to more precise or standard definitions for specific skin lesions. We have also developed image analysis techniques for quantitation of selected lesions that appear important in the tumorigenic process, e.g. epidermal hyperplasia.
TRPM-2 (testosterone repressed prostatic message) and TGA (transglutaminase) are genes expressed in cells during "programmed cell death" (apoptosis). The mRNA production by these genes was evaluated in the testes of rats glycol monomethyl ether (EGME) as part of an effort to evaluate the utility of these probes in the study of toxicologic disease processes. Young adult male rats were given daily oral gavage doses of 250 mg/kg EGME and evaluated 24 hours after 1, 2, 3, or 4 doses. The morphologic changes in the testes were evaluated using formalin-fixed, methacrylate embedded sections. The expression of TRPM-2 and TGA was evaluated in situ using an S0 labelled cDNA probe on frozen sections. Morphologic changes were related primarily to degeneration of premeiotic (pachyteny) and meiotic spermatocytes. TRPM-2 was expressed by Sertoli cells and epithelial cells of the rete testes of both treated and control rats. EGME treatment caused increased TRPM-2 expression, reaching a maximum after 3 to 4 days. Little or no TGA expression was evident in normal seminiferous epithelium. However, expression of TGA also increased during EGME treatment, reaching a maximum after 3 days.

THE DISTRIBUTION OF CYTOSKELETAL PROTEINS IN 2,5-Hexane-dione-TREATED RAT TESTES. E. Hall and K. Boekelheide. Brown University, Providence, RI

2,5-Hexanediene (2,5-HD) alters microtubule assembly dynamics in rat testes (Boekelheide, 1987) and induces testicular atrophy. Microtubules are normally arranged in parallel bundles between the base of the Sertoli cell and the elongating spermatids. These microtubules are probably involved in intercellular transport and anchoring of the developing germ cells. Other cytoskeletal proteins which are evident within Sertoli cells include vimentin and actin. Vimentin is also found between the developing spermatids and the base of the Sertoli cell while actin is an important component of the ectoplasmic specializations which anchor the spermatids to the Sertoli cells. We examined the distribution of these proteins within the testes of 2,5-HD treated rats. Adult Sprague-Dawley rats were treated for 5 weeks with 1% 2,5-HD in their drinking water. At 0, 3, 4, 5 and 8 weeks animals were killed and their testes were removed and processed for histological and immunohistological evaluation. Formalin fixed tissue was used for PAS/hematoxylin staining and morphological evaluations. Tubulin was detected in Bouin's fixed sections using monoclonal antibodies. Vimentin and actin were examined in fresh, frozen sections using monoclonal antibodies and TRITC labeled phalloidin, respectively. After 3 weeks, small changes in immunostaining and morphology were evident. By 8 weeks the testes were completely devoid of elongate spermatids, however, many tubules retained intense tubulin, actin and vimentin staining within Sertoli cells which was highly disorganized in appearance.

1,3-DINITROBENZENE TOXICITY IN STAGE-SYNCHRONIZED TESTICULAR TISSUE. C. Brown and M. G. Miller. Dept. Env. Tox., Univ. of California, Davis, CA. Sponsor: B. D. Halseh

The testicular lesion induced by 1,3-dinitrobenzene (1,3-DNB) in the rat is found only in seminiferous tubules at certain stages of spermatogenesis. Little is known about the cellular mechanisms underlying this stage-specific toxicity. With this in mind, the present studies will investigate 1,3-DNB toxicity in testicular tissue which has been synchronized to contain only 2-3 closely related stages. Rats were synchronized by the vitamin A depletion/repletion protocol outlined by Morales et al (Mol. Endocrinol. 3, 1989). In the whole animal, administration of 1,3-DNB (25 mg/kg, ip) elicited a marked testicular lesion which was present in all synchronized tubules even in those at stages which had been reported to lack susceptibility to toxicity in unsynchronized tissue. The lesion was also more pronounced than that found in susceptible stages with control animals. The differences in toxicity could be the result of either 1) toxicokinetic changes in 1,3-DNB metabolism and clearance or 2) inherent cellular sensitivity. Preliminary data would indicate that there are major differences in the capacity of the synchronized animals to metabolize and clear 1,3-DNB. Whether this alone accounts for the altered susceptibility to testicular toxicity or if cellular changes also are important will be investigated using freshly isolated seminiferous tubules obtained from stage-synchronized animals. (NIH ES-04699).
930 EFFECTS OF THEOBROMINE (TB) AND A COCOA EXTRACT (CE) ON THE TESTIS MORPHOLOGY AND BIOCHEMICAL PARAMETERS IN RAT TESTES. Y Wang and D P Waller. Program for Collaborative Research in Pharmaceutical Sciences and Department of Pharmacodynamics, Univ of Illinois at Chicago, Chicago, IL.

TB is the primary methylxanthine present in cocoa consumed in foods and beverages. Large oral doses of TB or CE for 4 weeks or longer cause damage to rat testes. Our current efforts evaluate early morphological and biochemical alterations in the testes after exposure to TB or CE. Male SD rats were treated by oral gavage with vehicle, TB (500 mg/kg) or CE (428 mg/kg containing 500 mg/kg TB) for 3 days and sacrificed 8 days later. One testes and epididymis were removed and weighed and the cauda epididymis used for sperm counts in all rats. The hemicastrate rats were either: 1. perfused with saline and buffered glutaraldehyde and the fixed testis removed, embedded in epoxy and sections cut on an ultramicrotome for microscopic examination; or 2. used to prepare a testis homogenate (TH) and seminiferous tubule fluid (STF). The supernatant of TH was used to determine sorbitol dehydrogenase (DH), lactate DH, glycerophosphate DH and isocitrate DH activities. The STF was used to determine lactate and androgen binding protein (ABP) content. Enzyme and lactate levels were determined by spectrophotometric methods. ABP was measured using polyclonal antibodies with labelled testosterone. A decrease in ABP was observed in the TB and CE treated groups. TB caused morphological alteration of the testes such as abnormally shaped spermatids and vacuolation in the seminiferous tubules. No changes in the other measured parameters were observed. Impairment of Sertoli cell function induced by TB could account for the observed alterations in testicular parameters.

931 EFFECTS OF METHANOL VAPORS (MV) ON THE MALE REPRODUCTIVE SYSTEM IN RATS: TESTIS MORPHOLOGY. W Lee and A N Brady. General Motors Research Laboratories, Warren, MI.

Effects of MV on testes morphology were examined in both folate sufficient (FS) and folate reduced (FR) LE rats in two age groups. Rats were exposed to MV for 20 hr/day for 13 wks. At the end of the exposure, the younger group was 10 months old and had subgroups exposed to 0, 50, 200, or 800 ppm while the older group was 18 months old and had subgroups of 0 or 800 ppm. No gross changes were observed in testes from either age group for any of the exposure categories. Microscopically, testes from younger FS rats did not display structural change at any exposure condition. On the other hand, 40% of the older FS rats exposed to air showed an early sign of testicular degeneration (TG), i.e., subcapsular presence of vacuoles in the germinal epithelium. The 800 ppm MV did not enhance the incidence of the age-related TG in the older FS rats (38%). Some (17%) of the younger FR rats exposed to MV showed signs of early TG but the incidence was not dose related. However, in the older FR rats, the cases of TG in the 800 ppm MV-exposed rats were increased from 25% (air) to 62%. Similar to FS rats, testicular weights of FR rats were not affected by a 13-week exposure to 800 ppm MV. Thus, these data suggest that MV have no inhibitory effect on testosterone synthesis but may have the potential to accelerate the normal age-related TG process in FR rats that respond like primates to acute methanol administration.


Acute administration of EDS rapidly reduces production of testosterone (T) by Leydig cells to undetectable levels and fertility is reduced until Leydig cell function and spermatogenesis are restored. In the current study, a time series analysis was conducted of the effects of an injection of EDS (65 mg/kg) on mating behavior, T and sperm measures in LE-hooded rats. Mating behavior and ejaculated sperm counts were determined prior to, and at 4, 10, 25, 39 and 53 d after treatment, when selected rats were necropsied. In EDS-treated rats, serum T, interstitial fluid T, seminiferous tubular fluid T, in vitro T production (with and without hCG), and seminal vesicle weight were dramatically reduced at 4 and 10 d. These measures returned nearly to normal levels by 39 d. Testes and epididymal weights were reduced only at d 10 and cauda epididymal sperm numbers were reduced by 75% at d 10, while the numbers of ejaculated sperm were reduced to less than 1% of pretreatment levels at this time. In contrast, T-dependent mating behavior was relatively unaffected. The information from this investigation will be used to design a study that correlates ejaculated sperm numbers and fertility in EDS-treated rats.

933 EVALUATION OF REPRODUCTIVE PARAMETERS IN ADULT MALE SWISS MICE AFTER CHRONIC EXPOSURE TO URANIUM. A. Ortega, JJ Sirvent, JM Llobet, JL Domingo and J Cortella. Laboratory of Toxicology and Biochemistry, School of Medicine, University of Barcelona, Quins, Spain.

Relatively few data are available concerning the reproductive and developmental toxicity of uranium. The present study was designed to evaluate its reproductive effects in male Swiss mice. The animals were treated with uranyl acetate dihydrate (UA) at doses of 0, 10, 20, 40 and 80 mg/kg/day given in the drinking water for 64 days. To evaluate the fertility of the UA-treated males, mice were mated with untreated females for four days. There was a significant but dose-related decrease in the pregnancy rate of these animals. Body weights were only significantly depressed in the 80 mg/kg/day group. Testicular function/spermatogenesis was not affected by UA at any dose, as evidenced by normal testes and epididymal weights and normal spermatogenesis, whereas interstitial alterations and vacuolization of Leydig cells were seen at 80 mg/kg/day. The results of this investigation indicate that UA does not cause any adverse effect on testicular function in mice at the concentrations usually ingested in the diet and drinking water, with a safety factor of more than 1000. However, UA administration produced a significant decrease in the pregnancy rate at 10, 20, 40 or 80 mg/kg/day.
Mutation-induced reproductive effects of MBT were assessed in Sprague-Dawley rats as a satellite of a reproductive toxicity study. MBT was administered to 28 rats/group in diets containing 0, 2500, 8750, and 15000 ppm. Diet was provided ad libitum for 13 weeks prior to cohabitation, for 16 hours/day during the cohabitation period, and ad libitum again until scheduled sacrifice. Positive control males (28) were administered by gavage 100 mg/kg Cytoxan® one week prior to mating. Each male was housed with 2 untreated virgin females/week for two consecutive weeks. Uterine examinations were conducted 13 days post-mating. Survival, clinical signs, and reproductive indices were comparable for control and MBT-treated males. Mids and high dose males gained significantly less weight than controls during the pre-mating treatment period. No evidence of a dominant lethal effect was seen in untreated females mated with MBT-treated males; numbers of early resorptions and viable embryos were similar in MBT and control groups. Significant increases in embryonic death were found in females treated with positive control males. Under the conditions of this study, MBT did not induce dominant lethal mutations in the sperm of males treated with doses which produced signs of toxicity. (Supported by the GNA Rubber Additives Panel)

**NEGATIVE DOMINANT LETHAL STUDY OF LEWISITE IN CD-RATS. R M Parker, T J Bucco, K H Denny and J C Dacre, Pathology Associates, Inc., National Center for Toxicological Research, Jefferson, AR. *USABRDL, Fort Detrick, Frederick, MD.**

Lewisite (2-Chlorovinylidichloroarsine, Agent L) was investigated as part of the US Army Toxicological Program on Chemical Agents. Twenty male CD-Rats per dose group received by gavage 1.5, 0.75 or 0.375 mg/Kg Lewisite or vehicle control (1 ml sesame seed oil) daily for 5 days. Positive control males were given 1 ml sesame seed oil by gavage [days 1-4] and on day 5 they were given an i.p injection of 100 mg/Kg ethyl methanesulphonate, a positive control mutagen. Each male was mated to 2 virgin females (12 weeks of age) per week for the next 10 weeks. Females were killed on Gestational Day 14. At necropsy, the corpora lutea were counted and the uteri and contents were examined. Implantation sites were categorized as live/dead fetuses or early/late resorptions. No significant differences in reproductive indices were seen between treatment groups and the control group with the exception of the positive control. Males were killed during week 13 and necropsied. Sperm morphology/motility statistical analysis, testicular histopathology evaluation and morphometric analysis of seminiferous tubule cross-sections are underway and will be reported. [Supported by US Army Medical Research and Development Command, APO#88PP8660]


Conventional dish cultures of testicular Sertoli cells and Sertoli/germ cell cocultures have been widely used to examine the toxicity of a number of known in vivo testicular toxins. However, the morphological appearance of these cultures is different to that of Sertoli/germ cells in vivo and the technique does not readily allow the effects of metabolic activation on toxicity to be examined. The two-compartment culture system may overcome these deficiencies. Mixed Sertoli/germ cell cocultures from 28-day old Sprague Dawley rats were established in fibronectin-coated Millipore (90nm diam). After 5 days the cultures were treated with a range of doses of 6 known in vivo Sertoli cell toxins and 3 related non-toxins. Toxicity was assessed after 24 and 48hr exposure by measuring germ cell detachment (GCD) and effect on protein synthesis. Of the known toxins, mono-2-ethylhexylphthalate (MEHP), AP131/T5, Gossypol and 1,3-dinitrobenzene (1,3-DNB) produced a dose-related increase in the number of germ cells detached, mono-2-butyl phthalate (MNB) was also effective but only at high doses whereas 2,5-hexanediol had no effect. Treatment with 2 of the 3 non-toxins (1,4-dinitrobenzene and 2,4-hexanediol) also resulted in an increase in GCD, the latter only at high doses. The third non-toxin, mono-2-butyl phthalate (MNB) had no effect. Over the same dose ranges used to assess GCD, protein synthesis was reduced following treatment with Gossypol and at high doses with 2,4-hexanediol and increased after exposure to 1,3 and 1,4-dinitrobenzene and MNB. No significant effect was seen with MEHP, AP131/T5, 2,5-hexanediol or MNB. Thus a dose related increase in GCD would appear to be a better indicator of toxicity in this system than effect on protein synthesis. (Supported by UK Ministry of Agriculture, Fisheries and Food)

**THE USE OF THE MOUSE ChIMERA ASSAY TO DETECT EARLY EMBRYOTOMIC DAMAGE FROM MALE MICE EXPOSED TO ETHYLENE GLYCOL MONOMETHYL ETHER (GME). D Oudix, K J Walsh, L Wiley. *Calif. Dept. Health Services, Sacramento, CA and Dept. Ob/Gyn, Univ. of Calif., Davis, CA.**

Mouse chimeras are aggregations of two 4-cell embryos and are used to detect subtle, non-lethal changes either directly to the embryos or through the germ cells, which are expressed as a cell proliferative disadvantage. One of the embryos is labelled with a viable dye (FITC) to determine the relative cellular contribution of each embryo when the chimera is dissocitated 40 hours later. This proliferative disadvantage occurs when male mice are irradiated at 0.01 and 0.05 Gy. The current study explored the ability of the chimera assay to detect damage from a nonmutagenic, reproductive toxicant. Male mice were exposed to GME by gavage for 5 days with 0.750, or 1500 mg/Kg and serially mated with unexposed female mice for the next 7 weeks. Proliferation ratios were significantly decreased in the high dose group every week, except weeks 4 and 7. On week 4, there were not enough embryos to evaluate but by week 7 the ratios returned to control levels. Ratios decreased for the low dose group on weeks 2, 3, and 4. Week 4 produced the greatest effect in decreased proliferation ratios, and in embryo yield and quality. This week corresponds with the pachytene spermatocyte stage.
INHIBIN SECRETION AS A MARKER OF TESTICULAR TOXICITY IN ISOLATED SEMINIFEROUS TUBULES (ST), SERTOLI CELL (SC) CULTURES AND IN VIVO, G Allenby, PMD Foster and RM Sharpe. MRC. RB1, Edinburgh, Scotland, U.K.

Inhibin, a SC glycoprotein hormone secreted in response to FSH, represents a new and potentially useful marker of testicular (SC) toxicity. The effect of two known SC toxicants, meta dinobenzene (mDNB) and nitrobenzene (NB), and two possible testicular toxicants, 3- and 4-monooctylbenzene (3- and 4-MNB), were compared in ST and SC culture systems at concentrations of 10^{-7}, 10^{-5} or 10^{-3} M. Inhibin secretion under basal conditions and after maximal stimulation with rat FSH or dibutyryl cyclic AMP (db cAMP) was used as an index of toxicity. Adult rat ST or SC were isolated and cultured for 1 to 3 days in the absence or presence of toxicants and inhibin measured in spent medium. For in vivo studies rats received a single oral dose of compound, calculated to produce tissue levels equivalent to those used in vitro, and testicular interstitial fluid (IF) was collected at 1 and 3 days post-treatment. Addition of either mDNB or NB to ST cultures increased both basal and FSH or db cAMP stimulated secretion of inhibin by 2- to 4-fold on days 1, 2 and 3 of culture. Identification of mDNB and NB added to SC cultures also enhanced inhibin secretion although these effects were more variable and, smaller in magnitude than the effects on ST cultures. In vivo, at 1 and 3 days after treatment with mDNB or NB a 2- to 4-fold increase in IF inhibin levels was observed in combination with histological changes. In contrast, addition of 3- or 4-MNB to ST or SC cultures had no effect on basal or stimulated inhibin secretion and no significant alteration in IF inhibin levels or histological changes were observed in vivo at 1 and 3 days post-treatment. Data suggest that modulation of inhibin secretion by isolated ST's may have potential as a sensitive in vivo screening method for identifying early adverse effects of chemicals on spermatogenesis, however better validation using a wider range of chemicals is required.

GLUTATHIONE LOCALIZATION IN SERTOLI-GERM CELL CLOCULTURES, Lynn T Frame, Jay Gandy. Div. of Tox., Univ. of Ark. for Medical Sciences, Little Rock, AR

Sertoli cells define the blood-testes barrier and are believed to play an important role in the maintenance and protection of the male germ cell line. The purpose of this study was to explore the role of Sertoli-germ cell contact in maintaining levels of glutathiones (GSH) in spermatogenic cells. Using Sertoli-germ cell cocultures from 25-35 day old male Sprague-Dawley rats, a brief hypotonic treatment was employed to separate germ cells from Sertoli cells. The isolation was considered valid since spectrophotometric-based assays showed that lactate dehydrogenase-X activity (a specific germ cell marker by immunohistochemical localization analysis) was 260-fold greater in the germ cell supernatants, whereas gamma-glutamyl transpeptidase (a Sertoli cell marker) was detected only in Sertoli cell lysates. When total glutathione was measured, germ cells, allowed to maintain contact with Sertoli cells, showed higher concentrations of GSH (45-89 nmol/mg) than Sertoli cells (3-6 nmol/mg), viable detached germ cells (2-6 nmol/mg), or germ cells isolated by much longer procedures. These data support the hypothesis that Sertoli cell contact is required to maintain germ cell glutathione levels, and validate our experimental approach for further study of cell-contact mediated events. Germ cell GSH was comparable to values for 6-hr cultured hepatocytes, the cell type generally assumed to have the highest glutathione concentration. (USPHS grant ES 05368.)

C-MYC GENE EXPRESSION DURING AFB1-INDUCED HEPATOCARCINOCENESIS. P S Larson, Mallory Institute, Department of Pathology, Boston University School of Medicine, Boston, MA, and C N Wogan, Division of Toxicology, Whitaker School of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA. Sponsor: P M Newberne

We noted earlier that a single exposure of aflatoxin B1, elevated c-myc liver mRNA levels in the adult male Fischer rat. To investigate how chronic aflatoxin B1, exposure might influence c-myc gene expression during the hepatocarcinogenesis process, rats were treated with a carcinogenic dose of aflatoxin B1. Liver samples were obtained during exposure to AFB1 and following development of tumors. Characterization of the c-myc induction response during chronic aflatoxin B1 treatment revealed that an elevation of c-myc mRNA levels occurred during each day-dosing period for the first three weeks. At the end of the 6 week dosing period, levels of c-myc mRNA in the liver of treated animals were approximately 2-fold higher than in control animals. This level was maintained up to 50 weeks, at which time adenomas appeared and c-myc mRNA levels sharply increased. Emergence of hepatocellular carcinomas at 63 weeks and beyond was accompanied by steady-state c-myc mRNA levels that were 8 to 15-fold higher than those in vehicle-treated control animals.
SUPPRESSION OF ONCOGENE EXPRESSION BY THE CHEMOPREVENTIVE AGENT ANTIPAIN (AP). L R COX and S J GARTE. Institute of Environmental Medicine, New York University Medical Center, New York, NY.

The NIH/NI focus forming assay was used to investigate the mechanisms by which the protease inhibitor AP inhibits transformation by a mutationally-activated H-ras oncogene. Following transfaction of the H-ras oncogene, exposure to 50 μg/ml AP resulted in maximal inhibition of focus formation. Maximal inhibitory activity was observed when cells were exposed during the proliferative phase of the transfection protocol, demonstrating that AP is a stage-specific inhibitor of H-ras transformation. No effects of AP on genenic resistance were observed after transfaction with the selectable markers gene aph. When cells were co-transfected with H-ras and aph, fifty percent of genenic-resistant colonies failed to express the transformed phenotype (determined by their inability to grow in soft agar) as a result of AP treatment. Northern blot analysis of the transformed and non-transformed colonies showed that suppression of H-ras transformation by AP was associated with decreases in expression of both the exogenously transfected transforming gene H-ras and the endogenous oncogene c-myc. Southern blots revealed no effects of AP on incorporation or copy number of the H-ras gene. Supported by NIH grants CA-52925, CA-13343 and ES-00260.

NOVEL TANDEM-REPEAT TRANSFORMING MUTATION IN KRAS GENE FOLLOWING EXPOSURE TO FURAN. V L Burnett1,2, S H Reynolds1, J S Wiest1 and M W Anderson1. National Institute of Environmental Health Sciences1, RTP, NC and North Carolina State University2, Raleigh, NC

Sponsor: F Hudson2

Since first identified as transforming genes in humans in 1982, ras has been implicated in pancreatic, colon, and other tumors. Codons 12, 13 and 61 are the 'hot spots' for the transforming point mutations. Utilizing the polymerase chain reaction to amplify DNA followed by exon sequencing, a novel type of transforming mutation has been discovered in liver adenomas of furan treated B6C3F1 mice. Nine codons in the second exon of K-ras (near but not including the 51st codon) have been randomly repeated, preserving the normal reading frame to produce an aberrant protein. Immunoprecipitation of the protein revealed an altered mobility distinct from the normal or point mutated ras gene protein, indicating an altered protein structure. Furan is negative in the Ames test, yet has been shown to cause chromosomal aberrations in Chinese hamster ovary cells, which could be the mechanism of the tandem-repeat mutations. It remains to be seen how prevalent the mutation is, and if it is involved in human tumors. Care should be taken when analyzing ras transforming genes, as this novel tandem-repeat mutation would not have been discovered by the common experimental techniques of performing oligonucleotide mismatch hybridization or sequencing of only the preselected 'hot spot' areas.
946 IMMORTALIZATION AND ONCOGENE EXPRESSION OF HUMAN TRACHEAL GLAND EPITHELIAL CELLS. DP Chopra, AP Jolainik, GM Taylor and PA Mathieu. Institute of Chemical Toxicology, Wayne State University, Detroit, MI. 
Sponsor: R.F. Novak.

Recently human tracheal gland epithelial cells that secrete mucin-like glycoproteins have been propagated in serum-free medium. The cultures however, undergo senescence after a few passages, which precludes their usefulness to study functional differentiation that requires large numbers of cells. We have immortalized the tracheal gland cells with Adovonirus12-SV40 hybrid virus. The immortalized cells exhibit many characteristics of epithelial cells including: microvilli on cell surfaces, desmosomes-like junctions between cells, cytokeratin positive filaments and the continued secretion of mucin glycoproteins. Transformed cells have altered growth characteristics compared to the normal cells including: shorter cell population doubling time, a requirement for collagen substrate for attachment, the ability to grow in suspension, and an apparent unlimited life span. Most cells were hypotetraploid with karyotype abnormalities similar to immortalized cells of other tissues. In addition, the cells have an increased expression of erbB-2 and decreased expression of c-myc genes as compared to the untransformed cells. No tumors were formed after subcutaneous injection into nude mice. These cells should be very useful for studying biochemical and molecular mechanisms of mucin synthesis and secretion. Supported by NIH NHLBI 41979 and 33142

947 HAMSTER RAS FAMILY GENE EXPRESSION DETECTED BY THE POLYMERASE CHAIN REACTION. V I C Orecchio P H Gumerlock and E P Witschi, Institute of Toxicology & Environ Health and School of Medicine, Univ. of California, Davis, CA.

The ras proto-oncogene family is highly conserved across species lines and its members (c-Ha-ras 1, c-Ki-ras-2 and c-N-ras) have been implicated in the carcinogenic process due to point mutations that activate them to transforming oncogenes. Experimental chemical carcinogenesis studies have consistently identified specific mutations at the 12th, 13th, or 61st codon as a direct result of treatment. We chose to examine the ras genes in our own model of Syrian golden hamster lung carcinogenesis. In this model, SCLC were produced in hamsters treated with N-nitrosodimethylamine (DEN) (Carcinogenesis 2: 293-296, 1988) or 4-(methylamino)-1-(3-pyridyl)-1-butanone (NNK) (Cancer Research 30: 1960-1965, 1990) and hyperoxia. Treated animals maintained in ambient air developed non-SCLC. The polymerase chain reaction (PCR) specifically amplifies target DNA sequences within genomic DNA and has recently been adapted to the study of RNA (RNA/PCR). We have utilized human sequence designed oligonucleotide primers to amplify messages from the hamster ras genes. We have confirmed the PCR products by (1) predicted size analysis and (2) by probing with non-radioactive (biotin labeled) oligonucleotides internal to the primer pairs. Furthermore, this approach has allowed us to generate preliminary sequence data from the hamster genes which will allow us to probe for activating point mutations in these tumors and premalignant lesions.

Supported by Center for Indoor Air Research (CIAR) and University of California Tobacco Related Disease Research Program.

948 DIRECT INDUCTION OF PROTOONCOGENES c-fos AND c-myc mRNAs BY CYSTEINE CONJUGATES AND OTHER TOXINS. KF Yu, Q Chen and JL Stevens. M. Alton Jones Cell Science Center, Lake Placid, NY.

The expression of protooncogenes c-fos and c-myc is an early event when growth arrested cells enter the cell cycle (G0→G1) after stimulation by serum or growth factor. However, we show that nephrotoxic cysteine conjugates directly induce the expression of c-fos and c-myc genes in confluent LLC-PK1 cells. Level of c-fos mRNA increased with increasing S-(1,2-dichlorovinyl)-L-cysteine (DCVC; 0.05 - 0.5 mM). c-Myc mRNA was higher at lower dose of DCVC and decreased with high doses of DCVC. The increase of c-fos and c-myc mRNAs appeared after treating cells with DCVC for 2 hrs, well before cell death occurs. The induction of c-fos and c-myc was blocked by preventing transcription with actinomycin D, blocking DCVC metabolism with AOA or inhibiting DCVC toxicity with the antioxidant DPPD. The protein synthesis inhibitor cycloheximide reduced the expression of c-myc but not c-fos mRNA. Both c-fos and c-myc mRNAs were also induced by the alkylating agent lodoacetamide and toxic doses of the organic peroxidant t-butylhydroperoxide. The data indicate that expression of protooncogene c-fos and c-myc is directly induced by cytotoxic cysteine conjugates and other toxins. c-fos may be an immediate early response gene for toxicity while c-myc may be a secondary gene which requires the action of another early response gene.

949 ANEUPLOIDY DETECTION IN INTERPHASE HUMAN LYMPHOCYTES USING CHROMOSOME-SPECIFIC DNA PROBES FOLLOWING TREATMENT WITH THE BENZENE METABOLITE, HYDROQUINONE. P A Eastmond and L Hasegawa. Environmental Toxicology Graduate Program, University of California, Riverside, CA.

Structural and numerical chromosomal aberrations have been observed at increased frequencies in the blood cells of workers occupationally exposed to benzene. Recent evidence suggests that these aberrations may be involved in the development of leukemia. Fluorescence in situ hybridization with chromosome-specific DNA probes offers an unique approach to investigate the metabolites and mechanisms involved in benzene-induced genotoxicity and leukemia. Hybridization with chromosome-specific DNA sequences results in brightly fluorescent spots in interphase nuclei which correspond to the position of the target DNA sequences. The number of hybridization regions in a nucleus should therefore indicate the number of copies of a chromosome that is present. Using a chromosome 9-specific centromeric probe, a dose-related increase in the frequency of cells containing 3 or more hybridization regions was observed following in vitro exposure of PHA-stimulated human lymphocytes to hydroquinone. Significant increases in cells containing multiple hybridization sites were observed at hydroquinone concentrations greater than 75 μM. The use of centromeric probes for other chromosomes indicated considerable variability in the frequency of cells containing multiple hybridization regions. Studies are currently underway to investigate the basis for this chromosome to chromosome variability and understand its significance in hydroquinone and benzene-induced genotoxicity. Supported by the University of California Tobacco-related Disease Research Program and the Universitywide Energy Research Group.

The natural product mutagens safrole, estragole, and eugenol are chemically related to allylsybenzene, and form reactive 2',3'-epoxides at the allylic double bond. We have studied the formation of covalent adducts of allylsybenzene 2',3'-oxide (ABO), or estragole 2',3'-oxide with DNA or individual deoxynucleotides. The 2 major ABO-DNA adducts are formed with dG; one adduct with N7 of dG has been identified and characterized. Formation of these major adducts and other minor adducts is strongly inhibited by both microsomal and cytosolic epoxide hydrolases in vitro. We conclude that either epoxide hydrolase can largely prevent the formation of these adducts in vivo. Supported by PHS CA34455.

AN EVALUATION OF THE CAPABILITY OF TRIHALOMETHANES TO INDUCE DNA STRAND BREAKS IN RATS AND MICE AND IN CULTURED CELLS. A.B. Prakash, L.W. Chang, C.L. Potter, and F.B. Daniel. U.S. EPA, Cincinnati, OH.

The trihalomethanes (THMs) are drinking water contaminants that were found to be carcinogens in rats and/or mice. Prior to starting chronic exposures of rodents to the THMs, drinking water was analyzed to determine the ability of the THMs to induce DNA damage when administered by this route was tested. Male B6C3F1 mice and F344 rats were given THMs (0.3 or 0.6 mmole/kg; 36-150 mg/kg) by gavage in 4% Emulphor suspension. Pharmacokinetic studies have shown that absorption of THMs from 4% Emulphor closely resembles absorption from drinking water. Dimethyltrinitrosamine (DNMA) was the positive control. Trichloromethane (chloroform), bromodichloromethane, dibromochloromethane, and tribromomethane (bromoform) were ineffective in inducing DNA strand breaks in the liver, kidney, or bone marrow of rats and mice. The 4 THMs (1-20 mCi) also failed to induce DNA strand breaks in rat hepatocytes treated in primary culture for 24 hours. The bromine containing THMs, however, did induce DNA strand breaks in C3H/10T1/2 cells, a human lymphoblastic cell line which detects direct-acting genotoxic agents. The number of strand breaks was positively related to the number of bromine atoms in the THM. These data demonstrate a direct-acting genotoxicity of the brominated THMs, and suggest that cellular metabolism of the THMs may be protective. This abstract does not necessarily reflect EPA policy.

REPAIR OF DOUBLE- AND SINGLE-DNA BREAKS IN CULTURED HUMAN CELLS IN VITRO INDUCED BY BLEOMYCIN. M.C. Cunningham, J.G. Peak and M.J. Peak. NIEHS. Research Triangle Park, NC and Argonne National Laboratory, Argonne, IL

As part of our ongoing studies to understand how chemicals can be mutagenic in vitro, we have been investigating the capacity of mammalian cells to repair DNA damage. Human P3 cells (epithelial, larynx carcinoma derived) were exposed to bleomycin (BLM), a radiomimetic agent, or X-rays at 40°C or 37°C at various doses and times, and the DNA analyzed for single (ssDNA) and double (dsDNA) strand breaks by alkaline and neutral elution techniques, respectively. Further studies were performed by allowing cells to repair BLM-induced DNA breaks at 37°C followed by analysis of the repair (repair) of DNA damage. Dose-dependent ssDNA breaks were observed from 0.25-2.0 mg/ml BLM. The high dose produced ssDNA damage equivalent to 3 Gray of X-rays; dsDNA damage was linear from 10-100 mg/ml BLM. Maximum DNA damage was observed by 30 minutes of exposure to BLM. Only cells dosed with BLM or X-rays at 40°C demonstrated DNA damage; cells dosed at 37°C did not, indicating that the damage was repaired as rapidly as it was produced at 37°C but not at 40°C. Repair of ssDNA or dsDNA breaks induced by BLM proceeded much slower than repair of damage induced by X-rays. X-ray damage was 100% repaired by 30 minutes, whereas only 75% of the damage induced by BLM was repaired by 2 hours. These data indicate that DNA damage induced by BLM exhibits different repair kinetics and may be qualitatively different than DNA damage induced by X-rays. [DOE W-31-109-ENG-38].


Benzamide (BA), a nicotinamide analog, inhibits the NAD-dependent DNA excision repair enzyme, ADP-ribosyltransferase (ADPRT). BA increases sister chromatid exchanges, presumably by inhibiting DNA repair (Natarajan et al., 1981, Mutat Res. 84:125). Rat hepatocyte nuclear ADPRT has been shown to be effectively inhibited in the presence of 10-6M BA (Oikawa et al., 1980, Biochem. Biophys. Res. Commun. 97:1311). This study has examined whether BA is genotoxic at both cytotoxic and non-cytotoxic concentrations. BA was not mutagenic in either the Ames or CHO/HGFR assays at concentrations of 25mM or higher. BA increased the percentage of cells with aberrations, including both simple and complex aberrations, at very high concentrations (25mM) only; BA also delayed the cell cycle and was toxic to at least 25% of the cells. Thus BA is not mutagenic but affects chromosomal integrity only at concentrations greatly exceeding those necessary for ADPRT inhibition.

Ames assays are routinely used to monitor the mutagenicity of cigarette smoke and cigarette smoke condensate. The KIR4F cigarette is considered to be representative of full-flavor low-tar cigarettes and is designed to serve as a reference standard for comparative studies on chemical and biological activities. The objective of this study was to determine if Ames mutagenic activity is comparable for mainstream smoke condensates from KIR4F cigarettes and cigarettes representative of the U.S. market. Mainstream smoke condensates from the KIR4F and 73 brand styles (encompassing >70% of the total U.S. cigarette market) were assayed using Salmonella typhimurium TA98 and TA100 (+S9) at concentrations of 0, 25, 50, 75, 100, 125 and 250 μg/plate. Revertants/μg condensate were estimated by calculating the slopes of the dose-response curves using linear and nonlinear models. The two models gave similar results. No significant differences (p>0.05) were observed between the average mutagenicities of mainstream cigarette smoke condensates when the KIR4F was compared to the U.S. market. We conclude that the KIR4F is a representative model for the U.S. cigarette market in comparative Ames mutagenicity studies using mainstream smoke condensates.

OUABAIN RESISTANT (Na+,K+)-ATPase ENZYME ACTIVITY IN CHEMICALLY INDUCED OUABAIN RESISTANT CSH/10T1/2 CELLS. M. Shibuya, T. Miura, J. R. Liiehauag, R. A. Farley, and R. L. Landolph, Deps. of Microbiology, Pathology, Biochemistry, and Norris Cancer Hospital and Research Institute, University of Southern California, Los Angeles, CA.

To define chemically induced ouabain resistance, we characterized (Na+,K+)-ATPase activity in plasma membranes of wild-type and ouabain-resistant (Oua') 10T1/2 cells and showed it was inhibited in a dose-dependent manner by ouabain and completely inhibited by 2.4 mM ouabain. 10%--45% of (Na+,K+)-ATPase activity from three Oua' 10T1/2 cell lines was resistant to 2.4 mM ouabain. Resistance of (Na+,K+)-ATPase activity in plasma membranes of Oua' cells and resistance of cultured Oua' cells to ouabain occurred over similar ouabain concentrations (0.1-3 mM). Two Oua' cell lines demonstrated an increased fraction of Oua' enzyme activity when cultured in ouabain. Thermal denaturation and pH dependence profiles of (Na+,K+)-ATPase activity in plasma membranes from wild-type and Oua' 10T1/2 cells were identical. A 3.9 kb (Na+,K+)-ATPase α-subunit mRNA transcript was found in 10T1/2 and Oua' MNNG C2 cells cultured in the presence or absence of ouabain. There was no amplification of the gene coding for the α-subunit of (Na+,K+)-ATPase in the Oua' MNNG C2 cell line cultured in the presence or absence of ouabain. These studies provide further evidence that the Oua' phenotype derives from Oua' (Na+,K+)-ATPase activity which increases further in some cell lines cultured in ouabain.

HYDROXYL RADICAL MEDIATED DNA STRAND BREAKAGE AND VIRUS INACTIVATION BY HYDRAZALINE. J. L. Poyer, J. E. Schneider, J. R. McManus, and R. A. Floyd. Molecular Toxicology Research Program, Oklahoma Med. Res. Foundation, Oklahoma City, OK.

The capacity of the hypotensive agent hydralazine to catalyze a Fenton reaction was measured by single strand break (SSB) formation in supercoiled plasmid DNA and by inactivation of the bacteriophage R17. SSB formation by hydralazine in the presence of phosphate buffer and iron was demonstrated to be dependent upon oxygen, iron, and temperature, and was inhibited by scavengers such as mannitol, benzoate and thiourea. Phage inactivation by the hydralazine-catalyzed reaction showed similar properties as did SSB formation and, in addition, allowed greater sensitivity and more precise quantitation of the active species. Salicylate was substituted for DNA or phage in the reaction and hydroxyl radical (·OH) was measured by the ability of ·OH to form dihydroxy benzoic acid from its reaction with salicylate. These data provide strong evidence that hydralazine is able to act as a reducing agent to generate ·OH from a Fenton reaction that damages genetic material. Although the chemical event measured by phage inactivation is not known, phage inactivation is shown to be a very sensitive indicator of the generation of chemical species that damage macromolecules. This work was sponsored in part by Oklahoma Center for Applied Science & Technology Grant (No. 3502).

Oleylamine is an 18-carbon fatty amine produced from naturally occurring animal or vegetable oils or triglycerides. Oleylamine is used as a cationic surface-active agent or in the manufacturing of other cationic surfactants. The potential genotoxicity of oleylamine was tested in vitro using the Ames test, the mouse lymphoma tk+/tk− forward mutation assay, the CHO/HGPRT mutation assay, and the CHO chromosomal aberration assay. In vivo, oleylamine was tested for induction of chromosome aberrations in mouse bone marrow cells. Oleylamine was tested in the Ames assay in strains TA98, TA100, TA1535, TA1537 and TA1538 both with and without metabolic activation at concentrations ranging from 100 to 0.2 μg/plate. For the mouse lymphoma, CHO/HGPRT and CHO chromosomal aberration assays, in the absence of metabolic activation, concentrations of 1.8 - 0.13 nM/ml, 2.0 - 0.1 nM/ml and 1.5 - 0.05 nM/ml were tested, respectively. In the presence of an Aroclor-induced rat liver S-9 mix, concentrations tested in these assays were 13.0 - 1.3 nM/ml, 10.8 - 0.8 nM/ml and 20.0 - 0.6 nM/ml, respectively. No significant or reproducible increases in mutant or chromosomal aberration frequencies were noted in any of the assays. For the in vivo cytogenetics assay, male and female ICR mice were treated by gavage with 500, 2500 and 5000 mg oleylamine in corn oil/kg body weight. Bone marrow cells, arrested in metaphase were collected 6, 12 and 24 hr after treatment. No statistically significant increases in percentage of aberrant cells were observed in the treated animals. These results indicate that under the conditions tested, oleylamine was neither mutagenic nor clastogenic. (Sponsored by CMA oleylamine program).

BACTERIAL MUTAGENICITY OF HYDRAZINE (HDZ). C. A. McQueen and B. M. Way, American Health Foundation, Valhalla, NY.

HDZ is an antihypertensive agent with limited evidence of carcinogenicity in animals. HDZ is genotoxic to mammalian cells and mutagenic in bacteria. There is evidence that HDZ induces DNA damage by interacting with pyrimidines. Greater reactivity has been observed with thymine compared to deoxyguanosine. As part of an ongoing study to investigate the interaction between HDZ and DNA, studies were initiated to evaluate mutagenicity in Salmonella tester strains TA 100 and TA 102. HDZ has been reported to be mutagenic to TA 100 which has G.C base pairs at the site of reversion. TA 102 has A.T base pairs at that site. The bacteria were exposed to HDZ by the plate incorporation method with and without S-9 from Aroclor-induced rat liver for 72 hours. HDZ induced mutants in both TA 100 and TA 102 in the presence of S-9, with differences being observed in the shape of the dose-response curve. Maximum response was induced at concentrations greater than 2.5 mg HDZ/plate for TA 100 and 1 mg for TA 102. This induction of mutants by HDZ in TA 102 is consistent with thymine being a target for HDZ. (Supported by NIH GM 39390).

THE BINDING OF TRANS,TRANS-MUCONALDEHYDE TO DEOXYGUANOSINE AND CALF THYMUS DNA. E. Schatz-Kornbrust, B. D. Goldstein, and G. Witg. Joint Graduate Program in Toxicology, UMDNJ-Robert Wood Johnson Medical School/Rutgers University, Piscataway, N.J.

In previous studies, trans,trans-mucosaldehyde (MUC) was found to be genotoxic to bone marrow cells of mice in vivo and to mammalian and bacterial cells in vitro. In order to elucidate the chemical interactions between MUC and genetic material, studies were undertaken on the binding of MUC to deoxyguanosine (dG) and calf thymus DNA. Gradient reverse phase HPLC analysis of MUC (10−3 M) reacted with dG (10−3 M) for 6 hr at 37° in 10 mM phosphate buffer, pH 7.4, showed a complex pattern of peaks using detection at 265 nm. Four of these peaks were also detected upon scanning the eluent at 340 nm, a new absorption present after reaction of MUC with dG. The enzymatic digest of calf thymus DNA (0.5 mg/ml) reacted with MUC (10−3 M) for 6 hr at 37° showed multiple HPLC peaks, four of which matched those identified as common peaks in the MUC-dG reaction mixture analyzed at 265 and 340 nm. The chromatogram of the digest scanned at 265 nm contained an additional peak which was also present in the MUC-dG reaction mixture scanned at 265 nm, but not at 340 nm. These studies suggest that interaction of MUC with DNA results in 4-5 products possibly formed by reaction of MUC with guanine residues. Supported by NIH grant ES02558 and EPA Assistance Agreement CR-815936.


A short-term, in vivo mutation assay utilizing transgenic mice containing a Lambda Zap™ lacI shuttle vector has been developed. The shuttle vector contains a lacI target gene and a lacZ reporter gene. Mutations in the lacI target gene inactivate the repressor activity allowing expression of the lacZ gene. The B-galactosidase protein expression results in blue plaques when plated on indicator agar plates containing X-gal, while non-mutant plaques remain colorless. To perform the mutation assay, transgenic mice were treated with suspect mutants by a single or five daily IP injections. Tissues were collected three days after the last administration and genomic DNA was extracted. The transgene was recovered by exposing the genomic DNA to in vitro packaging extracts. The resultant viable lambda phage particles containing the shuttle vector were plated onto an E. coli bacterial lawn and allowed to form plaques. Treatment of transgenic mice with the known mutagens N-ethyl-N-nitrosourea, cyclophosphamide, and benz(a)pyrene resulted in a dose dependent increase in mutant plaques over control animals in several tissues, including a purified population of germinal cells. The mutated target gene can be easily excised from the lambda phage into a plasmid through the use of partial filamentous phage origins. Since the lacI target is only 1.1Kb in size, it can be characterized by sequencing using a series of strategically spaced oligo primers. A number of mutant clones have been sequenced to determine mutational specificity. This assay system should be valuable as a short-term method of detecting mutations in vivo. (Supported by NIH Contract 101-ES-95252).
CONFIRMATION OF DNA INTERCALATION BY CGS 20928A USING AN IN VITRO DNA UNWINDING ASSAY W B Matthes, J C Kappehhain', E R Lasinski', S D O'Lein', E C Furt' and D W Matheson. CIBA-GEIGY, Farmington, CT. 'CIBA-GEIGY, Summit, NJ.

A compound's mutagenicity in different Salmonella tester strains can be indicative of its mechanism of reaction with DNA. However, confirmation of that mechanism through in vitro studies, particularly with an assay that can yield binding constants, can be crucial in establishing structure-activity relationships. Previously it was found that CGS 20928A, an adenine antagonist, produced an increase in revertants only for Salmonella tester strain TA1537, and only without metabolic activation. These data indicated that the compound was a direct acting frameshift mutagen, and possibly intercalated into DNA. Using a rapid, in vitro test for DNA unwinding we have confirmed that at concentrations of >0.5 mM CGS 20928A behaves like known intercalating compounds in that it unwinds DNA. These concentrations of compound are comparable to those found mutagenic in Salmonella tester strains, and known intercalating compounds such as 9-aminacridine and ICR-191 unwind DNA in this assay in a dose dependent fashion in the 5-15 \( \mu \text{M} \) range. A structurally related compound, which was not mutagenic in Salmonella tester strains, did not produce any DNA unwinding even at 10 mM. Because the assay uses microgram quantities of material it is ideal for screening congeneric series suspected of frameshift mutagenicity.

DNA FRAGMENTATION PRECEDES DIMETHYL-NITROSAMINE CYTOTOXICITY IN CULTURED MOUSE HEPATOCYTES. LM Kamendulis, W Shen, SD Ray, and GB Corcoran. Toxicology Program, University of New Mexico College of Pharmacy, Albuquerque, NM.

Chronic dimethyl nitrosamine (DMN) exposure produces hepatic tumors through recurrent DNA alkylation, whereas acute exposure can cause florid liver necrosis through mechanisms that are unknown. We recently found early DNA damage during DMN-induced necrosis (Corcoran et al., SOT 1991). A challenge to interpreting these in vivo results is that up to 30% of liver cells are non-parenchymal. In the present study, we have examined whether DMN induces genomic DNA damage and cytotoxicity in a homogeneous population of hepatocytes. Cells isolated from male ICR mice were cultured in Williams' E media containing 0-25 mM DMN. Cytotoxicity was determined from release of lactate dehydrogenase or adenine nucleotides from cells pre-labeled with \(^3\)H-adenine. DNA damage was assessed from % fragmentation following sedimentation, and from agarose gel electrophoresis. DMN produced a 2-fold increase in LDH and \(^3\)H-adenine release after 10-25 mM for 36 hr, whereas increased DNA fragmentation appeared by 2 hr (187% of control). Addition of aurintricarbonylic acid, a C'-endo nucleoside inhibitor, prevented DNA fragmentation and virtually abolished cytotoxicity. Because DNA fragmentation preceded but paralleled acute cell death in vitro, our results suggest that similar DNA changes may contribute to DMN-induced hepatocellular necrosis in vivo. (Supported in part by NIH GM41564)

RAPID IN VITRO ASSAY FOR DETECTING ALKYLATION AT GUANINE-NT SITES IN DNA W C Gunther and W B Matthes. CIBA-GEIGY, Farmington, CT.

Compounds can be mutagenic in short-term tests by a variety of mechanisms. We have developed an assay that detects chemically induced piperidine-labile sites in DNA. Such sites include alkylation at the N7 position of guanine as well as abasic sites. These sites are converted to DNA strand breaks by treatment with aqueous piperidine. DNA is denatured, and the terminal of the single strands are dephosphorylated with alkaline phosphatase, then labeled with T4 polynucleotide kinase. Incorporation of label into acid-precipitable material (DNA) is proportional to the production of strand breaks and hence to the dose of the DNA damaging chemical. When supercoiled pBR322 DNA is treated with the alkylating agent dimethyl sulfate (DMS), a linear incorporation of label is seen as the DMS concentration is increased from 0.1 to 2.5 mM. Because the assay does not require radioactive compound, and uses microgram quantities of material it is ideal for screening congeneric series suspected of DNA alkylating activity.

EFFECTS OF ANTIOXIDANTS ON MUTAGEN FORMATION DURING FRYING OF GROUND BEEF C Chen, A M Pearson and J I Gray. Center for Environmental Toxicology and Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI. Sponsor: W N Choy

2-Amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-Amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ), 2-Amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQX) and 2-Amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-DiMeIQx) are potent bacterial mutagens. They are found in beef extract and fried beef (Felton et al., 1984). The effects that BHA, BHT, PG and Tenox 4 have on mutagen formation during frying of ground beef were investigated. Ground beef patties (0.5 cm in thickness, 100g) were fried in a frying pan set at 215°C for 9 min. per side. The fried patties were extracted using modifications of the methods of Pariza et al. (1979) and Felton et al. (1981). The antioxidants were added to the ground beef before frying. Results demonstrated that the basic fraction of fried ground beef was mutagenic toward Salmonella TAA9 or TA 100 + S-9. BHA, PG and Tenox 4 decreased mutagenicity by inhibiting formation of IQ, MeIQ and 4,8-DiMeIQX. However, BHT enhanced mutagenicity and increased the amount of 4,8-DiMeIQX by 4-fold.

Our studies in short-term tracheal explants have shown significant species differences in metabolic activation and susceptibility to the effects of aflatoxin B1 (AFB1). In the present study, we examined the in vivo formation of AFB1-DNA adducts and ultrastructural effects of a single intratracheal dose of AFB1 (1.5 mg/kg; 43 ml) in Syrian hamster and Fischer 344 rats. Bromodeoxyuridine was also given 1 hr prior to sampling to examine cell division rates. Various tissues were collected 1, 4, 12, 24 and 48 hr post injection. AFB1-DNA binding was higher in trachea, liver and kidney in the rat, but was similar for both species in lung. In both species, there was a decrease in AFB1-DNA adducts over time, with most removed by 48 hr. In rats, multifocal erosions of the bronchial epithelium occurred, due to an apparent non-selective epithelial cell necrosis and an increased rate of cell division. Airways and lungs of hamster showed no evidence of any proliferative changes, although by 24 hr, many hypertrophy of the PC cells occurred due to hypertrophy of the smooth endoplastic reticulum. These results indicate significant differences with respect to airway activation and ultrastructural effects of AFB1, in these two species which are not quantitatively predictable based on airway morphology (Supported in part by ES 04812).

AROCOLOR 1254 ALTERS INTESTINAL ENZYME ACTIVITY AND BIOTRANSFORMATION OF 2,6-DINITROTOLUENE IN RATS. RW Chadwick, SE George, J Chang, MJ Kron, J Allison, Y Hayes, and E Crownover. USEPA, HERL, RTP, NC; UNC, Chapel Hill, NC; EHRRT, RTP, NC.

Pretreatment of rats with pentachlorophenol potentiates the genotoxicity of the industrial toxicant, 2,6-dinitrotoluene (DNT). To determine whether or not Arococolor 1254, alters the genotoxicity of DNT, male Fischer 344 rats were dosed daily by gavage with 25 mg/kg Arococolor 1254 for 4 weeks. At 1, 2, and 4 weeks select rats were dosed by gavage with 75 mg/kg DNT, were transferred to metabolism cages, and urine was collected for 24 hours. The urine was tested for mutagenicity. Intestinal Nitroreductase (NR), azo reductase (AR), dechlorinase (DC), dehydrochlorinase (DH) and β-glucuronidase (BG) activity was determined. A significant increase in urine mutagenicity was observed at all times in rats treated with Arococolor 1254 and DNT when compared to rats dosed with DNT only. After 2 weeks, Arococolor 1254 significantly reduced NR, AR and DC activity in the small intestine as well as azo reductase in the cecum. After 4 weeks, Arococolor 1254 significantly increased β-glucuronidase activity in the small intestine and cecum and reduced dechlorinase activity in the cecum. Arococolor 1254 is more potent than pentachlorophenol in potentiating the genotoxicity of DNT. Abstract does not reflect EPA policy.

TEMPERATURE MODULATED HEPATIC DISPOSITION AND DNA ADDUCTION OF AFLATOXIN B1 (AFB1) IN RAINBOW TROUT. L R Curtis, Q Zhang, D E Brock and K Suorsa-Supper, Oak Creek Laboratory of Biology, Oregon State University, Corvallis, OR.

Temperature influenced chemically-induced tumor incidence in fish after immersion exposures (NCI Monogr. 65:129-137; 337-344). The present study investigated whether this was explained by carcinogen disposition to a target organ (liver) or bioactivation and subsequent genotoxicity of given target organ doses. Rainbow trout fry (2-5 g) and juveniles (130-250 g) were acclimated to 10 or 18°C for 4 weeks. Fry were immersed in 0.07-0.12 ppm [3H]AFB1 for 30 min at their respective acclimation temperatures or 14°C (acute temperature shifts). Immediately after immersion in 0.1 ppm [3H]AFB1, livers of 18°C acclimated and exposed fish contained 50% more genotoxin than 10°C fish, while liver concentrations of 10 and 18°C acclimated fish exposed at 14°C were similar. At all temperatures, at this time, about 75% of the total [3H]AFB1 in liver was identified as parent compound by HPLC. [3H]AFB1 DNA adduction was up to 2.5-fold higher in 10°C fish shifted to 14°C than 18°C acclimated fish shifted to 14°C. At 16 hr after injection of juvenile fish with 0.4 μg [3H]AFB1/kg there were no temperature effects on liver genotoxin concentration. Acute temperature shift from 18 to 14°C significantly reduced DNA adduction. Biochemical adaptations associated with temperature acclimation of fish can be exploited to modulate genotoxicity (Supported by ES03850 and ES04766).

EFFECT OF DNA STRUCTURE MODIFYING AGENT HOECHST 33342 ON DNA REPAIR AND MUTATION RATE. C Shi and P C Keng. Department of Biophysics and Cancer Center, University of Rochester School of Medicine, Rochester, NY. Sponsor: V C Lattes.

It has been previously demonstrated that Hoechst 33342, an A-T region specific DNA binding agent, is capable of relaxing DNA supercoiling structure. Hoechst 33342 is employed in this study to investigate the effect of DNA structural modification on mutation rate as well as the repair of DNA strand breaks. Ionizing radiation (6 Gy) was used to induce DNA strand breakage and mutation in cultured Chinese Hamster Ovary cells pretreated with 50 μM Hoechst 33342. The single- and double-stranded DNA breaks and their repair kinetics were measured using the alkaline and neutral elution techniques, respectively. Mutation frequencies were determined by the HPRT gene mutation assay. Our results showed that most single- and double strand breaks were repaired over a period of 60 min in both treated and control cells. The repair rate of single-strand breaks was not altered in treated cells. However, the initial repair rate of double-strand breaks was increased significantly in Hoechst 33342-pretreated cells, with the half-times being 4.2 min in treated cells and 10.5 min in control cells. Further, it was shown that the mutation frequency of the HPRT locus was reduced in Hoechst-treated cells by more than 50%. These evidence suggest that the relaxation of DNA supercoiled structure may not only facilitate the repair of DNA double-strand breaks, but also increase the overall fidelity of the repair, which, among other factors, decreases the mutation frequency at the HPRT gene locus. (Supported by NIH CA44723 and CA11051)
970 CYTOGENETIC EFFECTS OF NETOBINIM AND ALBENDAZOLE IN MOUSE MICRONUCLEUS AND CHINESE HAMSTER Ovary (CHO) CELL ASSAY. R S Lake, C B McCullough, E J Mirro and H E Black. Schering-Plough Research, Lafayette, NJ.

Netobimin (NB) is a guanidine carbamate produe of the antihelmintic benzimidazole Albendazole (ABZ). Both compounds were investigated in mouse bone marrow micronucleus and in vitro CHO chromosome aberration assays. Male and female mice were orally dosed with NB (5.7 g/kg) and ABZ (3.2 g/kg) along with appropriate vehicle and positive controls. Bone marrow was sampled at 24, 48 and/or 72 hours after dosing. The proportion of micronucleated erythrocytes was elevated over controls in mice sampled at 24 hours with both NB and ABZ. NB at dose levels of 200, 1000 and 2000 


The LS178Y mouse lymphoma (tk<sup>−</sup> to tk<sup>+</sup>) assay has features suggesting that it detects a range of genetic alterations. To study the type of events detected, trifluorothymidine-resistant cells were isolated after treatment with either ethyl methanesulphonate (EMS) or mitomycin C (MMC) and the DNA analysed by Southern blotting. As described by others, two types of mutant colony (large and small) were distinguishable after mutagen treatment. EMS induced both types of colony in approximately equal numbers while MMC induced mainly small colony mutants. No alterations in restriction fragment patterns were seen for most enzymes tested with either colony type. However for the enzyme NcoI a minority of EMS-induced mutants and the majority of MMC-induced mutants had lost a 6.3kb fragment. Loss of this fragment was associated more frequently with small-colony mutants and appears to represent deletion of the entire tk<sup>−</sup> allele. Spontaneous mutants also frequently lost the 6.3kb NcoI band. Mutants showing no fragment changes with any enzyme tested are presumed to be due to point mutations. These results agree with those of Clive et al (Mutagenesis 5, 191-197, 1990) and suggest that mutations may arise by a variety of mechanisms in this system. (Supported by UK Ministry of Agriculture, Fisheries and Food)

972 SPECIES DIFFERENCES IN THE COVALENT BINDING OF TRICHLOROETHYLENE (TRE) TO TISSUE PROTEINS. R J Eyre, D K Stevens and R J Bull. Pharmacology/Toxicology Program, Washington State University, Pullman, WA.

We are investigating the relative levels of covalent binding of TRE to hepatic (HP) vs. renal (RP) proteins in rats and mice as a measure of tissue specific generation of reactive intermediates that might be responsible for species specificity in carcinogenic responses. 3<sup>14</sup>C(UL)-TRE was administered p.o. to male Fischer 344 rats and male B6C3F1 mice at 20, 45, or 100 mg/kg. At intervals over a 5 day period HP and RP were isolated and covalent association of radiolabel determined. Association of radiolabel with proteins is complicated by significant metabolic incorporation of label as glycine. After correction for glycine incorporation, disappearance of radiolabel in HP was approximately first order. Peak association of radiolabel with HP was equivalent in the rats and mice. Radiolabel associated with RP was about twice greater in rats than in mice. Association of radiolabel with HP and RP was linear up to 45 mg/kg but was less than linear at 100 mg/kg. We conclude that the pathways responsible for generating reactive metabolites in the rat and mouse liver and kidney are saturated at low doses of TRE. The species differences seen in association of radiolabel to RP suggest the generation of a metabolite(s) that is related to the renal carcinogenicity of TRE in male Fischer 344 rats. This may be due to species differences in renal metabolism and/or the activity of cysteine conjugate β-lyase. Binding to RP does not appear to be related to species differences in sensitivity to TRE in the liver. (Supported by EPA CR-815216)

973 IN VITRO EVIDENCE FOR 1,3-DICHLOROACETONE AS A REACTIVE INTERMEDIATE OF 1,2,3-TRICHLOROPROPANE. J G Sipes and G L Weber. Dept. Pharm/Tox, University of Arizona, Tucson, AZ.

1,2,3-Trichloropropene (TCP) is used as an intermediate in the manufacturing of pesticides and polysulfide rubbers. TCP has been reported to be tumorigenic by the National Toxicology Program and is mutagenic in the Ames test in the presence of rat liver S9 or microsomes. Identification of TCP microsomal metabolite(s) would help in understanding the role of metabolism in the bioactivation of TCP. TCP was incubated with hepatic microsomes, from phenobarbital pretreated F-344 rats, in the presence of NADPH and N-acetylcycteine (NAC). Reactive intermediates of TCP were trapped through conjugation with NAC and purified by preparative HPLC. The major product was identified as 1,3-(2-propanone)-bis-N-acetylcycteine (PDM) by co-chromatography, mass spectrometry and NMR comparisons with an authentic synthesized standard. The formation of PDM demonstrates C2 oxidation of TCP by cytochromes P450 leading to the formation of 1,3-dichloroacetone (DCA). DCA is subsequently conjugated with NAC forming PDM. Since the formation of PDM accounted for nearly all of the TCP microsomal metabolism and the fact that DCA is a known direct acting mutagen, we suggest that DCA is the mutagenic metabolite formed from TCP by rat hepatic microsomes. Supported by NTP ES-85230.
MECHANISMS OF ISOTHIAZOLINONES BINDING TO PROTEIN. D. W. Potter and Y. G. Whittle. Rohm and Haas Co., Spring House, PA. Sponsor: C. B. Frederick

Although 2-methyl-4-isothiazolin-3-one (MI) and 5-chloro-2-methyl-4-isothiazolin-3-one (CMI) are effective biocides, high concentrations (in excess of typical use levels) may cause allergic contact hypersensitivity. In vitro experiments have been performed to better understand the mechanisms of protein binding. [14C]MI and [14C]CMI were reacted with protein and binding was quantified by the amount of radioactivity associated with protein after exhaustive dialysis. Protein adduct formation was also evaluated by HPLC after protein hydrolysis with pepsin and pronase. Neither MI nor CMI were activated to protein binding intermediates during microsomal incubations. Glutathione (GSH) activated CMI at a ratio less than 2 mol GSH/mol CMI, while higher concentrations of GSH decreased protein binding. In contrast, MI was not activated by GSH. However, both MI and CMI were shown to bind to thiol-rich proteins such as metallothionein without appreciable binding to casein, BSA, which lacks accessible thiols. HPLC analyses indicate that CMI reacts with multiple protein binding sites while MI binding is limited. These data suggest that CMI and MI bind to protein by different mechanisms. Whereas CMI appears to be activated by thiols to intermediates that bind to protein, MI appears to bind directly, but at low levels, to protein thiols.

POSSIBLE CELLULAR TARGET(S) FOR 2-BROMOHYDROQUINONE NEPHROTOXICITY. M. L. Rivera, T. L. Monks, and S. S. Lan. Div. of Pharmacol/Toxicol., College of Pharmacy, The Univ. of Texas at Austin, TX.

We have previously shown that administration of 2-bromo hydroquinone (2-BrHQ; 0.8 mmol/kg) to male Sprague Dawley rats causes extensive coagulative necrosis of renal tubular cells. To determine possible subcellular targets for 2-BrHQ in the kidney, we have now investigated the effects of 2-BrHQ (0.8 mmol/kg) on enzymes associated with specific subcellular organelles. The activity of γ-glutamyl transpeptidase (γ-GT), NADPH cytochrome c-reductase, succinate and lactate dehydrogenases were used to determine the effects of 2-BrHQ on the plasma membrane, endoplasmic reticulum, mitochondria, and cytosol, respectively. All enzyme activity remained unchanged 12 hr after 2-BrHQ administration. At 24 hr, only the activity of γ-GT was significantly decreased (3.63 ± 0.065 units/mg protein in treated vs. 6.01 ± 0.28 units/mg protein in control rats). The decrease in γ-GT activity corresponds to the previously observed increase in the urinary excretion of γ-GT following 2-BrHQ intoxication. The effects of 2-BrHQ on renal mitochondrial function, in vivo, was also determined. There was no difference in either state 3 or state 4 respiration 12 and 24 hr after 2-BrHQ administration between treated and control rats. The lack of effect on mitochondrial function at 24 hr is surprising since at this time blood urea nitrogen levels are significantly elevated (57.6 ± 13.4 vs. 10.8 ± 1.9 mg/100ml in controls). Although the data suggest that the plasma membrane might be a possible target of 2-BrHQ, the primary subcellular target(s) responsible for 2-BrHQ-induced nephrotoxicity remains to be determined. (GM13270, GM39338, ES04662).


Four different methylated thiocochelates have been previously reported as urinary metabolites of bromobenzene (BB) by the guinea pig. Their GC/MS properties of 3- and 5-isomers derived indicates that the thiol and the hydroxyl groups are adjacent on BB ring, and they were believed to be four different isomers. A new isomer (S-methylated)thiocochelates. Recently, we reported that they were also derived from metabolites of the 3- and 4-S- and 3,4,5-trisubstituted acids (Pher acids) of BB by the isolated guinea pig kidney-S. Detection of four different (S-methylated)thiocochelates, instead of two, from the 3- and 4-S- Pher acids experiment prompted us to re-examine the structure of these metabolites. In the presence study, the investigation was carried out by using urinary extract of neutral and phenolic metabolites of BB by the guinea pig. A different derivative, methyl ester-trimethylsilyl ether, was made. Analytical GC and MS indicated that the four isomeric methylated thiocochelates were converted to two major products. This result indicated that the four methylated thiocochelates are not the 2,3-isomeric (S-methylated)thiocochelates but they are the 5-methyl ether and 5- methyl ether of the 3,4-bromothiocochelates which have the following structures:

![Structure](https://example.com/structure.png)

From these evidences, it shows that thiochelates and/or thioquinones do exist in the wholebody as metabolites, and not only as metabolic intermediates. Thiochelates are now known to be formed in the kidney, an information may be important for kidney target organ toxicity by BB under high dosage condition. (Supported by ES04857)

EVIDENCE FOR A ROLE OF PROSTAGLANDIN H SYNTHASE (PHS) IN CYCLOPHOSPHAMIDE (CP) TOXICITY IN MICE. L. Frazier and J. F. Kohner. Division of Pharmacology & Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX.

CP is an antineoplastic agent commonly used in multi-drug regimens for the treatment of various cancers and as an immunosuppressant in organ transplants. CP chemotherapy has been associated with considerable organ-specific toxicity including lung injury and hemorrhagic cystitis. It is commonly accepted that CP requires metabolic activation for both its therapeutic and toxic effects. Oxidation mediated by mixed-function oxidase (MFO) enzymes has been believed to be the sole pathway for the metabolic activation of cyclophosphamide. In vivo studies performed previously in this lab showed that pretreatment of mice with aspirin, indomethacin or nordihydroguaiaretic acid (NDGA), inhibitors of PHS, significantly reduced cell proliferation and fibrosis after CP-induced lung injury. In this study, the interactions of CP with PHS and MFO enzymes in mouse lung and liver were investigated by measuring initial oxygen consumption rates. The addition of CP to lung microsomes resulted in both a NADPH- and an arachidonic acid-dependent increase in oxygen consumption. These increases were inhibited by piperonyl butoxide or indomethacin and NDGA, respectively. It has been suggested that the reactive metabolite(s) responsible for CP-induced bladder toxicity may be generated to some extent by the kidney. NADPH- and arachidonic acid-dependent bioactivation of CP were compared using covalent binding of [ring-4,14C]CP to kidney microsomal preparations. Preliminary studies indicated that this reaction was derived from whole kidney were incapable of metabolizing CP to a reactive metabolite labeled at the ring-4 position (probably acrolein) in the presence of either NADPH or arachidonic acid. However, in vivo studies indicated that CP-induced hemorrhagic cystitis could be significantly reduced, as indicated by bladder wet and dry weights, when mice were pretreated with aspirin. These results suggest that PHS may serve as an alternate bioactivation pathway for CP. (Supported by NIH grant HL35869.)
FORMATION AND REACTIVITY OF QUINONE METHIDES DERIVED FROM ALKYL PHENOLS.
J. L. Bolton and L. A. Thompson. Molecular Toxicology and Environmental Health Sciences Program, University of Colorado, Boulder, CO.

Oxidation of alkyl phenols to 1,4-quinone methides (QM's) by cytochrome P-450 may be an important metabolic pathway contributing to various toxicities reported for the parent compounds. BHT (2,6-di-tert-butyl-4-methylphenol) for example, is believed to be activated to a mouse lung toxin by such a process. Little is known, however, about relationships between structures of alkyl phenols and rates of P-450 catalyzed QM formation, and about the effects of QM structure on electrophilic reactivity. A series of QM's were synthesized by chemical oxidation of variously substituted phenols. The addition of glutathione to each QM produced conjugates which were characterized by 1H-NMR and titromoscap LC-MS analysis, and HPLC assays were developed to quanitate enzymatic formation of QM's as GSH adducts. With liver microsomal P-450, the formation rates were dependent on the extent of alkyl substitution, with the more highly substituted phenols oxidized most rapidly. 2,6-di-tert-butyl substituents caused a 65-fold increase in rate relative to that measured with the unsubstituted analog. Replacing the 4-methyl with an ethyl substituent had little affect on rates. Kinetics of QM reactivities with nucleophiles were investigated also. Bulky substituents resulted in low-r reactivity; the 2,6-di-methyl QM reacted 120-fold faster with water than 2,6-di-tert-butyl QM. Substituents on the exocyclic methylene group decreased electrophilic reactivity. The formation and reactivities of QM's, therefore, are affected differently by alkyl substitution, and the role of QM's in phenol toxicity depends on the balance of these properties. (Supported by NIH Grant CA33497).


After hepatotoxic doses of APAP, arylated proteins of 44 and 56 kDa were the major protein targets detected by immunochemical analysis. This study was conduct- ed to characterize the 44 kDa protein (P44), the earliest detectable target. When S DS-PAGE separated liver proteins from APAP dosed mice (600mg/kg, po) were subjected to Western blot analysis with affinity purified a-APAP antibody, arylated P44 was detected in the microsomes by 30 min, and in the cytosol by 1 hr. Salt and detergent extraction studies indicate that P44 is a peripheral membrane protein associated with the ER. Cytosolic and microsomal P44 are part of a larger protein complex of about 350 kD and consist of 2 isoforms with P of 7.0 and 7.1 on 2-D gels. Upon incubation of control liver fractions with APAP electrophile, NAPQI, or upon APAP activation by S-9, the arylated P44 complex appeared to be of microsomal origin with some released into the cytosol. Thus, APAP arylated complex altered the subcellular distribution of P44. (N.I.H. GM31460 and ES07163).

ARACHIDONIC ACID-DEPENDENT METABOLIC ACTIVATION OF CYCLOPHOSPHAMIDE. S. Kaneko and J. P. Keen. Division of Pharmacology & Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX.

Chemotherapy with cyclophosphamide (CP), a drug extensively used for immunosuppression and the treatment of cancer, is complicated by the development of acute and chronic lung injury. Although CP requires metabolic activation to exert therapeutic and toxic effects, the relative roles of mixed function oxidase (MFO) and prostaglandin H synthase (PHS) systems in the biotransformation of CP are unclear. Our previous work showed that inhibitors of PHS, but not MFOs, can prevent CP-induced lung injury in mice. Studies examining covalent binding of ring-4-14C-CP in pulmonary and hepatic microsomes isolated from mouse strains susceptible (ICR) and resistant (C57) to CP-induced lung fibrosis were performed to elucidate the role of the PHS pathway in CP bioactivation. NADPH-catalyzed covalent binding of ring-4-14C-CP (metabolites probably acrolein) was significantly higher in C57 hepatic and pulmonary microsomes compared to ICR's (C57 liver: 3.46; ICR liver: 1.91; C57 lung: 0.49; ICR lung: 0.37 n mole/mg protein/20 min). In both strains, NADPH-mediated binding to liver microsomal proteins was greater than to lung. In contrast, arachidonic acid-catalyzed covalent binding was higher in pulmonary than hepatic microsomes in both strains (C57 lung: 0.22; C57 liver: 0.11; ICR lung: 0.25; ICR liver: 0.07 n mole/mg protein/15 min). Although binding was time-dependent, arachidonate-mediated binding was maximal by 5 min and NADPH-catalyzed binding was linear up to 30 min. Piperonyl butoxide inhibited 90% of NADPH- and indomethacin inhibited 50% of arachidonate-mediated microsomal binding. These data demonstrate that CP can be metabolically activated by an arachidonate-dependent pathway in the lung. The self inactivation of PHS with time makes quantitative comparisons to NADPH-mediated metabolism difficult. However, it would appear that lung metabolism is significant and that strain differences in susceptibility to CP-induced lung fibrosis may be due to greater hepatic metabolism in resistant animals. (Supported by NIH grant HL35689).

Previous studies with piperonyl butoxide suggested that late production of electrophile was important in mediating APAP hepatotoxicity. To further test this the effect of delayed, irreversible P450 inhibition by CLTZ on APAP hepatotoxicity was studied. CLTZ (75mg/kg in 30% PEG 400) was given, po., to fasted, 3-4 month old male CD-1 mice either 1 hr before, or 2, 4 or 6 hr after APAP. APAP (600mg/kg) was administered, ip, in 50% propylene glycol, and mice were killed 12 hr later for collection of blood and liver. In control mice given APAP, plasma SDH was elevated to 3885 ± 620 U/ml (Control mean ± SE = 16 ± 11 U/ml). One hour pretreatment with CLTZ completely prevented liver damage, while both 2 and 4 hr post-treatments with CLTZ resulted in partial protection with the SDH increase being blocked by 75% and 50%, respectively. By contrast the 6 hr post treatment resulted in no apparent protection. The SDH data were confirmed by histopathology. These results indicate that the formation of the reactive metabolite continues for at least 4 hr after the administration of APAP and that the late production of electrophile is important in hepatotoxicity. (Supported by NIH GM31460 and ES07163)


Male B6C3F1 mice (25-30 g) were administered 1% acetone (AC) in drinking water for 10 d, fasted (F) 48 h, or given streptozotocin 180 mg/kg in citrate buffer (pH 4.5) ip 12 d earlier to precipitate diabetes (D: mean serum glucose 285 mg/dl at death), to induce P450IIE1, or no pretreatment (C). An hepatotoxic dose of AP (500 mg/kg in saline) or saline was given at 9 am. Mice (3-4/group) were killed 6 hr later: clinical signs (AC moribund) >> F > C > D (normal); serum ALT, as multiples of no AP (x): C (300x) > AC (150x) > > F (20x) > D (2x); liver/body wt ratio, as multiples of no AP (x): AC/F (1.5x) > C (1.25x) > D (0.9x); GSH (μmol/g liver, % loss from no AP): AC/F (97%) > C (83%) >> D (21%); hepatic 3-(cystein-S-yl)protein adducts (3-Cys/A) by immunohistochemistry (area and intensity): AC/F > C > D; histopathology (extent and severity of necrosis): AC/F > C > D. Histology and 3-Cys-A suggest loss of 3-Cys-A into serum from centrilobular and fasciculus areas. Loss of tissue adducts increased with severity of necrosis (AC/F > C). The clinical signs of toxicity in AC mice were far greater than could be expected if relative GSH loss, hepatic 3-Cys-A or induction of P450IIE1 and suggests injury to hepatocytes by an additional mechanism.


Male B6C3F1 mice (25-30 g) were administered 1% acetone (AC) in drinking water for 10 d or fasted (F) 48 h to induce cyt. P450IIE1, or no pretreatment (C). Induction of P450IIE1 by AC and F relative to C was confirmed by immunohistochemistry. A non-hepatotoxic dose of AP, 300 mg/kg ip in saline, or saline only, was given at 9 am. Mice (3-4/group) were killed 2 hr later with the following results: serum ALT, as a multiple of no AP (x): AC (150x) >> F (20x) > C (3x); GSH (μmol/g liver, % loss from no AP): AC/F = C (75-77%); hepatic 3-(cystein-S-yl)protein adducts (3-Cys-A) by immunohistochemistry (area and intensity): F > AC > C; histopathology (extent and severity): AC/F >> C. In C mice, the minor histological changes and 3-Cys-A were centrolobular (CL). In AC and F mice, early necrotic changes and 3-Cys-A were present CL, but 3-Cys-A was most intense mizondally, at the leading edge of necrosis. Pretreatment with AC and F, which induce P450IIE1, potentiated AP hepatotoxicity. At 2 hr, an increase in ALT and in necrosis, but not tissue 3-Cys-A or GSH loss correlated with severity of hepatotoxicity. 3-Cys-A may be lost from necrotic tissue. Since AC and F exhibited greater necrosis than C, greater loss of 3-Cys-A in AC and F than in C would account for the lack of correlation between tissue 3-Cys-A and histopathology.


Because of the insolvability of acetaminophen (APAP), various solvents including propylene glycol (PG) have been used to solubilize the drug in animal models of APAP toxicity. To investigate the possibility that this vehicle alters the hepatotoxicity of APAP, B6C3F1 male mice (5 per group) were fasted 15 hours, treated orally with either PG (50% in water, 0.01 ml/g body weight) or water, and dosed by the ip route with APAP in saline. PG treatment abrogated or diminished APAP toxicity at 4 hours (lower liver/body weight ratios, conservation of hepatic GSH, lower serum ALT, and decreased hepatic 3-(cystein-S-yl)APAP protein adducts (3-Cys-A)). In vitro, PG competitively inhibited (P450IIE1-dependent) 4-nitrophenol hydroxylase activity in mouse hepatic microsomes.

APAP Oral Liver/Body GSH ALT 3-Cys-A (mg/kg) tmt. (μmol/g) ALT 3-Cys-A

<table>
<thead>
<tr>
<th>Tmt.</th>
<th>(x100)</th>
<th>(μmol/g)</th>
<th>IU</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 water</td>
<td>4.04</td>
<td>2.00</td>
<td>38</td>
</tr>
<tr>
<td>PG</td>
<td>4.16</td>
<td>2.06</td>
<td>34</td>
</tr>
<tr>
<td>200 water</td>
<td>5.85</td>
<td>0.60</td>
<td>8668</td>
</tr>
<tr>
<td>PG</td>
<td>4.00</td>
<td>1.26</td>
<td>34</td>
</tr>
<tr>
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<td>0.35</td>
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</tr>
<tr>
<td>PG</td>
<td>4.33</td>
<td>0.45</td>
<td>1057</td>
</tr>
</tbody>
</table>

The data demonstrate that PG is a competitive inhibitor of APAP metabolism which modifies toxicity.
The metabolism of 2-hydroxyacetanilide (2HA), a non-hepato-
toxic isomer of acetaminophen (4HAH) was studied in rats, mice, and
hamsters. Administration of 2H-(aceetyl)-2HAH (20mg/kg) to
animals pretreated with BNPP led to greater than 85% recovery of
the administered radioactivity as urinary metabolites. Reverse
phase HPLC analysis indicated that the major urinary metabolites
included glucuronide (~ 85%) and sulfate (~ 8%) conjugates of
the parent compound while unchanged 2HAH represented less than
2% of the dose. Although 2HAH depleted hepatic glutathione,
mercaptopuric acids of 2H-2HAH could not be detected in the urine,
even when the urine was spiked with non-radioactive 2HAH-
mercaptopurcates standards. Pretreatment with 3-MC, known to
potentiate 4HAA-hepatotoxicity and to increase urinary 4HAA-
mercaptopurcates, also failed to give rise to urinary 2HAH-
mercaptopurcates. Three additional minor metabolites, representing
5-7% of the dose, yielded phenolic upon hydrolysis with gl-
ucuronidase and arylsulfatase. These phenolic metabolites were
identified as 2,4- and 2,5-dihydroxyacetanilide by GCMS
and proton NMR using synthetic 2,4- and 2,5-dihydroxyacetanilide
standards. Like 4HAH, 2HAH is known to bind covalently to
liver proteins in vivo. In order to characterize the nature of
2HAH covalent binding, livers of 2H-2HAH-treated hamsters
were homogenized, washed, and subjected to protease digestion
to liberate free amino acids. After subsequent purification by
reverse phase HPLC, the 2H-2HAH adducts were derivatized and
identified as 2HAH-cysteinyl adducts by GCMS. Collectively, the
data indicate that 2HAH is metabolized in vivo to a chemically
reactive metabolite(s) that binds covalently to cysteinyl groups
of proteins yet does not bind to the cysteinyl group of glutathione.

PURIFICATION, PARTIAL PEPTIDE SEQUENCING AND
GENETIC CLONING OF THE 58 KD ACETAMINOPHEN
(APAP) BINDING PROTEIN. D A Rochefort, J B
Bartolone, R B Birge, S D Cohen and E A Khairallah
Toxicology Program, Deps. of Molc. & Cell Biol. and
Pharmacol. & Toxicol., Univ. of Connecticut, Storrs, CT.

APAP hepatotoxicity in mice and humans is associated
with selective aroylation of a cytosolic 58 Kd protein
(P58). To further characterize the P58, livers from
APAP-treated (600 mg/kg, po, 4hr) and control mice
were pooled and P58 purified by gel permeation
chromatography, preparative isoelectric focusing and
2-D PAGE. The protein was found to be a cytosolic
monomer, consisting of 4 isoforms with F1 from 6.2 to
6.5. Amino acid analysis revealed a low cysteine
content (1.4%), making it unlikely that the protein is a
major site for sequestering electrophilic. Peptide
fragments from P58 were generated by cyanogen bromide and trypsin treatment. Sequence analysis of
two of these fragments (9 and 19 amino acids) did not
show homology with known proteins. Two degenerate
oligonucleotides were made from the amino acid
sequence of these fragments and used to probe a mouse cDNA library. Two clones hybridizing to the
32P-end labelled oligonucleotides were identified and
will permit identification of the P58 gene. (Supported
by the Center for Biochemical Toxicology and NIH
GM31480 and ES07163)

TCDD is a potent hepatocarcinogen in female rats but not male rats in chronic carcinogen bioassays. Our studies have investigated this finding by evaluating histological and biochemical parameters in a two-stage model for hepatocarcinogenesis in female Sprague-Dawley rats (intact and ovariectomized) using diethylaminoethanol (DEN) as the initiating agent and TCDD as the promoting agent. Increases in gamma glutamyl transpeptidase (GGT) positive foci were greater in intact female rats than in ovariectomized (OVX) animals. For example, in intact rats receiving both DEN and TCDD the percentage of liver occupied by GGT positive foci was 0.57 compared to 0.08 in OVX rats. Cell proliferation data, obtained using BRDU in osmotic mini pumps, were consistent with preneoplastic foci data in that the hepatocysta labeling index was increased in DEN/TCDD intact rats but not in DEN/TCDD OVX rats. Analysis of data from individual animals revealed a strong correlation (P<0.01) between cell proliferation and GGT foci/cm² in liver. Hepatic cytochrome P-450d (IA2) was induced approximately 6-8-fold in all TCDD-treated groups and the magnitude of induction was not influenced by ovariectomy. This cytochrome efficiently catalyzes 17β-estradiol metabolism to catechol estrogens. We also observed that TCDD down-regulated the EGFR in intact rats but not OVX rats. This pathway is known to stimulate mitogenesis in liver. EGFR data is compared to effects on EGFR stimulated autophosphorylation. Our data suggest that ovarian hormones (probably estrogens) play a significant role in hepatocarcinogenic actions of TCDD and that indirect genotoxic events as well as cell proliferative events may be involved.

DETERMINATION OF AH RECEPTOR SYNTHESIS AND TURNOVER IN HEPA I CELLS. H. I. Swanson and G. H. Perdew. Department of Foods and Nutrition, Purdue University, West Lafayette, IN.

The Ah receptor (AhR) mediates transcriptional activation of cytochrome P450AI by binding to an appropriate agonist, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin or β-naphthoflavone (βNF), followed by subsequent binding to DNA. Although many studies have focused on the AhR-DNA interaction, little work has been done to study the mechanisms of control of AhR levels. This study utilizes the density shift method to determine the turnover of the AhR in Hepa 1 cells during unliganded conditions and during ligand occupation. The turnover of the unliganded AhR was determined to be approximately six hours but decreases significantly during maximum occupation with the agonist, βNF. Agonist occupation also appears to facilitate AhR down regulation as determined by time course experiments. In Hepa 1 cells, a 12 hour exposure to βNF resulted in a 62% decrease of the original AhR level. In contrast, the same treatment with the partial agonist α-naphthoflavone (αNF) resulted in a 14% decrease. In αNF-treated cells, the AhR nuclear translocation mutant, a 12 hour exposure to βNF resulted in a 44% reduction of the original level, whereas exposure to αNF resulted in a 5% decrease. This would suggest that the control of AhR levels in Hepa 1 cells is dependent on two mechanisms; one which is dictated by the type of ligand bound to the AhR and the other which is independent of AhR translocation. (Supported by NIH ES04869.)


The comparative antiestrogenic effects of 6-methyl-1,3,8-trichlorodibenzo-furan (MCDF), 6-buty1,3,8-trichlorodibenzo-furan (trCDF) and 6-cyclohexyl-1,3,8-trCDF were determined in immature female Sprague-Dawley rats. Treatment of the animals with 17β-estradiol (5 μg/rat X 2) caused an increase in uterine cytosolic and nuclear estrogen and progesterone receptor levels, uterine wet weights and uterine epidermal growth factor (EGF) receptor binding activity and steady state EGF receptor mRNA levels. MCDF and 6-buty1,3,8-trICDF (100 μmol/kg) two compounds which exhibit moderate acute hydrocarbons (AB) receptor binding affinity decreased the constitutive and 17β-estradiol-induced responses noted above. In contrast, 6-cyclohexyl-1,3,8-trCDF, a congener which exhibits low Ah receptor binding, was inactive as an antiestrogen. These studies show that selected 6-alkyl-1,3,8-trCDFs elicit a broad spectrum of antiestrogenic activity in immature female rats. Moreover, in contrast to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) which also is a potent antiestrogen, the 6-alkyl-1,3,8-trCDFs are relatively nontoxic and can serve as prototypes for the future development of a new class of antiestrogens with potential for clinical applications. (Supported by N.I.H., ES04716.)
994 6-METHYL-1,3,8-TRICHLORODIBENZO[4,5-FURAN(MCDF) AS AN ANTIESTROGEN: EFFECTS ON NUCLEAR ESTROGEN RECEPTOR LEVELS, CELL GROWTH AND THE 17β-ESTRADIOL-INDUCED SECRETION OF PROTEINS IN MCF-7 CELLS. M. Harris, L. Biegel, T. Zacharewski and S. Safe. Dept. of Vet. Phys. & Pharm., Texas A&M Univ., College Station, TX.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) exhibits a broad spectrum of antiestrogenic effects in rodents and human breast cancer cell lines. MCDF is a relatively non-toxic analog of TCDD which is also being investigated as an antiestrogen with potential clinical use as an antitumorigenic drug. Treatment of MCF-7 cells with [3H]-17β-estradiol resulted in the rapid accumulation of nuclear estrogen receptor (ER) complex which is maximized within 1 hour and then rapidly decreased. Pretreatment of the cells with 100 nM MCDF 6 or 12 hours prior to the addition of [3H]-17β-estradiol resulted in a > 60% decrease in nuclear ER levels in these cells at all time points (1-3 hours). In a second series of experiments, the effects of MCDF or 17β-estradiol-induced growth and secretion of the 34, 52- and 160-kDa proteins was also determined in MCF-7 cells. MCDF (100 nM) significantly decreased the 17β-estradiol-induced growth of MCF-7 cells; in addition, MCDF caused a > 60% inhibition of the 17β-estradiol-induced secretion of the 34-, 52-, and 160-kDa proteins as determined by autoradiographic analysis of the 3H-labeled proteins which were separated by polyacrylamide gel electrophoresis. The results confirm the antiestrogenic activity of MCDF and suggests that this type of compound may be useful for further study as an antioestrogen agent. (Supported by N.I.H., ES04176).

996 EFFECTS OF PERINATAL EXPOSURE TO 2,3,7,8- TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON PRE- AND POSTNATAL PLASMA TESTOSTERONE (T) CONCENTRATIONS IN MALE RATS. T. A. Mably, R. W. Moore, and R. E. Peterson. School of Pharmacy, Univ. of Wash., Madison, WI.

Perinatal TCDD exposure masculinizes and feminizes male rats. Because T is required perinatally for sexual differentiation, effects of TCDD on perinatal plasma T concentrations and testicular T production were examined. Pregnant rats were given TCDD (1.0 μg/kg) or vehicle on gestation day (gd) 15. From gd 18-21, plasma T in control female fetuses was maintained at 0.5 ng/ml and was not affected by TCDD. Relative to control female fetuses, plasma T in control male fetuses was about 3-fold higher. TCDD exposure reduced this difference by 38%. Postnatally, control males exhibited a pronounced and transient increase in plasma T between 0 and 2 hr postpartum. In TCDD-exposed males the postnatal T surge did not peak until 4 hr postpartum and the magnitude of the increase was 50% of control. From 6 hr postpartum through Day 5, TCDD had little effect on plasma T. When fetal testes were incubated with the LH analog hCG, maximal T production was decreased 30-40% by TCDD on gd 18-21. Postnatal T production was decreased by TCDD in 1- and 5-day-old males by 45% and 38%, respectively. These results show that TCDD decreases perinatal plasma T in male rats, possibly by decreasing testicular responsiveness to LH. This perinatal decrease in plasma T may account for the masculinization and feminization in adult male rats caused by perinatal TCDD exposure. (NIH ES01332).


2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) decreases hepatic and uterine estrogen receptor (ER) levels in rats and mice. Because ER levels are under multihormonal control, we compared the effects of TCDD on hepatic and uterine estrogen receptor levels in intact, ovariectomized (Ovx) and hypophysectomized (Hpx) female CD-1 mice. Mice received either corn oil or 3, 10, 30 or 100 μg/kg of TCDD and were sacrificed 2 days later. Hepatic and uterine ER levels were determined using an equilibrium binding assay and hepatic cytosol. P-450 levels were determined by CO binding. Treatment with TCDD decreased hepatic and uterine ER levels to 50% of controls in intact mice. Ovx decreased hepatic ER levels and increased uterine ER levels compared to intact controls. TCDD decreased hepatic ER levels by 50% but did not affect uterine ER levels in Ovx mice. Hpx decreased hepatic and uterine ER levels to 40-50% of intact mice. Treatment of Hpx mice with TCDD did not alter hepatic or uterine ER levels. However, Hpx increased the lethality of TCDD compared to intact animals. Hepatic cytosol P-450 levels in intact mice were increased 3-5 fold by TCDD treatment. The increases in hepatic cytosol P-450 were not affected by either Ovx or Hpx at the doses tested. The results indicate that the endocrine status of the animals studied can alter the toxicity of TCDD and its ability to affect endocrine function. Supported by NIADDK grant C29510.


This laboratory has reported decreased serum testosterone and testicular P450α activities in TCDD-treated rats. This study investigated TCDD regulation of Leydig cell P450α, P450 and P450α,β activities (17α-hydroxylase and C17-20lyase) in vitro. Male Sprague-Dawley rats (200 - 240 g) were sacrificed, testes removed, and Leydig cells (LC) purified by Metrizamide gradient centrifugation. 2.5 X 10⁶ cells were cultured at 32°C (93% air/7% CO₂) in the presence of TCDD (10⁻⁷, 10⁻⁸ 10⁻⁹ for 10⁻14M) for up to 5 days. Media were collected for T determination and LC enzyme activities determined. T was decreased to 73 and 78% of control by 10⁻⁷ and 10⁻⁶ M TCDD, respectively at day 2. All doses of TCDD decreased T at days 3, 4 and 5 of culture. LC P450α,β activity was unaffected by TCDD at any dose during the 5 day period. 17α-hydroxylase activity was decreased at day 1 by 10⁻⁷ or 10⁻⁶ M TCDD by 79 and 84% of control, respectively. All doses of TCDD decreased 17α-hydroxylase activity after 3 days and continued to decrease activity for the 5 day period. 10⁻⁷ and 10⁻⁸ M TCDD decreased C17-20 lyase activity after 1 day and 10⁻⁷, 10⁻⁸ 10⁻⁹ M and 10⁻⁶ M TCDD decreased C17-20 lyase activity after 3 days. At 5 days of exposure to TCDD all doses decreased C17-20 lyase activity. The results of this study suggest that the decreased serum T observed in vivo is due to decreased LC T production. The mechanism by which TCDD decreased T is due to decreased P450α,β activities (17α-hydroxylase and C17-20 lyase) and not the decreased P450α,β activity. (Supported in part by T32 ES-07062).
OXIDATIVE STRESS INDUCED BY TCDD IS MEDIATED BY THE Ah-RECEPTOR COMPLEX. N.Z. Alsharif and S J Stohs. Creighton University Health Sciences Center, Omaha, NE.

The toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and its bioisosteres involves binding to a specific TCDD (Ah) receptor, interaction of this complex with chromatin, and the ultimate production of a pleiotropic response. The mechanism whereby these effects are produced following interaction of TCDD with the receptor complex is not known. Oxidative stress and the Ah-receptor complex may play important roles in expression of the toxic manifestations of TCDD. Thus, the dose and time dependent effects of TCDD on the production of superoxide anion by peritoneal exudate cells (primarily macrophage) from responsive C57BL/6J [bb] and non-responsive C57BL/6J [dd] mice were examined. One day after the administration of 5, 25, 50 or 125 μg TCDD/kg p.o. as a single dose, 1.4-, 1.7-, 4.3- and 3.5-fold increases, respectively, occurred in superoxide anion production by macrophage from the response [bb] strain. However, only 125 μg TCDD/kg produced a significant increase in superoxide anion formation with macrophage from the non-responsive [dd] strain (1.7-fold). These differences agree well with other indices of responsiveness to TCDD in the two strains of mice. No differences were observed with respect to dose and time in the composition of the peritoneal exudate cells. The results indicate that TCDD produces an oxidative stress in mice, and this effect is controlled in part by the Ah-receptor complex.


Male Sprague-Dawley rats (240-260 g) were gavaged with an LD50 of 2.3,7,8-TCDD, 2.3,4,7,8-PeCDD, and 2.3,4,6,7,8-HxCDD, respectively, in corn oil/acetone (95/5, v/v). Groups of 5 animals were sacrificed at two and eight days after dosing, livers removed and homogenized, 100,000 g supernatants prepared, and activities of phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate carboxylase (PC) determined spectrophotometrically. Results were compared to those obtained with ad libitum-fed controls. At two days after dosing PEPCK activity was reduced after all doses, but significant changes were observed after the highest dose only. At eight days after dosing PEPCK activity was dose-dependently reduced by each of the dioxin isomers. The reductions ranged from an average of 20% at the LD50 to an average of 45% at the LD50 of either dioxin isomer. There is a perfect correlation between the dose responses of acute toxicity, feed intake as well as body weight reduction, and inhibition of PEPCK activity after all three dioxin isomers. The activity of PC responded in a different fashion to the PCD-induced insult. Two days after dosing PC activity was reduced after either dose by about 20%, and dose response was suggestive. By eight days after dosing this enzyme's activity was reduced by about 55% after either dioxin isomer without an apparent dose response. The regulation of PC activity is not well understood and differs from the well-described regulation of PEPCK activity. The present results support this view by demonstrating differential PCD-induced responses. In addition, these data further strengthen the contention that polychlorinated aromatic hydrocarbons share a common mechanism of toxicity, viz., inhibition of gluconeogenesis.


The carcinogenicity of CDDs in humans has been controversial because thus far all attempts failed to establish such a link. Extrapolation of animal data to humans has also caused much discord among scientists and regulators because of great uncertainties about the validity of methods applied. A survey of the literature and our own data indicate that the relative acute potency of several CDD isomers is about the same in guinea pigs and in rats. More importantly, the relative acute potency in rats is quite predictive for subchronic and chronic toxicity potencies (carcinogenicity), as shown in the table.

<table>
<thead>
<tr>
<th>Isomer of CDD</th>
<th>LD50 Subchronic Carcinogenicity/Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LD50LD50</td>
</tr>
<tr>
<td>2,3,7,8</td>
<td>1</td>
</tr>
<tr>
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<tr>
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</tr>
<tr>
<td>1,2,3,4,6,7,9</td>
<td>0.0014(1)</td>
</tr>
</tbody>
</table>

ND is not determined * Estimated

Recently, the acute LD50s of TCDD in humans was estimated at about 5,000 to 6,000 μg/kg. Assuming that the relative acute potency of CDDs in rats is predictive for humans, as is the guinea pig's for the rat, and provided that acute potency in humans predicts subchronic and chronic toxicity potencies as is the case with rats, would suggest that humans are among the least sensitive species to CDDs regarding subchronic and chronic toxicities. As we found strict additivity in the acute toxicity of a mixture of 4 CDD isomers, it is suggested based on the potency predictions that subchronic and chronic toxicities of CDDs will also be additive.
ASSESSING DIOXIN-LIKE ACTIVITY OF CHLORINATED THIATHRENES


2,3,7,8-Tetrachlorothiophane (TCT), the sulfur analog of 2,3,7,8-tetrachlorobifenzo-p-dioxin (TCDD), and 2,7-dimethyl-3,8-dichlorothiophane (DMDC) were investigated for dioxin-like activity in male Wistar rats. Six groups of 6 rats received TCT (1, 50, 1000 ug/kg) or DMDC (50, 500,000 ug/kg) in corn oil or corn oil alone (control) for 14 consecutive days and were sacrificed 24 hours later. Another group received TCDD (13 ug/kg, po) on day 1, and was sacrificed on day 15. Significant decreases in the rate of body weight gain and in thymus and spleen weights were observed at the high doses of TCT and DMDC. Significant increases in liver microsomal cytochrome P-450 content and ethoxyresorufin O-deethylase activity were observed in the TCDD group and in the high dose TCT and DMDC groups. Benzphetamine N-demethylation was not increased in any group. No-effect levels for TCT and DMDC were 1 ug/kg x 14d and 50 ug/kg x 14d, respectively. Toxicity equivalence factors (TEFs) are used to assess the relative potency of chemicals with dioxin-like activity, with TCDD having a TEF of 1.0. The results indicate that TCT and DMDC have TEFs ranging from 0.0023 to 0.0001 for TCT and 0.0004 to 0.0000017 for DMDC. Thus, by replacing the oxygen in TCDD with sulfur, as in TCT, the biological activity is markedly reduced. Similarly, by replacing two chlorines in TCT with methyl groups, as in DMDC, biological activity is further reduced.

SCREENING METHOD FOR ESTIMATION OF POTENTIAL FISH AND SHELLFISH CONTAMINATION FROM METALS EMISSIONS. H. M. Goeden and A. H. Smith, Department of Biomedical & Environmental Health Sciences, University California, Berkeley, CA.

Fish and shellfish uptake of metals from sediment or water can be influenced by many factors including: the individual metal, metal concentration, seasonal variations, organism size, available substrates, water salinity, temperature, pH, and the position of the organism in the aquatic environment. At present there is no approach by which one can predict the fraction of metal which is bioavailable for a given set of conditions. Chemical indices that predict bioaccumulation potential (e.g. log Kow) have not yet been developed for trace metals. Consumption of contaminated fish and shellfish may be an exposure pathway for emitted pollutants in human populations. We propose a method for estimating the upper bound of possible metal fish and shellfish contamination. The method relies on the fact that if we relate average air levels to average contamination in some other medium, only part of which originates from air, then we can derive a ceiling estimate for contamination originating from the air. Average background air and fish or shellfish tissue levels were determined from the published literature. The ratio of background tissue levels to the background air levels gives a prediction of average tissue contamination from any given air level, assuming that airborne sources were the sole source of contamination. Since there are other sources, e.g. industrial discharge, the ratio of background tissue to air levels will produce a conservative estimate of tissue contamination from a given air level. This method can be used in a screening analysis to estimate upper bounds of contamination.

QUANTITATIVE RISK ASSESSMENT FOR ACUTE EXPOSURES TO HYDROGEN FLUORIDE. C. V. Alexeff, B. C. Lewis and N. Eagle, California Department of Health Services, Berkeley, CA.

Hydrogen fluoride (HF), a skin, eye and lung irritant, was released in 1987 from a Texas City facility and resulted in over 900 hospital visits. Exposure guideline levels were calculated for routine and accidental HF emissions with a model using existing animal lethality and human irritation data. Using log-probit extrapolation and concentration-response data, the model calculated the 95% Lower Confidence Limit on the toxic concentration producing a 1% response (LC50), the defined practical threshold. To this value, species-specific and chemical-specific adjustment factors were applied to take into account response variation among species, the range of effects over various concentrations, the conversion of concentrations to equivalent 60-minute exposures and the expected irritation-response slope. To prevent irritation from a one hour exposure to routine emissions a 0.08 ppm level was obtained from human data and a range of 3 to 13 ppm from rodent data. In contrast, application of 10-fold uncertainty factors would result in levels ranging from 0.1 to 0.3 ppm. For emergency planning hazard analysis a level of 0.5 ppm from the human data and a range of 2 to 30 ppm from the animal data were calculated.

USE OF DATA ON CARCINOGENIC METABOLITES IN ASSESSING CANCER RISK FROM PARENT CHEMICALS. M. El-Naggar, Chemical Risk Management, Midwest Research Institute, Kansas City, MO.

The process of identifying and regulating chemical carcinogens remain problematic. One unresolved issue that was debated during evaluation of chemicals e.g., dimethylnitrosamide, styrene, and benzidine-based dyes is how to use metabolic data in assessing cancer risk. This study evaluated how the available policies addressed the role of these metabolites and outlined a framework when it would be appropriate to incorporate metabolic data in risk analysis. Most policies require the use of these data in evaluating carcinogens but primarily to support results from human and long-term animal studies. OSHA would consider a substance to be carcinogenic if it is metabolized into one or more occupational carcinogens. None of the carcinogenic chemicals appear to have been identified based on data from their metabolites. These data, however, were used by EPA in IARC to modify the weight-of-evidence classification for certain chemicals. For many substances that generate carcinogenic metabolites, there are sufficient data to allow for a qualitative review in the hazard identification process but detailed information that can be used in quantitative assessments are lacking. Data on the biological disposition and stability of the active metabolites and on the pharmacokinetic interrelationship among organs of formation, organs of elimination, and target sites are essential. This information would allow for defining tissue exposure to the carcinogenic metabolite and assist in determining whether such exposure would lead to a carcinogenic response for the parent chemical.
CAN EPA CONDUCT ALL RISK ASSESSMENTS?
B Molholt, ERM, Inc., Exton, PA
Sponsor: A De Caprio
Recently EPA has issued guidelines for assessing public health and ecological risks from hazardous wastes at both active facilities (RCRA) and inactive sites (CERCLA). In each case, according to these guidelines, EPA will conduct the risk assessments rather than owners or potentially responsible parties. However, an examination of EPA’s resources suggests that they cannot accurately assess risk at all RCRA facilities and CERCLA sites in a site-specific manner. If EPA will conduct all future RCRA- and CERCLA-driven risk assessments, then less attention will be paid to specific site conditions which greatly affect risk, such as fate and transport modeling, identification of potential receptor populations and frequency of exposure. Only the most conservative risk assessment methodologies will be applied by EPA regional offices if they are to handle risk assessments at a rate equivalent to or greater than that of the past five years. The overall result of generic rather than site-specific risk assessments will be to increase uncertainty. This runs counter to EPA’s RURA program which attempts to reduce uncertainties in risk assessment.

UTILITY OF RISK ASSESSMENT IN EVALUATING REMEDIAL OPTIONS ON A SITE-SPECIFIC BASIS.
Risk assessment methodology can be incorporated into the site remediation process by addressing the risk reduction afforded by each remedial option along with cost and traditional engineering considerations, thus producing enhanced decision-making tools for a direct comparison of cost-effectiveness of the options in terms of risk reduction provided by each. A risk reduction/cost relationship was established for four remedial alternatives at an operating chemical manufacturing facility. Risk assessment determined the “risk-sensitive” steps of the various process trains, and allowed for an iterative risk assessment/engineering process to ultimately design an alternative that was both risk and cost-effective, as well as feasible to implement. Evaluation of the alternatives necessitated innovative assessment methods for determination of potential exposures resulting from implementation of such remedial technologies as bioremediation, incineration, and stabilization. The analysis also included determination of subchronic and chronic toxicity indices for relatively uncharacterized compounds and chemical mixtures. The result is a supporting argument for the selected remedial option that is credible, scientifically defensible, and effective.

Toxicokinetics were used in the assessment of systemic safety of 3 topical medicated products:
1. A chronic use antimicrobial product.
2. A short term use (several weeks) antimicrobial for use on wounds.
3. A potent pharmaceutical for single application.
In example 1, a 6 hour dermal exposure to 13 healthy volunteers resulted in mean absorption of 1.63±0.59 (±SD) mg of drug. Even when worst case absorption (4xSD above mean) was considered in comparison with chronic toxicity data in rats and baboons, safety margins ≥ 250 were obtained. Worst case predictions from plasma levels confirmed safety margins. In example 2, 14C-drug in solution was injected i.v. to 1 group of rats and administered to a full thickness wound in other groups. 14C was measured in urine, faeces, carcass, wound tissue, wound dressing and blood for up to 8 weeks post-dose. Absorption through wounds was 75% of the applied dose and elimination half live of 14 days indicated potential risk of accumulation. Quantitative risk assessment and metabolic fate is required. In example 3, percutaneous absorption and excretion of the drug in mini-pigs was compared with blood levels and excretion after i.v. injection to the same mini-pigs. Bioavailability was 20% and a 50 fold safety margin achieved. This toxicokinetic approach has demonstrated adequate safety margins and eliminated the need for further chronic toxicology studies.

ACUTE AND SUBCHRONIC, TOXICITY OF DETERGENT ENZYMES. L J Sauers1, G R Cookan2, and R D Laurit1, The Procter & Gamble Company, Cincinnati, OH1, and New Castle Upon Tyne, England2
Proteases and amylases increase the effectiveness of laundry detergents in the cleaning of protein and starch based soils. As part of the human risk assessment process, four proteases and two amylases, all isolated from Bacillus, have been evaluated in a series of acute and subchronic toxicity studies. These studies were conducted by the suppliers of these enzymes over the past several years using a variety of protocols. The results of these studies demonstrated that the use of proteases and amylases in laundry products does not represent a human safety concern. For all enzymes tested, the acute oral LD50 was greater than 2.75 g/kg and the inhalation LC50 was greater than 0.2 mg/l. Both classes of enzymes were shown to be mild skin and eye irritants when tested under exaggerated exposure conditions. In addition, the enzymes were evaluated for toxicity following subchronic oral exposure. No effect levels were observed at the highest dose tested in all studies. Due to their low order of toxicity, the extensive database that has been accumulated, and the minimal oral exposure that occurs, acute and subchronic oral toxicity studies of amylases and proteases are no longer conducted to support consumer exposure to enzyme-containing laundry products. In addition, the number of inhalation and irritation studies have been reduced. These studies are only conducted when the specific activity of a new protease or amylase is higher than that previously tested.

Under contract to the Air Force Western Space and Missile Center, Vandenberg Air Force Base, ACTA Inc. is developing an integrated model for assessing the potential health risks to the adjacent public from the toxic effluents of nominal and failed rocket launches. The health risks are represented probabilistically as the expected numbers of individuals in an exposed population suffering unacceptable health effects. Different susceptibilities among the individuals, different levels of effect, and uncertainties in the levels of exposure and levels of effect, are taken into account.

The presentation outlines the steps of the model from the launch event, with a probability of occurrence of an accident; through the development and dispersion over the Vandenberg-area terrain of a toxic cloud; to the imposition of time mean concentrations on the Vandenberg-area population and the resulting possible levels of health effect. By translation of concentrations to probabilities of health effect, a probit dose-response model is employed, with parameters quantified from expert estimates of relevant percentiles. Illustrative results using the REED model to estimate concentrations from a launch explosion and the development and dispersion of a toxic cloud are presented.

THE CONTRIBUTION OF INDIRECT EXPOSURE PATHWAYS TO HEALTH RISKS FROM HAZARDOUS WASTE INCINERATORS. S T Cragg, S Carg, M T Alberts, Radian Corp, Herndon, VA, USEPA, Washington, DC, Radian Corp, Sacramento, CA.

Ten risk assessments of hazardous waste incinerators were compared to evaluate the relative contribution of indirect (i.e., non-inhalation) exposure pathways to overall cancer risk. Indirect pathways include: 1) incidental ingestion of soil contaminated by emission from a nearby incinerator, 2) dermal absorption from contaminated soil, 3) ingestion of contaminated vegetable matter, 4) ingestion of contaminated meat or milk from livestock grazing in the vicinity of an incinerator, 5) ingestion of contaminated surface waters and fish from such waters, and other pathways. In 5 of the 10 risk assessments, the indirect pathways contributed at least 40% of the overall risks. Of these five, the produce, meat, and dairy pathways contributed the largest portions of the risk. In the remaining risk assessments the inhalation pathway contributed most of the risk. Differences in the relative contribution of inhalation and indirect pathways have been examined and may be attributed both to methodological and site-specific differences among the risk assessments. Recommendations are made that would standardize assumptions used for the various pathways. Greater methodological consistency would permit more consistency among risk assessments of hazardous waste incinerators for purposes of site permitting and risk prioritization.

METHOD FOR SETTING RISK-DRIVEN REMEDIAL GOALS IN A MULTI-SCENARIO, MULTI-CHEMICAL RISK ASSESSMENT. D J Sullivan, Envirotologic Data, Inc., Ventura, CA.

The calculation of risk-based remedial goals for multi-scenario and multi-chemical sites should consider the respective contribution of given scenarios and chemicals to the overall risk. The methodology presented here provides for a site-specific analysis in the setting of these remedial goals. The methodology can be used to evaluate sites having one chemical and multiple exposure scenarios, multiple chemicals and one exposure scenario or multiple chemicals in multiple exposure scenarios. The 6-step methodology is based on first independently estimating the percentage contribution of each chemical and scenario to the total risk. These percentages are used to allocate the risk (for carcinogens) or hazard index (for non-carcinogens) between chemicals and scenarios. In the final step, the allocated risk or hazard index is used to estimate allowable concentration for all chemicals so that total exposure through all scenarios does not exceed what is allowable. This methodology has shown to be effective in setting site-specific remedial goals for multi-chemical and multi-scenario sites.


Our laboratory has evaluated isolated hepatocytes as an in vitro model of xenobiotic metabolism by laboratory species and humans, to provide comparative data for use in the early stages of new drug and chemical development. Hepatocytes are isolated from various species, including human by collagenase perfusion and incubated in suspension or monolayer culture in serum-free medium for 4 hr. Metabolite profiles are determined by hplc, gc and tlc techniques. Studies to date have been performed on: tolbutamide, acetaminophen, amphetamine, benzo(a)pyrene, trichloroethylene, caffeine, benzidine, atrazine, 7-ethoxycoumarin, aldrin, 4 glycol ethers and 2,3,7,8-tetrachlorodibenzofuran. In all cases, the principal metabolites found were in agreement with in vivo data except that 5-acetylamino-6-aminomethyluracil, a major urinary metabolite of caffeine in humans, was not detected in human hepatocytes. Rate data generated in these experiments indicate that, depending on the chemical, rodent, rabbit, dog, monkey or human liver tissue is the most active for a particular pathway. Studies on the glycol ethers also demonstrated that isolated hepatocytes can be used to generate apparent kinetic constants that can applied to human risk assessments. (Supported in part by ES-55109, DK-8-2235, and GM-20158)
Sucrose is a non-nutritive sweetener 600 times sweeter than sucrose. Made by selectively substituting chlorine for three hydroxyl groups in sucrose, sucrose is a hydrophilic, water soluble (25% at 22°C), white crystalline solid and is remarkably stable. In all species tested, an oral dose of sucrose is poorly absorbed (typically 10% to 35%) and excreted unchanged in the feces. The amount that is absorbed is excreted in the urine mainly as unchanged sucrose. Metabolism is apparently limited to Phase II pathways and there is no evidence for either dechlorination or hydrolysis of sucrose in vivo. Acute toxicity studies in mice and rats showed no toxicity at 15 g/kg and 10 g/kg, respectively. Range-finding and subchronic studies in mice and rats demonstrated that dietary concentrations higher than 3% led to physiological effects caused by either the high volume of the additive in the GI tract or by decreased food consumption and the instability of diets containing the sweetener. Sucrose was not teratogenic in either rats or rabbits. Multigeneration reproduction studies in rats, chronic toxicity studies in dogs and rats and carcinogenicity studies in mice and rats demonstrated that sucrose was neither toxic nor carcinogenic and that a 3% dietary concentration was the MTD. These studies demonstrated a HELD of 1500 mg/kg/day for both rats and mice.

We have reported a guinea pig model for allergic respiratory sensitization to industrial/environmental chemicals. The guinea pig produces two major types of cytolytic antibody, IgG, and IgE. In efforts to probe the mechanism(s) underlying immunologic sensitization, we developed methodology to separate and isolate these two classes of antibody. To stimulate IgE antibody production, Strain 13 guinea pigs were injected with cyclophosphamide followed by Staphylococcus aureus enterotoxin. Serum was obtained and the globulin fraction isolated by addition of caprylic acid then ammonium sulfate. Immunoglobulins were separated into classes using FPLC employing a Mono Q column and a linear gradient of 0.01-0.3 M Na,K phosphate buffer, pH 7.5 (buffer B). IgG was eluted with 8-18% buffer B. IgE was eluted later, emerging in the 19-32% buffer B fraction with lesser amounts in the 33-63% fraction. These immunoglobulins were clearly distinguished by heat lability. IgE lost all activity after heating at 56°C for 4 hr whereas IgG, retained full activity. This chromatographic procedure enabled rapid isolation of immunoglobulin classes from guinea pig sera and effectively separated the two classes of antibody associated in the guinea pig with hypersensitivity reactions. Supported by NIHES #01532, PPG Industries and the Procter and Gamble Co.

The purpose of the study described was to define the immunological characteristics of the reduced distinguish chemical respiratory allergens from contact allergens. Mice were exposed topically to either trimecillin anhydride (TMA, a respiratory allergen) or 2,4-dinitrochlorobenzene (DNCB, a contact allergen which lacks the capacity for respiratory sensitization). Under conditions where TMA and DNCB elicited equivalent levels of lymph node activation and T lymphocyte proliferation, exposure only to TMA resulted in an IgE response. DNCB and TMA also induced divergent IgG anti-hapten antibody responses. DNCB resulted in a strong IgG2a anti-dinitrophenol (DNp) response, while anti-TMA antibodies were primarily of IgG1 and IgG2b subclasses. Identical results were obtained with other pairs of respiratory and contact allergens. Taken together the evidence suggests that different types of chemical allergens induce divergent immune responses; observations which may reflect a differential activation of helper (Th1) cell sub-sets. Thus, interferon-g (IFN-g), a product of Th1 cells promotes IgG2a responses and inhibits IgE antibody production, while interleukin 4 (IL-4) secreted by Th2 cells stimulates IgE responses.

IgG, ANTIBODY MEDIATES IMMEDIATE AND LATE-ONSET PULMONARY SENSITIVITY RESPONSES IN THE GUINEA PIG. D Griffiths-Johnson, R Jin and M H Karol. Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA

Pulmonary allergic responses are frequently classified by their time of onset. Those occurring within one hour of exposure to the allergen are designated immediate-onset airway responses (IAR) and those occurring after 1 hr as late-onset responses (LAR). We developed a guinea pig model for pulmonary hypersensitivity to chemicals. Animals demonstrate both IAR and LAR upon appropriate sensitization and challenge exposures. To probe mechanisms of response, we hypothesized immunoglobulin would mediate both IAR and LAR. Using FPLC and a Mono Q column, IgG, was purified from serum of animals sensitized to ovalbumin (OA). The IgG, was transferred into naive guinea pigs with each animal receiving 1.4 mg. Animals were challenged 24 hr later by inhalation of 25% OA solution. Each of the 4 recipient animals demonstrated severe IAR with 2 resulting in fatality. The remaining two animals demonstrated LAR, maximum 5-6 hr after the inhalation challenge. LAR was documented by sustained increases in breathing frequency and a coincident febrile reaction. These data demonstrate that IgG, antibody can mediate both IAR and LAR in the guinea pig model. The ability of additional immunologic components, including IgE, to mediate these responses must also be considered. Supported by NIHES #01532.
The binding affinity of antibody refers to the strength of the interaction between an antibody combining site and a determinate of its epitope. High affinity antibodies mediate different biological effects than do low affinity antibodies. Affinity also influences the detectability of antibodies by a number of methodologies including ELISA. For these reasons, studies were undertaken to assess the affinity of antibodies produced in guinea pigs in response to isocyanate exposure. The possible change in antibody affinity as a result of repeated exposure was studied in animals exposed to diphenylethylene disocyanate (MDI). Guinea pigs were injected with 10 mg MDI on day 1 and received booster immunization on days 26 and 36. Blood was drawn on days 20 and 50. Antibody affinity was determined by comparing ELISA results in the presence and absence of 0.5 M guanidine HCl. The leftward shift in the concentration-response curve in the presence of the salt is inversely related to the antibody affinity. The affinity of the antibodies obtained on day 50 was considerably higher than that obtained on day 20; i.e., 0.22 dissociation units on day 50 compared with 1.2 units on day 20 (where one unit is one doubling serum dilution). Knowledge of antibody affinity will indicate the likelihood of biologic significance of antibody titers. Supported by NIHES #01532.

We previously isolated and characterized a trifluoroacetylated 58 kDa protein that reacts specifically with antibodies in the sera of halothane hepatitis patients. This protein was tentatively identified as phospholipase C isozyme I α (PI-PLC I α), because the amino acid sequences of its NH2-terminal and several internal peptides showed 99% homology with the deduced amino acid sequence of a cDNA that was purported to encode for this enzyme. In the present report we provide evidence that the 58 kDa protein is not PI-PLC I α. Instead, the protein was concentrated within the ER of liver cells whereas most of PI-PLC I α activity is present in the cytosol. Second, the purified 58 kDa protein did not have PI-PLC activity. Third, immunoprecipitation of 67% of the 58 kDa protein from an extract of RBL-2H3 cells did not reduce PI-PLC activity or more than that observed with an unrelated antibody. Finally, because the reported cDNA of PI-PLC I α has not yet been shown to express PI-PLC activity, we conclude that this clone and our 58 kDa protein do not correspond to PI-PLC I α and their identities remain to be determined.

A trifluorocetlated 82 kDa stress protein appears to be a neogantien associated with halothane hepatitis. M C. Davila, N R Pumford, B M Martin, D Thomassen, J L Martin, and J R Pohl. NHBRI and NIHM, NIH, Bethesda, MD, and the Dept. of Anesthesiology, Johns Hopkins Medical Inst., Baltimore, MD.

In previous reports we have shown that serum antibodies of patients with halothane hepatitis recognized a trifluoroacetylated (TFA) 80 kDa protein. This protein was mainly present within the microsomal fraction and was identified as ERp72. We have now observed by immunoblotting that several patients' antibodies react instead with another TFA protein in the 80 kDa region of the immunoblot. This was demonstrated by removing the TFA-ERp72 from a partially purified fraction of liver microsomes of the halothane-treated rats with a specific antibody, prior to immunoblotting. It was found that 7 of 40 halothane-hepatitis patients had serum antibodies that reacted with a TFA protein of 82 kDa. The N-terminal amino acid sequence of the protein immunoblotted to a PVDF membrane indicated that the protein might be glucose-regulated protein GRP78 which appears to be identical to the immunoglobulin heavy chain binding protein BIP. These proteins are localized in the lumen of the endoplasmic reticulum and are believed to have a role in the protein folding. The results of this study suggest that in susceptible individuals, the TFA 82 kDa is immunogenic and may have a role in the pathogenicity of halothane hepatitis.
The safety of rhIL-4 was evaluated in cynomolgus monkeys (five/sex/group) by twice daily subcutaneous injection of 0, 0.5, 2.5, or 25 microg (daily dosage) for one-month. Clinical signs of muscular weakness and pale coloration occurred in the high dosage group during the final week of dosing. This was associated with the moribundity of several animals and the death of one high dosage female. Statistically significant alterations occurred in various clinical pathologic parameters which were consistent with a consumptive coagulopathy, decreased erythrocyte mass, lymphoid stimulation and increased neutrophil production. Histopathologic evaluation of tissues from animals necropsied following treatment revealed increased bone marrow granulopoiesis, tubular atrophy of the testes and a variety of proliferative and inflammatory vascular lesions associated with an infiltration of eosinophils. Following a two-week recovery period, clinical chemistry and hematology parameters were returning toward pretreatment values, bone marrow appeared normal, tubular atrophy of the testes remained unchanged, and vascular lesions were decreased in incidence and were in the process of healing. It is unclear at the present time whether all of the effects seen in this study were a direct result of rhIL-4 administration or were secondary to the regulatory activity of this molecule on the immune system.

An ELISA for detection of antibodies to rhIL-4 was used to analyze serum samples collected during a one-month safety study in order to assess the immunogenicity of three doses (0.5, 2.5 and 12.5 mcg/kg, BID) of test article plus a vehicle control. Five animals/sex/group were dosed twice daily by subcutaneous injection for 28 consecutive days (necropsy at end of Day 29). This was followed by a 14 day recovery period. Serum samples were collected pretreatment on Day 1 and prior to the a.m. dose on Days 8, 22, 28 and 42. Two monkeys in the control group, seven in the 0.5 mcg/kg group and ten each in the 2.5 and 12.5 mcg/kg groups had antibody titers greater than 1:50 on at least one study day. Significant titers were seen as early as Day 8 in four monkeys which received test article. Responses in the vehicle control-treated monkeys, were not considered biologically relevant. Increases in titer were dose-related for the combined sexes (no difference between sexes) on Days 22, 28 and 42. The antibody response was specific for the test article as determined by competitive binding assays using serum samples collected on Day 28 from two 12.5 mcg/kg group monkeys. The data obtained in this study was valuable in both understanding the various clinical and pathologic alterations which occurred during this study and in the conduct of the to assess the safety of the to assess the safety of the combined sexes (no difference between sexes) on Days 22, 28 and 42. The antibody response was specific for the test article as determined by competitive binding assays using serum samples collected on Day 28 from two 12.5 mcg/kg group monkeys. The data obtained in this study was valuable in both understanding the various clinical and pathologic alterations which occurred during this study and in the conduct of the safety study and designing a rational approach to dose and schedule of test article administration in future safety studies.

Nitrogen dioxide (NO2) is a major environmental, occupational, and indoor air pollutant. There is widespread exposure of human beings to NO2. Previous studies from our laboratory showed significant reductions in murine T lymphocyte subpopulations and a lower rate of progression of a murine T-cell leukemia/lymphoma following exposure to ambient levels of NO2. To study the consequences of six months of intermittent exposure to 0.35 ppm NO2 on lymphocyte function, C57BL/6 mice were induced to express interleukin 2 receptor (IL-2R) in vivo by IV injection of con A. Expression of IL-2R on spleen cells was detected and quantitated by flow cytometry using a rat IgM monoclonal antibody to murine IL-2R (7D4) purified by fast protein liquid chromatography. Splenectomized animals exposed to NO2 showed a 43.1% decrease in the percentage of IL-2R+ cells (P<0.02). When the spleen cells were gated according to size, animals exposed to NO2 showed decreases in large and small IL-2R+ cells of 50.9% and 33.1%, respectively (P<0.005). Expression of the IL-2R is one of the early events of T-lymphocyte activation. Proliferation of T-lymphocytes occurs upon IL-2R is expressed and interaction between IL-2 and its receptor takes place. From our results, it is clear that chronic exposure to an ambient level of NO2 reduces the ability of the mouse to activate T lymphocytes in response to an immunologic challenge. Reduced IL-2R expression in NO2-exposed animals may explain, in part, the reduced rate of progression of the T-cell leukemia/lymphoma and the lower percentages of T lymphocyte subpopulations following exposure of mice to NO2.


Methylated polycyclic aromatic hydrocarbons, such as 7,12-dimethylbenz(a)anthracene (DMBA), are known to be cytotoxic to lymphoid cells. Recent studies in our laboratory have shown that DMBA produces alterations in Ca2+ homoeostasis in lymphocytes. The purpose of the present study was to determine if DMBA produces an elevation of baseline intracellular free Ca2+ leading to DNA fragmentation and cell injury. Female B6C3F1 mice were dosed daily for 14 days with DMBA in corn oil via gavage at total cumulative doses of 14, 42, and 140 mg/kg. We found that the total number of viable lymphoid cells recovered from the spleen, thymus, Peyer's patches, and mesenteric lymph nodes (MLNs) was greatly decreased at the 42 and 140 mg/kg doses of DMBA. Lymphoid cell necrosis was detected histopathologically by light microscopy for each of these tissues after the mid- and high-dose treatments. No necrosis was detected in the liver or kidneys of treated mice. Free intracellular Ca2+ in lymphoid cells, detected using Indo-1 and flow cytometry, was mildly elevated in splenic B cells and T cells, as well as in the Peyer's patches and thymus. Electrophoretic analysis of DNA in agarose gels revealed a dose-dependent fragmentation of chromatin by DMBA in lymphoid organs and the thymus, but not in the kidney. These results demonstrate that DMBA may induce 'apoptosis-like' phenomena in lymphoid cells and tissues, in a manner that may resemble what has been reported for glucocorticoids and dioxins.
Infinite dose skin permeation studies of TCDD. M S Rahman*, J L Zatz*, T H Umbreit, and M A Gallo. UMDD-J-Robert Wood Johnson Medical School, and *College of Pharmacy, Rutgers University, Piscataway, NJ.

The permeation of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) through female dorsal hairless mouse skin was investigated using the infinite dose technique. Nine solvents were evaluated as donor media using caffeine as a reference compound and preserved saline as receptor fluid. Steady-state flux of caffeine (J) from each of these solutions was determined and flux at saturation (J*) was calculated using the relationship: J* = (J S) / C, where S is the solubility and C is the concentration of caffeine in a particular solvent. Light mineral oil yielded the lowest value of J*, suggesting a very weak interaction between this solvent and mouse skin. Infinite dose skin permeation studies were carried out with TCDD in light mineral oil as donor at concentrations of 1.15, 5.04 and 11.2 μg/ml respectively and steady-state flux was determined. 5% polysorbate 80 in preserved saline was used as receptor solution. A linear relationship was observed between steady-state flux and donor TCDD concentration. Comparison of the permeabilities of TCDD through full-thickness and stripped skin revealed that the viable tissues of mouse skin provided the greater resistance to TCDD permeation. (Supported by NIDEP Grant #C29510)

Penetration of TCDD into pig skin in vitro - lack of influence of viability. LWD Weber and K Norman. Dept. of Pharmacology, Toxicology & Therapeutics, University of Kansas Medical Center, Kansas City, KS (USA), and Gesellschaft für Strahlen- und Umweltforschung München, Neuhemberg (FRG).

Porcine skin was chosen because of its morphological similarity to human skin. It was obtained fresh from a local meat pack and kept on ice-cold "medium" (MEM, Earle's salts, L-glutamine, D-valine, 10% fetal bovine serum, 0.01% gentamicin). Most of subcutaneous fat was cut off immediately prior to use or freezing. The stratum corneum was removed by 30 times stripping with adhesive tape. TCDD was used at 65 ng/cm² dissolved in either acetone or mineral oil. Incubation was performed in organ culture dishes with "medium" at 32°C under 40% O₂, 5% CO₂, 55% N₂. Metabolic viability of the skin was assessed by ¹⁴CO₂-release from ¹⁴C-glucose or lactate formation. Pig skin kept at +4°C in "medium" retained viability for at least 48 hr. Exposure to TCDD for up to 1,000 min under the above conditions did not affect viability. About 5 times the amount of TCDD dissolved in acetone was absorbed, as compared to TCDD dissolved in mineral oil. In either case TCDD formed a steep concentration gradient (over 3 orders of magnitude) through the upper 800 μm of skin, with essentially no change thereafter, or a slight increase in remnants of subcutaneous fat. Removal of stratum corneum increased TCDD concentrations in skin 2- to 10-fold. Loss of viability (freezing of the skin) caused little change in dermal penetration of TCDD, with a tendency to somewhat higher TCDD concentrations after long-term exposure. These results are very similar to those obtained previously with human cadaver skin. It can be concluded that porcine skin is a valuable model to study dermal penetration of TCDD, and that the use of previously frozen skin can lead to a slight overestimation of rates of dermal penetration of TCDD. This work was supported by USEPA # 814751.

Dermal absorption and disposition of 2,3,7,8-tetrabromobenzodioxin-p-dioxin (TBDD) in rats. J A Jackson, J J Diiiberto, L P Kedderis, L S Birnbaum HERL, US EPA, RTP, NC; Curriculum in Toxicology, UNC-CH, Chapel Hill, NC.

Polybrominated dibenzodioxins are contaminants in certain brominated flame retardants used in a variety of commercial materials. They exhibit toxicities similar to those of their chlorinated analogues. The skin is an important route of exposure to consider for these environmentally persistent chemicals. In order to examine the potential for systemic exposure to TBDD following low dose dermal exposure, the absorption, distribution and elimination of the compound were evaluated in 10-wk-old male Fischer 344 rats. The dose (0.2 nmol/1.8 cm² = 1 nmol/kg) of ³H-TBDD in 60 μl of acetone was applied to the intrascapular region and covered with a perforated cap. Animals were housed in individual metabolism cages for 3 days, and radioactivity was determined in tissues and excreta. The application site was swabbed with acetone prior to excision; ~13% of the administered dose remained in the application site after swabbing. Another ~12% was absorbed (translocated into the tissues or excreta). Major tissue depots were the liver, adipose tissue and skin, with a 3:2 liver to adipose tissue concentration ratio. Only ~17% of the absorbed dose was eliminated, primarily in the feces. Results from this study and those from other studies in our laboratory indicate that, at equimolar doses, only 30-40% as much TBDD is absorbed through rat skin as is TCDD. (This abstract does not necessarily reflect EPA policy.)
EFFECTS OF TAMOXIFEN ON CYTOCHROME P450 ACTIVITY AND TCDD TOXICITY. SA MacKenzie, TH Umbriet, T Thomas and MA Gallo. Joint Graduate Program in Toxicology, University of UMDNJ-RW Johnson Medical School, Piscataway, NJ.

Tamoxifen (TMX) potentiated the lethality of an intraperitoneal (i.p.) dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) when coadministered to female CD-1 mice. This effect did not appear to be mediated by interactions at either the Ah or the estrogen receptor. TMX alone had minimal effect on liver microsomal aryl hydrocarbon hydroxylase (AHH) activity but it decreased the TCDD-induced elevation in AHH activity by 35%. This lower activity was not accompanied by a decrease in cytochrome P450IA1 protein, as illustrated by Western blot of liver microsomes. Preincubation of control or TCDD-induced liver microsomes with either TMX or its active metabolite 4-hydroxytamoxifen decreased AHH activity with Ki values of 15-55 μM. TMX reduced enzyme activity to 10% of vehicle control while 4-hydroxytamoxifen only reduced activity to 50% of vehicle control. Coadministration of TMX and 14-C TCDD increased the retention of TCDD in the liver and fat. These results suggest that TMX interacts with cytochrome P450IA1, decreasing its enzymatic activity. This decreased activity may interfere with TCDD metabolism and/or elimination, thus exposing target organs to higher concentrations of TCDD. This increase in the biologically effective dose may be responsible for the TMX potentiation of TCDD toxicity. (Research supported by NJ DEP Grant C29510).

EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON HEPATIC PROTEIN BOUND (PB) AND NON-PROTEIN BOUND (NPB) SULFHYDRL (-SH) GROUPS, AND REDUCED PYRIDINE NUCLEOTIDES IN FEMALE RATS. S J Sibhs and M A Shara. Creighton University Health Sciences Center and University of Nebraska Medical Center, Omaha, NE.

Evidence suggests that TCDD induces oxidative stress in rats. Alterations in the status of thiol groups and pyridine nucleotides are indicators of oxidative stress. Therefore, the effects of TCDD on PB and NPB sulfhydryl groups as well as NADH and NADPH in hepatic mitochondria (MT) and cytosol (CT) were examined. TCDD (100 μg/kg) was administered orally to female Sprague-Dawley rats as a single dose. A 50-76% decrease in MT NPB-SH was observed between days 3 and 10. PB-SH of MT increased 40% on day 5 and returned to control levels by day 10. Cytosolic NPB-SH decreased 40-44% on days 3-5 post-treatment. A gradual decrease of 18-45% in MT NADPH was observed between days 7 and 18 post-treatment. Increases in MT NADPH ranged from 40% (day 5) to 118% (day 13). After day 13, MT NADPH remained elevated at about 50% above control values until death. Cytosolic NADPH increased by 20% on day 3, reaching a maximum of 70% increase on day 7 with a gradual decline to 65% below controls by day 18. Thus, TCDD induces marked alterations in NADPH, NPB-SH and NADPH homeostasis. The increase in NADH may be due to a previously demonstrated induction of aldehyde dehydrogenase. These findings support the hypothesis that TCDD induces an oxidative stress which may contribute to manifestation of the toxic effects.

EXCRETION OF FORMALDEHYDE, ACETALDEHYDE, MALONDIALDEHYDE AND ACETONE IN THE URINE OF TCDD-TREATED RATS. M A Shara and S J Sibhs. Creighton University Health Sciences Center and University of Nebraska Medical Center, Omaha, NE.

2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces enhanced lipid peroxidation as evidenced by increased formation and content of thioribarbituric acid reactive substances. In order to verify that enhanced lipid peroxidation was occurring, the time-dependent excretion of formaldehyde [FA], acetaldehyde [ACT], malondialdehyde [MDA] and acetone [ACON] was determined in the urine of rats which were ad-libitum fed, pair-fed and TCDD-treated (100 μg/kg as a single oral dose). The metabolites were quantitated by HPLC of the 2,4-dinitrophenylhydrazine derivatives, and identification was verified by GC-mass spectroscopy. Five days after the administration of TCDD, the excretion of MDA, FA, ACT, and ACON increased by 5.7-, 2.7-, 213-, and 29.3-fold, respectively, as compared to ad-libitum fed animals, while excretion of the metabolites in pair-fed animals increased by 2.4-, 1.5-, 4.3-, and 9.3-fold, respectively. The excretion of these four fatty acid metabolites further increased with time. The increase in the excretion of these products following the administration of TCDD could not be attributed entirely to hypophagia. The results clearly demonstrate a marked increase in lipid metabolites in response to TCDD-induced oxidative stress. The HPLC system which has been developed for quantitating these metabolites has widespread applicability to the investigation of lipid metabolism and exposure to environmental pollutants.
Inhalation is an important route of systemic exposure for airborne contaminants. TBDD is present in emissions from waste incinerators and leaded gasoline fueled cars and may be formed in plastic manufacturing atmospheres. The transpulmonary absorption of TBDD was assessed following intratracheal instillation. Male Fischer 344 rats (~250g) were treated with 1 nmol (0.5 μg/kg) of $^{3}H$-TBDD (~2 μCi) in 250 μl (1:1:3:: ethanol:Emulphor:water), then housed in individual metabolism cages for 3 days. Following termination, radioactivity was measured in tissues and excreta. Feces was the major route of excretion; by 72 hrs, ~37% of the total dose was excreted. Most of the body burden was found in the liver with ~18% of the administered dose and adipose tissue with ~22% of the administered dose. These results indicate that pulmonary bioavailability of TBDD is similar to that observed following oral exposure. Thus, inhalation needs to be considered in risk assessments. (This abstract does not necessarily reflect EPA policy.)
A COMPARISON OF GAP JUNCTION EXPRESSION IN VITRO AND IN VIVO DURING NORMAL AND NEOPLASTIC HEPATIC DEVELOPMENT. M J Neveu, J R Hully, G L Satler, D C Paul, H C Pitot, McArdle Laboratory and Environmental Toxicology, Univ. of Wisconsin, Madison, WI, Harvard Univ., Boston, MA

Altered cell-to-cell communication studied in vitro has been proposed as a potential assay for the detection of tumor promoters. However, studies on gap junction expression during rat hepatocarcinogenesis in vivo suggest that data from in vitro studies may be incommensurate with the action of rat liver tumor promoters in vivo. Previously, we demonstrated that tumorigenic and non-tumorigenic cell lines in vitro either express the heart-like gap junctional protein connexin 43 (Cx43) or are gap junction-deficient. Although the normal hepatocyte gap junctional protein, connxin 32 (Cx32), is dramatically decreased in most preneoplastic and neoplastic hepatocytes, these cells do not express Cx43 mRNA or protein. Using immunocytochemistry, Western and Northern blot analyses we have demonstrated that Cx43 is not expressed in hepatocytes during fetal development, regenerative or adaptive hyperplasia. However, our results indicate that several non-parenchymal liver cells including bile duct cells, oval cells and Glisson's capsule do express Cx43. Analysis of serially transplanted hepatomas demonstrated that some cell lines that express Cx43 mRNA and protein in vitro shift to Cx32 mRNA expression in vivo. The disparate Cx expression patterns observed in vitro compared with those in vivo, coupled with the observation that gap junctions composed of Cx32 and Cx43 have different gating properties, indicates that caution should be used in evaluating the relevance of in vitro cell-to-cell communication assays for human carcinogenic risk assessment.

GAP JUNCTION PROTEIN EXPRESSION IN NORMAL, PRENEOPLASTIC AND NEOPLASTIC MURINE LIVER. X Wang, J Cao, Z Xie, E Dupont, J E Trosko, and J E Klauinig. Medical College of Ohio, Toledo, OH and Michigan State Univ, East Lansing, MI

Previous studies have shown a modification of the expression of the gap junction (GJ) protein connexin 32 in neoplastic rat liver. In this study, we have examined the expression of three GJ proteins (connexins) in normal, preneoplastic and neoplastic mouse liver during tumorigenesis. GJ protein expression was determined by immunohistochemistry. Normal murine liver expressed connexins 32 (cx32) and 26 (cx26) in hepatocytes and connexin 43 (cx43) in nonparenchymal tissue. In neoplastic liver (adenomas), cx expression of cx32 and cx26 was significantly decreased. mRNA for these connexins was also decreased in the adenomas. In preneoplastic foci two patterns of expression were evident. In one set of foci cx26 and cx32 were expressed as in the normal liver. In a second set of foci, expression of both cx32 and cx26 was significantly decreased. Cx43 expression was not seen in adenomas or foci. Expression of GJ protein may be a potential marker for those foci that progress to neoplasia.

GAP JUNCTIONAL INTERCELLULAR COMMUNICATION (GJIC) AND GAP JUNCTION (GJ) PROTEIN EXPRESSION IN RODENT AND HUMAN HEPATOCYTES. L E Schutrum, E. Dupont, J Cao, Z Xie, J H Resau, J R Cottrell, and J E Klauinig. Medical College of Ohio, Toledo, OH, Michigan State Univ., East Lansing, MI, and Univ. of Md, Balt., MD.

The down regulation of GJIC in cells following exposure to toxic agents has been well documented. In this study we examined the expression of GJIC, GJ protein (connexin) expression, and GJ mRNA expression in human liver and compared it with rodent liver. GJIC was evaluated by dye coupling in precision sliced livers and in primary cultured hepatocytes. Untreated human liver slices and hepatocytes exhibited similar patterns of dye spread to that seen in rat and mouse liver. Human, rat, and mouse liver expressed mRNA and connexin 32 protein. Connexin 26 protein was also expressed, with greatest expression in the mouse, less in rat, and much less in the human hepatocytes. Connexin 43 was expressed only in nonparenchymal cells. Treatment of hepatocytes with PB inhibited GJIC in rodent hepatocytes in a dose dependent manner but had no effect on GJIC in the human hepatocytes.

Protein kinase C translocation during mezerein-inhibited intercellular communication in vitro. Heather L. Rupp, J E (Trosko) and B V Madukar, Dept. of Pediatrics/Human Development and Institute for Environmental Toxicology, Michigan State University, E. Lansing, MI

A major epigenetic modulation induced by many tumor promoters both in vivo and in vitro is inhibition of gap junctional intercellular communication (GJIC). In the present study we investigated the correlation between inhibition of GJIC by mezerein, a stage II tumor promoter, and the membrane translocation of the calcium and phospholipid dependent protein kinase C (PKC) in rat liver epithelial culture model WE-F344. The results indicated that mezerein induced the translocation of PKC to the plasma membrane in a time and dose dependent manner (0.1 - 10 ng/ml). However, the membrane association of PKC was reversed after 6 hours and no PKC activity was observed either in the membrane or the cytosol at 24 hours. Under similar experimental conditions, we observed that GJIC was abolished in mezerein-treated cells in a time and dose dependent manner.

The calcium antagonist, TM-8, (50 µm) transiently prevented mezerein induced translocation of PKC and down-regulation of GJIC. The data suggested that PKC translocation (activation) is directly involved in the down-regulation of GJIC. (Supported by Grants from NIEMS (ES04911) and USAF OSR (89-0325)).
1053 **XENOBIOIC MODULATION OF GAP JUNCTIONAL COMMUNICATION OF RAT PANCREATIC EPITHELIAL CELLS IN VITRO.** B.V. Madhukar, Heather L. Rupp, William J. Paradis and James E. Troeko, Dept. of Pediatrics/Human Development and Institute of Environmental Toxicology, Michigan State University, E. Lansing, MI

In our continuing efforts to develop in vitro systems to study chemical modulators of gap junctional intercellular communication (GJIC), we have investigated the action of several environmental xenobiotics to inhibit GJIC in rat pancreatic epithelial cells. The results indicated that many chlorinated pesticides, the phorbol ester tumor promoter, TPA, and a number of PCBs inhibited GJIC of these cells. The inhibitory action of these chemicals was time and dose dependent. However, the inhibitory action of TPA was transient (less than 6 hours) while that of DDT, dieldrin or heptachlor epoxide was sustained suggesting possible differences in the action of these agents. Concentrations at which TPA (0.1 ng/ml) and dieldrin (1 μg/ml) had minimal effect on inhibition of GJIC, these two chemicals showed synergism. These observations suggested that GJIC is modulated by more than one mechanism. TPA induced the translocation of the calcium and phospholipid dependent protein kinase C while DDT, dieldrin or heptachlor epoxide induced a transient increase in intracellular free calcium. (Supported by Grants from NIHRS (ES04911) and USAF-OSR (89-0325))

1054 **INCREASED GAP JUNCTION-MEDIATED INTERCELLULAR COMMUNICATION IN SK-UT-1 CELLS EXPOSED TO NICKEL (II) CHLORIDE IN VITRO.** M. S. Fahl and R. Louis Czuran, Toxicology Program, Dept. of Environmental and Industrial Health, The University of Michigan, Ann Arbor, MI.

Nickel has been demonstrated to increase contractility in numerous smooth muscle tissues, including uterus, but its mechanism is not well defined. A human uterine leiomyosarcoma cell line, SK-UT-1 (ATCC HTB 114), was used to study the effects of nickel (II) chloride (NiCl₂) on gap junction-mediated intercellular communication. SK-UT-1 cells were cultured in vitro with 25, 50, or 100 μM NiCl₂ for 24 hours or with 100 μM NiCl₂ for 12 hours prior to microinjection. To evaluate the presence of functional gap junctions, SK-UT-1 cells were simultaneously microinjected with 0.1% propidium iodide (permanent marker) and 1.0% Lucifer yellow dyes, then observed under epifluorescence conditions for evidence of Lucifer yellow transfer. The percentage of gap junctional communication between SK-UT-1 cells increased in a dose-dependent fashion. No signs of cytotoxicity were observed under the experimental conditions. These findings suggest a possible mechanism whereby NiCl₂ increases smooth muscle contractility by increasing the amount of intercellular coupling via gap junctions.

1055 **REGULATION OF MYOCYTE GAP JUNCTIONAL INTERCELLULAR COMMUNICATION (GJIC) BY INTRACELLULAR CALCIUM.** Z. Xie, A Askari, and J. E. Klaunig. Dept. of Pharmacology, Medical College of Ohio, Toledo, OH.

The blockage of GJIC by toxic chemicals and during ischemic injury may be through modification of free intracellular calcium ([Ca²⁺]). In the present study, the effect of changes in [Ca²⁺] on GJIC in cultured neonatal rat myocytes was examined by dye coupling. [Ca²⁺] in living cells was determined by the spectrofluorometric measurement of fura-2. The resting [Ca²⁺] in spontaneous beating myocytes was about 0.1 μM under control conditions. Exposure to ouabain or a Na free media increased the resting [Ca²⁺] to about 0.8 μM. Under these conditions, the myocytes maintained GJIC but exhibited arrhythmic beating at [Ca²⁺] above 0.6 μM. Increasing [Ca²⁺] above 1 μM resulted in both an inhibition of GJIC and arrhythmic beating. We also examined the possible modification of intracellular pH (pHi) measured spectrofluorometrically with BCECF, on [Ca²⁺] induced inhibition of GJIC. These results showed that while pHi modified [Ca²⁺] by H⁺ - Ca²⁺ interaction, it had no effect on [Ca²⁺] induced inhibition of GJIC in cultured myocytes.


R-134a (1,1,1,2 tetrafluoroethane) is presently being considered as a replacement refrigerant for the currently used R-12 (dichlorodifluoromethane). The present study was undertaken to examine the hepatotoxic and cardiotoxic effects of R-134a, two structurally related compounds (R-132b, 1,2-dichloro-1,1-difluoroethane; and R-133a, 2,2,2-trifluoroethylchloride), and a known hepatotoxic/cardiotoxic agent (halothane). Compounds were administered to primary cultured rat (F344) and mouse (B6C3F1) hepatocytes and rat myocytes in gaseous form in modular incubators. GJIC and LDH leakage were evaluated following 4 and 24 hrs of exposure to each compound at various concentrations. Halothane, R-133a, and R-132b exhibited a dose and time dependent inhibition of GJIC in both hepatocytes and myocytes at sublethal concentrations. R-134a produced no toxic effects in either hepatocytes or myocytes at concentrations up to 750,000 ppm.
ANALYSIS OF PATULIN TOXICITY WITH IN VITRO FLUORESCENCE ASSAYS. R C Burghardt, R Mounehme, E J Lewis, R H Bailey, and T D Philips. Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX.

Fluorescence based vital assays are increasingly being developed to measure cellular mechanisms of chemically induced toxicity. In the present study, two assays were employed to examine the cellular effects of exposure to patulin (a cyclic gamma lactone). An immortalized granulosa cell line was used to examine the effect of this mycotoxin on two physiological parameters: gap junction mediated intercellular communication and mitochondrial membrane potential. Dose-dependent reduction in intercellular communication was observed following incubation of cells for 2 hr in patulin at doses of 10 μM or greater as determined by measurement of the rate of fluorescence recovery after carboxyfluorescein photobleaching. Rhodamine-123 fluorescence provided a more sensitive index of patulin-induced cytotoxicity. At doses between 0.1 and 1.0 μM, reduction in mitochondrial membrane potential was indicated by a depression of rhodamine-123 fluorescence compared to controls. Further, at doses of 10 μM or greater, significant increases in both mitochondrial and cellular fluorescence suggested irreversible damage to mitochondrial function. These studies support the use of vital fluorescence assays as rapid and sensitive screening approaches to assess cellular toxicity (Supported by the Texas Agricultural Experiment Station and TAES Project H6215).


Toxicokinetic studies of p-chloro-α,α,α-trifluorololene (CTFT) after administration as an aqueous α-cyclodextrin (CD) molecular encapsulated suspension or as a corn oil (CO) solution have been shown to be bioequivalent. The toxicity of CTFT was assessed in F344 rats and B6C3F1 mice administered CTFT daily for 14 days by the gavage route using both the cyclodextrin and corn oil as vehicles. Body and organ weights, clinical chemistry, hematology, and a complete histopathology examination was performed. Tissue levels of CTFT in both rats and mice were determined for kidney, liver and blood and α-2u globulin concentration in the kidney of male rats was also measured. For both vehicles there were treatment-related changes in both rats and mice in serum chemistry (increased bile acids, cholesterol, triglycerides, & phospholipids) and histopathologic effects in liver (hyperplasia) and adrenal cortex (cytoplasmic vacuolation). However, the primary toxic effect was seen in the kidney of male rats. A dose-related increase in the severity of a toxic nephropathy having morphologic features consistent with those described as a "hydrocarbon or hydrocarbon derivative nephropathy" was present, in a dose dependent manner, in male rats administered CTFT in both the CD and CO vehicle. Nephrotoxicity, as demonstrated by light and electron microscopy, increased with concentration of CTFT and α-2u globulin in kidneys of dosed male rats. These results demonstrate the utility of using α-cyclodextrin as a substitute for corn oil in toxicity studies of CTFT.

INTRACELLULAR GLUTATHIONE CONTENT CORRELATES WITH THE EXTENT OF GAP JUNCTIONAL INTERCELLULAR COMMUNICATION IN RAT-1 FIBROBLAST VARIANTS WITH ABNORMAL GLUTATHIONE CONTENT. S E Masta, G Raghu, G M Hrad, J K Kadish, and G W Bednarski. Departments of Medicine, Environmental Health, and Pathology, Univ. of Washington, Seattle, WA. Sponsor: E M Faustman.

Gap junctional intercellular communication (GJIC) is a form of cell-cell communication thought to be important in development and differentiation, and in the control of cell proliferation, as many teratogens and tumor promoters block GJIC. Previous work with a Chinese hamster V79 cell line selected for deficiency in GJIC revealed an abnormally low glutathione (GSH) level when these cells were replated from confluence. We have isolated Rat-1 fibroblast variants with alterations in intracellular GSH content using fluorescence activated cell sorting and the GSH viable stain monochlorobimane (MCB). We decided to test whether these variants would display abnormal GJIC. We assessed GJIC by the scrape loading dye transfer technique. Five out of seven variants with GSH content higher than the parental cells underwent GJIC to a greater extent than Rat-1 cells, one displayed similar GJIC and one had lower GJIC than the Rat-1 cells. Of four cell lines tested with low GSH, two showed virtually no GJIC and two were similar to the Rat-1 cells. These results signify a role for GSH or some covariant factor in the control of GJIC, and suggest that agents which perturb GSH may adversely impact upon the development, differentiation and the control of cell proliferation by interfering with GJIC.

CHARACTERIZATION OF LIMITING VOLUMES OF ISOTONIC SALINE OR DEXTROSE ADMINISTERED BY RAPID INTRAVENOUS INFUSION IN THE DOG. G Walzel, WA Mann, T Briner and LB Kinter. SmithKline Beecham Pharmaceuticals, Dept of Toxicology, King of Prussia, PA. Sponsor: T Leonard.

Toxication evaluations of i.v.-administered drugs are frequently limited by the volume of the drug solution to be infused. These studies were designed to determine maximum volumes of isotonic saline (0.9%) and dextrose (5%, DSW) that could be administered i.v. over 60 min, without contributing effects that might confound detection of drug toxicities. Following a 30 minute equilibration period, 18, 60, or 180 mg/kg of saline or DSW was infused over 60 minutes (0.3, 1.0, or 3.0 mL/min/kg) to 4 conscious beagle dogs (2M/2F) prepared for cardiovascular and renal monitoring; a 60 minute recovery period followed the infusion. Volume-dependent increases in urine flow rate and decreases in urine osmolality were associated with both saline and DSW, heart rate was increased up to 80% while arterial pressure was unchanged. Notably, serum osmolality decreased in a volume-dependent fashion with the DSW infusion only, and continued to decrease during the recovery period. Hematocrit decreased (-25%) during infusion of 180 mg/kg of saline and DSW; hematocrit remained depressed following the saline infusion but rebounded to elevated levels (+24%) following the DSW infusion. Adverse clinical signs occurred only with the higher DSW volumes and were consistent with the developing hyponatremia. We conclude: 1) In conscious dogs i.v. infusion of ≥60 mg/kg saline is associated with significant hemodilution and tachycardia while infusion of ≥18 mg/kg DSW is associated with the above plus a delayed hyponatremia associated with CNS disturbances; and 2) The presence of a metabolizable osmotic solute in the drug vehicle may present delayed toxic effects in studies in which large volumes are infused.
1061 VENOUS INFUSION: AN AMBULATORY SYSTEM FOR CONTINUOUS ADMINISTRATION IN DOGS. S C Denault, F Morril, G Haggerty and G Miller. G D Searle & Co., Skokie, IL.

There is an increasing need for the development of continuous infusion techniques in dogs and rats suitable for the early assessment of candidate intravenous agents. Proposed USDA regulations which require routine exercise for dogs will create additional challenges regarding the maintenance of in-dwelling catheters for extended periods in this species. A technique for implanting and maintaining intravenous catheters in dogs which incorporates the use of ambulatory infusion pumps has been developed at Searle to meet these needs. In a feasibility study, vascular access ports were implanted in twenty-four beagles (Hazleton). Customized "jackets" designed to house ambulatory pumps were fitted to each animal eliminating the need for restrictive tethers. Animals were continuously infused for four weeks following a three week post-surgical phase. Clinical chemistry, hematology, electrocardiography, physical examination and pathology evaluations were conducted on all catheterized animals.

1063 RAPID DETECTION OF ANTICHOLINESTERASES BY AN ENZYME BIOSENSOR. M E Eldefrawi, K R Rogers, C J Cao, J J Valdes and A T Eldefrawi. Dept. Pharmacol. & Exper. Ther., Univ. Maryland, School of Medicine, Baltimore, MD; Biotechnology Division, U.S. Army Research Development & Engineering Center, Edgewood, MD.

An optical sensor, constructed by immobilizing fluorescein isothiocyanate (FITC)-tagged eel acetylcholinesterase (AChE) on quartz fibers, was used to detect anticholinesterases (AntichEs). The pH-dependent fluorescent signal generated by FITC-AChE was quenched with protons produced by ACh hydrolysis. Analysis of the fluorescence response showed Michaelis-Menten kinetics with a Km of 0.42 mM for ACh hydrolysis. AntichEs interfered with Ebert's hydrolysis and quenching of the fluorescent signal. Reversible inhibitors gave fast inhibition and reactivation. Enzyme reactivation was slower with carbamates and very slow after exposure to organophosphates. The biosensor detected ppb to ppm concentrations of several carbamate and organophosphate insecticides. (Supported in part by U.S. Army CRDEC grant DACA15-89-C-0007. M.E.E.) and NIH grant ES02594 (A.T.E.).

1062 A COMPUTER PROGRAM FOR EASY CALCULATION OF THE MEDIAN-EFFECTIVE DOSE. R D Thompson, Y Alaie and M Schaper. University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA.

In 1947, W. R. Thompson developed the method of moving-averages interpolation (Bact. Rev. 11, 115-145) to estimate the median-effective dose (ED50 or ED50) for use in toxicity studies. In 1952, Thompson and Weil described the construction of tables to determine the ED50 and Weil published two extensive tables to simplify the calculation of the ED50. A computer program was recently written for use in our laboratory which calculates the ED50. It is also based upon the method of moving-average interpolation and follows the assumptions of Thompson. This program offers several advantages over the previously published tables. First, the program ED50 is short and easy to install on a personal computer and will also provide the 95% C.L. It is user-friendly and potential human calculation errors can be avoided. Secondly, the program can accept a larger number of doses and animals than those listed in the tables. This program may be helpful for other toxicologists and is available from us for free. Supported by UAW-GM.

1064 THE ELLMAN METHOD UNDERESTIMATES THE LEVEL OF CHOLINESTERASE (ChE) INHIBITION IN TISSUES FROM CARBARYL-TREATED RATS. J A Duncan1, E Benmat2, S Padilla1 and C Pope3, 1Neurotox. Div.US EPA and 2NSI Tech. Services, RTP, NC, and 3Sch. of Pharm., NLU, Monroe, LA.

Carbaryl, a carbamate pesticide, is a reversible inhibitor of cholinesterase (ChE) while paraoxon is an irreversible inhibitor. A commonly used spectrophotometric method for measurement of ChE activity (Ellman et al, Biochem. Pharmac. 7:88-95, 1961) necessitates extensive (≥1:125; v/v) dilution of the tissue samples; however, dilution may alter the dynamic interaction of a reversible inhibitor with ChE. A radiometric assay (Johnson and Russell, Anal. Biochem., 64:229-238, 1975), on the other hand, can be conducted with only minimal tissue dilution (≥1:1.25; v/v). Plasma and brain tissue from adult male rats treated with carbaryl (75 mg/kg, po, n=3) or paraoxon (0.34 mg/kg, sc, n=3) were used for ChE determinations using the two methods. No significant difference in ChE inhibition due to method was noted in brain or plasma from the paraoxon-treated rats. On the other hand, the radiometric method detected 73% (brain) and 60%±4% (plasma) ChE inhibition in the carbaryl-treated rats compared to 47%±2 (brain) and 43±3 (plasma) ChE inhibition using the spectrophotometric method. These results demonstrate that dilution of tissue samples underestimates the extent of ChE inhibition in tissues from carbamate-treated animals.
THE USE OF THE NEUTRAL RED BIOASSAY USING NORMAL HUMAN KERATINOCYTES TO DETECT MATERIALS REQUIRING METABOLIC ACTIVATION. R Curren, K Wallace, S Valone* AND J Harbell, Division of Toxicology and Division of Biotechnology Services, Microbiological Associates, Inc., Rockville, MD, and Clonetics Corporation, San Diego, CA. Sponsor: L Yang

Recently, several in vitro cell and/or organ based models have been proposed as potential replacement assays for acute eye and skin irritation tests. In general, validations of these systems have used direct acting toxicants - the type of materials generally encountered in the cosmetics and personal care products industries. However, unpurified industrial chemicals contain trace amounts of chemicals which require metabolic activation before becoming toxic. Therefore, we used one commercially available system, the Neutral Red Bioassay Kit supplied by Clonetics Corporation, San Diego, CA, with five model compounds requiring metabolic activation. Low passage (P2) normal human epidermal keratinocytes (NHEK) were grown in serum-free media. 7,12-dimethylbenzanthracene (DMBA), benzo(a)pyrene (BaP), 3-methylcholanthrene (3-MC), cyclophosphamide (CP), and 2-aminonaphthalene (2Aa) were applied to exponentially growing NHEKs for 48 hours. DMBA, a skin carcinogen in rodents, was very cytotoxic to the NHEK (NR50 = 0.2 µg/ml). BaP had an NR50 of ~10 µg/ml. The NR50’s for MCA, 2-AA and CP were all greater than 100 µg/ml. These results show that NHEK grown under these conditions are capable of detecting a subset of compounds which require metabolic activation to exhibit toxicity. These data suggest that some of the xenobiotic metabolizing enzymes present in vivo continue to be expressed by these cells in culture.

ALTERED RESTRICTION ENZYME PROCESSING AS A MEANS FOR DETECTING CHEMICAL-INDUCED DNA-PROTEIN COMPLEXES IN INTACT CELLS. Y Chen, M D Cohen, E T Snow and M Costa, Institute of Environmental Medicine, NYU Medical Center, New York, NY.

Most common methods to detect DNA-protein complexes (DPCs) include filter binding, alkaline elution, immunological assays and/or various blotting assays. We have developed a gel electrophoresis assay to detect DPCs based on differences in restriction enzyme processing (i.e. with HindIII or EcoRI) that can readily be observed in agarose gels by monitoring shifts in ethidium fluorescence staining of DNA. The DPCs were isolated by lysing whole nuclei in sodium dodecyl sulfate (SDS) and by ultracentrifugal sedimentation in the presence of 2% SDS. Potassium chromate, K2Cr2O7, has been shown to produce a time- and dose-dependent increase in DPCs in Chinese hamster ovary cells in vitro. DNA from cells treated with low concentrations of K2Cr2O7 also shows a dose-dependent decrease in restriction enzyme cleavage due to inhibition of the enzymes by the bound protein-Cr complexes. This method to detect DPCs is simple, rapid, and sensitive. We are currently investigating Southern blot hybridization as a means to further increase sensitivity and other crosslinking agents are being tested to determine the general applicability of the assay (This research was supported by grants #ES04895 and #ES04715).

GOOD PREDICTION OF ACUTE TOXIC BLOOD CONCENTRATIONS IN MAN BY 24h CYTOTOXICITY TO Hep-G2 AND HeLa CELLS FOR THE FIRST 10 MEIC CHEMICALS. B Ekwall, M J Gómez-Lechón*, S Hellberg and S Welum, Dept. Toxicology, Univ. Uppsala, Uppsala, Sweden and Hospital La Fe, Valencia, Spain.

To evaluate the relevance for human toxicity of in vitro tests, the Scandinavian Society of Cell Toxicology has organized a Multicenter Evaluation of In Vitro Cytotoxicity (MEIC). Presently, 40 international laboratories are testing a list of 50 chemicals. A preliminary evaluation of various forms of toxicity is performed with use of the first 10 chemicals (Acetaminophen, Aspirin, Ferrous Sulfate, Diazepam, Amitriptyline, Diphenhydramine, Ethylene Glycol, Methanol, Ethanol, Isopropyl alcohol) and the first submitted 14 cellular assays (rat hepatocytes, Y79, Hep-G2 and HeLa cells combined with several toxicity criteria) as training sets. The first study in this series demonstrated a good prediction by most assays of acute lethal blood concentrations in man, in parity with rodent median lethal dose (LD50) prediction (Ekwall et al., 1992). In the present, second study 24h IC10 (10% inhibitory conc.) from these assays was compared to single-dose, clinical 4 and 12h human blood concentrations corresponding to mild/moderate poisoning of the chemicals. Judged as the variance of a direct 1:1 correlation most assays resulted in good prediction. The 4 assays with human cell lines predicted toxic 4h concentrations in man in parity with the prediction of lethality.

AN IN VITRO FLUORESCENCE ASSAY FOR THE DETECTION OF DRUG-INDUCED CYTOPLASMIC LAMELLAR BODIES. K Kilgore, L Ginsberg, E Sun, R Ulrich. Dept. Biological Sciences, Western Michigan University; and Upjohn Laboratories, Kalamaoo, MI. Sponsor: T Parry

Many cationic amphiphilic drugs and aminocyclitol antibiotics produce lysosomal phospholipidosis in experimental animals with the potential to do so in man. This phenomenon, characterized by intracellular lamellar bodies composed principally of anionic phospholipids is a safety (and hence a regulatory) issue in the registration of new pharmaceuticals. It would be of benefit to assess similar drugs before in vivo safety studies are initiated. For this, we have developed an assay to detect lamellar body induction in vitro. Liposomes were made using a fluorescent analog of phosphatidylcholine (NBD-PC). Human fibroblasts (HFF) were labelled with NBD-PC liposomes during exposure to gentamicin, amikacin, or trospectomycin at non-cytotoxic concentrations. Gentamicin- and trospectomycin-treated, but not control or amikacin-treated cells, showed a distinct fluorescent labelling of lamellar bodies. Spectrophotometric measurements of deoxycholate extracts revealed a net increase in fluorescence in higher dose groups for gentamicin and trospectomycin-treated cells above control. Though more compounds need to be evaluated in order to validate this assay, we conclude that these compounds lamellar body induction is readily detected by fluorescence microscopy and spectrofluorimetry.
1069 INHIBITION OF PROTEIN SYNTHESIS IN VERO CELLS AS A DRUG-SCREENING ASSAY FOR RICIN TOXICITY. W L Thompson and J G Pace. USAECDCD, Frederick, MD. Sponsor: R. V. Venemaheer Jr.

Ricin, a toxic glycoprotein (MW = 65,000), isolated from castor beans (Ricinus communis) causes death in animals by inhibiting protein synthesis. An in vitro assay for inhibition of protein synthesis (PSI) in Vero cells was developed to test a wide variety of drugs for possible therapeutic use against ricin intoxication. A short toxin exposure time (4 hr) was used to decrease variability and minimize assay time. Two concentrations of ricin were used; a dose resulting in 50% PSI (ED50 = 7.5 ng/ml) and a dose which caused complete PSI (ED90 = 0.1 µg/ml). Most drugs tested had no effect on ricin-induced PSI. Some were toxic (quinacrine, cyclosporin A, nifedipine, verapamil, danysalcydavine, diamide) or enhanced the toxicity of ricin (chloroquine, nigericin, monoplatin, osininosine). Only brefladin A (BFA), galactose, lactose, and several derivatives of these sugars blocked the PSI effects of ricin. Concentrations of BFA greater than 0.5 µM caused about 50% PSI, but blocked any further PSI effects of ricin. Concentrations of BFA less than 0.1 µM, which had no effect on protein synthesis, did not significantly block the toxic effect of ricin. Galactose, lactose, and derivatives of each had 50% protection levels in a range of 1 to 12 µM against a PSI ED90 of ricin (0.1 µg/ml). None of these sugars alone had negative effects on PSI, even at concentrations above 10 µM.


Studies investigating the toxicity of polychlorinated biphenyls (PCBs) in aquatic organisms demand a reliable method to produce aqueous PCB solutions. We evaluate the generator column method not as a way of producing an aqueous solution containing a broad spectrum of PCB congeners. Solutions are created by pumping distilled water through a column packed with glass beads coated with a standard Aroclor mix (1221, 1016, 1254, and 1260, 1:1:1:1), which contains all of the PCB congeners known to have been dispersed into the environment. Water was passed through the column at a flow rate of 1.66 ml/min, well within the range of flow rates previously shown to produce saturated solutions for individual congeners. Solutions produced by the column were analyzed by high-resolution glass-capillary gas chromatography for 68 individual PCB containing peaks. The total PCB concentration in 4 replicate solutions averaged 442 ±12 µg/L. A total of 59 congeners were identified in the solution. The concentration of the PCB congeners decreased with increasing chlorine content. Two mono-chlorinated congeners (2 and 4 chlorobiphenyl) comprised greater than 65% of the total PCB concentration. The concentration of individual congeners in the solution were much lower than reported individual solubilities. This suggests that a partitioning of individual congeners between water and the oil, which is composed of the mixture of PCBs, is taking place rather than a simple dissolution. The generator column method is a rapid and reproducible technique to create aqueous solutions containing a high concentration and broad spectrum of PCB congeners. Supported in part by Superfund grant #IP02 ES04913-01(O4) to BIP.

1071 SYNCHRONOUS FLUORESCENCE AND GAS CHROMATOGRAPHY/ELECTRON CAPTURE (GC/ECD) MEASUREMENT OF PYRENE METABOLITES IN RAT URINE. E L C Lin, T V Reddy, C W Guion, J W Glass and F B Daniel. USEPA, HERL, Cincinnati, Ohio

Two quantitation methods, synchronous fluorescence scan and GC/ECD, were investigated as possible biomonitoring methods for exposure to pyrene, the most abundant representative of the environmentally occurring polycyclic aromatic hydrocarbons. Administration of pyrene to rats resulted in the excretion (16% of dose in 24 hr) of fluorescent metabolites in urine which were mostly conjugated 1-hydroxyppyrene (HP). These fluorescent metabolites were isolated by absorption to C18 Sep-pack cartridges and eluted with 50% MeOH. About 90% of the urinary radioactivity was recovered by this isolation method when urine of rats dosed with 3H-pyrene was used. Quantitation by synchronous fluorescence scan (λ=370nm) after dilution of at least 10-fold showed linear dose response between 0.1-10 µmol/kg. Treatment of urine with sulfatase/β-glucuronidase followed by isolation with C18 Sep-pack cartridges showed that about 60% of the radioactivity was isolated as HP. Derivatization with pentafluoropropionic anhydride to pentafluoropropionic acid ester and quantitation by GC/ECD gave linear response down to 0.1 µmol/kg of pyrene administered. In view of its simplicity, the synchronous fluorescence scan is the preferred detection method. (Abstract does not necessarily reflect EPA policy).

1072 METHOD DEVELOPMENT AND VALIDATION FOR THE DETERMINATION OF PPM LEVELS OF BORON IN RAT TISSUES. RF Moseman', RE Brink', CW Jameson', KA Treinen' and RE Chapin'. Radian Corporation, Research Triangle Park, NC. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Boric acid has been shown to adversely affect the reproductive organs of male rats causing testicular atrophy and cellular dystrophy. As part of an investigation to study this effect in male rats, an analytical method for the determination of ppm levels of boron in various tissues was developed and validated. Whole blood, plasma and four different types of tissue samples (epididymis, kidney, liver and testis) were digested with nitric acid in a microwave oven, and analyzed by inductively coupled argon plasma emission spectrometry. Recovery of boron from spiked blood and tissue samples ranged from 88% to 110%. Precision of the method at 3µg/gm up to 30 µg/gm was generally better than 6% relative standard deviation, with correlation coefficients better than 0.996. The accuracy of the method was confirmed by relative errors of generally less than 12%.

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Citral is a naturally occurring aliphatic aldehyde found in lemon grass and all citrus fruits and is widely used as a flavor and fragrance additive. Because of its volatile and reactive nature, the NTP has micro-encapsulated this chemical for use in prechronic and chronic toxicity studies. In order to evaluate the bioavailability of the microencapsulated material, a method to determine Hildebrandt acid, a major metabolite of Citral, in blood was developed and validated. Spiked blood samples (0.2 gm) were extracted with 5.8 mM NH₄OH, mixed with 4-butylbenzoic acid internal standard and analyzed by HPLC/UV. Recovery of the compound from spiked blood samples ranged from 90 to 97%. Precision of the method at 9 µg/gm and 95 µg/gm was better than 4% relative standard deviation, and the correlation coefficient over this range of spiked samples was 0.997. The accuracy of the method was confirmed by relative errors of less than 10%.

1074 TERTERATINE DETECTION BY IMMUNOASSAY IN EQUINE URINE. J Bowden, E W Woods, D Watt, H-H Tai, B Blake and T Tobin. Department of Veterinary Science and Graduate Center for Toxicology, University of Kentucky, Lexington, KY.

Terbutiline is a β₂-selective broncodilator which may be illegally used in racing horses. We have raised antibodies in a one step enzyme-linked immunosorbent assay (ELISA) for terbutiline as part of a panel of post race tests for drugs abused in racing horses. The terbutiline test shows high sensitivity to terbutiline with an I-50 of 0.8 ng/ml. Cross reactivity studies show that the terbutiline ELISA reacts well with salbutamol and clenbuterol in equine urine. Analysis of 40 post-race urine samples has shown background levels less than 0.9 ng/ml. After administration of 1.5 mg/horse of terbutiline by subcutaneous injection, the presence of parent drug or its metabolites are detected for 8 hours in equine urine. No sample treatment or minimal sample dilution of 1:2 is required for this assay. This terbutiline ELISA, therefore, readily detects terbutiline, salbutamol, and clenbuterol in samples from racing horses and as such will be useful in equine medication control. (Supported by Kentucky Equine Drug Research Council and Kentucky State Racing Commissions.)

1075 DETECTION AND METABOLISM OF DETOMIDINE IN THE HORSE BY ENZYME-LINKED IMMUNOSORBENT ASSAY. J M Yang, T Wood, H Tai, D Watt and T Tobin. The Graduate Center for Toxicology and Department of Veterinary Science, University of Kentucky, Lexington, KY.

Detomidine is a potent non-narcotic sedative/tranquilizer that has been approved for use in veterinary practice. Like all tranquilizers, detomidine can be used in small doses prior to racing to improperly influence performance. Because detomidine is highly potent, sensitive non-isotopic immunoassay based detection methods are required for effective clinical and forensic monitoring. Antibodies against detomidine carboxylic acid, a major detomidine metabolite, were raised and incorporated into an ELISA test. Using this test, plasma and urinary levels of detomidine and its metabolites were determined in post-race and dosed horse samples. The detection limit of this test in equine urine is 0.5 ng/ml, which allows detection of parent drug for up to 8 hours after a dose of 1 mg/horses. Specific extraction methods were developed to distinguish parent drug and metabolites. In vivo metabolic profiles of detomidine were analyzed by a combination of HPLC and ELISA. We propose that metabolism of detomidine in equine is mediated via the cytochrome P-450 system and that its major metabolites are detomidine carboxylic acid and hydroxydetomidine O-glucuronide.

Supported by American Horse Show Association and Kentucky Equine Drug Council.


The popular use of immunoassays for urine screening for amphetamine and its congeners produces complications due to the differential binding characteristics of the d- and l- isomer and concentration dependent crossreactivities. Two Abbott fluorescence polarization immunoassays (FPIA) and two Syva enzyme multiplied immunoassay technique (EMIT) assays were evaluated based on their response to standards containing d-amphetamine, d-methamphetamine or both drugs in combination. The Abbott ADx® and TDx® results were similar (crossreactivity of approximately 100% for d-amphetamine). d-Methamphetamine showed a greater than 110% crossreactivity at all concentrations tested and did not agree with d-methamphetamine crossreactivity data published by Abbott. Differences in optical purity of the d-methamphetamine standard may account for these inconsistencies. The ADx® and TDx® showed an enhanced response when d-amphetamine and d-methamphetamine were present in the same sample. Results between Syva assays were not comparable. Assuming the crossreactivity of the d-methamphetamine calibrator to be 100% for the Syva EMIT®-d.a.u.® monoclonal assay, crossreactivity at the assay cutoff for d-amphetamine was 1000%. d-Amphetamine and d-methamphetamine crossreactivity at concentrations near the assay cutoff for the EMIT®-d.a.u.® polyclonal kit was approximately 100%, with d-methamphetamine being slightly greater. The variations in the assay responses to different isomers (d- or l-) and different compounds (amphetamine or metamphetamine) may be due to functional differences in optical purity and antibody specificity. Caution is advised when relating positive/negative results to specific cutoff concentration.

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A FIELD PRACTICAL METHOD FOR RAPID DETECTION AND SEMI-QUANTITATION OF AFLATOXIN M1 AND SULFAMETHAZINE RESIDUES IN MILK. Z C Huang, C Y Hwang, B A Clement, R H Bailey, J A Ellis, and T D Phillips. Department of Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX.

Although several analytical procedures have previously been developed for the detection of mycotoxins, few of these procedures can be used to provide rapid and reliable results outside of a laboratory environment. In addition, none of the previous screening procedures could be used as a single test to screen for the presence of a mycotoxin and an antibiotic in a sample. The development of technology for the selective adsorption of mycotoxins (SAM) has provided a technically simple analytical test that can be used in the field for rapid preliminary screening of milk for the presence of aflatoxin M₁ (AFM1) and sulfamethazine (SMZ). Milk was passed through a C₁₈ SepPak, then AFM1 and SMZ were eluted with methanol/water. The eluant was treated with fluoresceinamine in order to derivatize SMZ. A binary SAM column was employed to detect AFM1 (bright blue fluorescence) and SMZ (intense yellow fluorescence). The assay requires less than 20 min to complete. Detection limits were < 0.5 ppb for AFM1 and SMZ (Supported by Texas ATP Project 3842, USAID Project 02-50305-2 and TAES H6215).


Lime processing of corn (moxatamalization), has resulted in an apparent reduction in the level of aflatoxin detected in the final product. Typical methods of detection of the aflatoxins are based on fluorescence observed when these chemicals are viewed under longwave UV light. This fluorescence is associated with the coumarin portion of the aflatoxin molecule. Alkaline treatment and amination, can temporarily open the lactone ring (by salt formation) and thereby terminate this fluorescence. This salt, which cannot be detected by traditional methods of analysis, may spontaneously reform aflatoxin upon acidification. This study was designed to determine: (i) the degree of reformation of aflatoxins upon acidification of the lime processed product, and (ii) the distribution and levels of aflatoxins in the different stages of processing. HPLC was used to quantify extractable aflatoxin (with and without acidification) at each stage of the process. Results demonstrated that different aflatoxins (representative of the B and G series) were unequally distributed throughout the various stages of the process. Acidification resulted in significant reformation of the aflatoxins. This work suggests that current methods of aflatoxin analysis should be modified for alkaline processed corn products, i.e., tortillas and chips (Supported by USAID Project 02-50305-2 and T AES H6215).

UTILIZATION OF IN VITRO BIOASSAYS TO ASSESS THE EFFECTIVENESS OF CHEMISORPTION OF AFLATOXIN B₁ BY HYDRATED SODIUM CALCIUM ALUMINOSILICATE. M D Machen, K Mayura, B A Clement, and T D Phillips. Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX.

Sufficient evidence exists to regard aflatoxin B₁ (AFB₁) as a potent chemical carcinogen and mutagen. Sorption of AFB₁ by different classes of phyllosilicates as a method to control contaminated foodstuffs has been proposed. This study was designed to evaluate the effectiveness of chemisorption by hydrated sodium calcium aluminosilicate (HSCAS) by the use of aflatoxin-sensitive microbial and developmental in vitro bioassays. These included: (i) Salmonella/histidine auxotroph (strains TA98 and TA100) for detection of frameshift and base-substitution mutagenic events (Ames Assay) (ii) Bacillus subtilis RecE bioassay for the detection of increased lethal damage to DNA (iii) cultures of Hydrobacter (HAB) and postimplantation rat embryos (WEC) to detect developmental toxicity. The effects of AFB₁ at doses ranging from 0.1-1.0 ug/plate (Ames), 10-50 ug/plate (Rec), 5-50 mg/L (HAB), and 5-25 mM (WEC) were established. Samples containing 8 ppm AFB₁ were treated with 0.02% HSCAS (w/w). HSCAS treatment resulted in a marked reduction in the diverse toxicities of AFB₁ (as measured by these in vitro bioassays) (Supported by USAID Project 02-50305-2 and T AES H6215).

The fresh water coelenterate Hydra attenuata is used for a developmental toxicity screening assay and, in a modified form, an aquatic toxicity assessment of effluents. Because Hydra are markedly insensitive to UV irradiation it occurred that it might also be used as a photosensitization assay. There are but few in vitro phototoxicity assays available and if one could be devised with Hydra, marked efficiency would result from use of the same animal for multiple assays. Preliminary studies used the known photosensitizers 6-Methoxypsoralen (8-MOP) and anthracene, and the non-sensitizers colchicine and sodium cyclamate to explore the potential and feasibility of using hydra for prescreening assessments of phototoxicity. Adult Hydra were exposed to a series of concentrations of each chemical for 24 hrs followed by 24 hrs in standard hydra medium. One-half of the test populations received exposure of UVA radiation during the first 16 hrs of chemical exposure. Except during actual UV exposure periods, all animals were maintained in total darkness. Nonaqueous chemicals were dissolved in ethanol (final conc. < 1%). A Blakray lamp (20mW/cm² with peak emission at 365nm) was placed 29 cm from the test dishes. A phototoxicity index was determined through the comparison of light and dark NOAE concentrations. Results. Adult Hydra in their usual media are relatively insensitive to UVA radiation. Exposure to the known photocarcinogens 8-MOP and anthracene resulted in a 100X increase in toxicity in the presence of UVA. The same UVA exposure with sodium cyclamate and colchicine resulted in little or no change suggesting that phototoxicity assessment may be possible using Hydra attenuata.

A METHOD TO MEASURE SKIN:AIR PARTITION COEFFICIENTS FOR VOLATILE CHEMICALS. D R Mattie, G D Bates, G W Jepson, and J N McDougal. Toxic Hazards Division, Armstrong Aerospace Medical Research Laboratory, Wright-Patterson AFB OH.

The affinity of a chemical for the skin is an important factor affecting the dermal penetration of chemicals. The partition coefficient (PC), a measure of the affinity of a chemical for a tissue, is the ratio of concentrations between the tissue and an adjacent media, such as air, water or other vehicle at equilibrium. Tissue:air PCs for volatile chemicals have been measured via headspace analysis of a homogenate in saline. Skin does not homogenize easily; therefore, the goal of this study was to develop a simple, rapid, alternative method to measure skin:air PCs. Important considerations in the development of this method were effect of size and shape of skin sample, dependence on concentration, and time to equilibrium. Shaved, whole thickness skin was obtained from the dorsal surface of 11-16 week old male F-344 rats. After removal of the hypodermis, skin was cut into strips and placed on the side of a glass vial. Dibromomethane vapor (207ppm) was introduced into the sealed sample vial, and a corresponding reference vial, which were equilibrated at 32°C. Gas chromatographic determination of headspace concentrations resulted in a skin:air PC of 68.3. This method provides useful predictive information about skin penetration of volatile chemicals.

ASSESSING THE MILDNESS OF ANIONIC SURFACTANT SYSTEMS WITH THE MICROTOX™ LUMINESCENT BACTERIA TEST (LBT), THE TETRAHYMENA MOTILITY ASSAY AND TESTSKIN™ LIVING DERMAL EQUIVALENT (LDE) MULTIPLE ENDPOINT TESTS. J E Heinze, T J Stephens, T D Swedland and P M Sliper. Vista Chemical Company, Austin, TX and In Vitro Alternatives, Inc. Dallas, TX.

The comparative irritancies of surfactants are usually established by applying occlusive patches of test materials to the skin of animal or human subjects. This research was undertaken to see if the Microtox™ LBT, the Tetrahydrena motility assay, and the TESTSKIN™ LDE cytotoxicity/Inflammatory mediator release test could predict the irritation potential of five surfactants commonly used in consumer products. Test materials consisted of purified sodium lauryl sulfate, sodium C12-14 alcohol sulfate, sodium C12-14 alcohol ether (3) sulfate, sodium C12-14 ether (7) sulfate, and sodium coco isethionate. Test materials were coded and tested in duplicate. All three in vitro tests successfully discriminated between the toxicity of the test materials. The Microtox™ LBT and TESTSKIN™ LDE multiple endpoint assays provided results which were most consistent with published data on the mildness of these five surfactants. Therefore, in vitro tests appear to be useful screening tools for evaluating the mildness of anionic surfactants.

EVALUATION OF THE EYTEX™ SYSTEM, A n IN VITRO OCULAR IRRITATION ASSAY. CP Sabatis and SKJ Leong. The Upjohn Co., Kalamazoo, MI.

The EYTEX™ System is a commercially available in vitro assay for primary eye irritation. An EYTEX™, Draize equivalent score (EDF), which is theoretically equal to a 24 hour in vivo Draize score, and pH values were determined for 15 compounds. These compounds were previously classified by the Upjohn modified in vivo Draize test (UJMOT) scores at peak response. The UJMOT uses only 1 or 2 animals instead of the standard 6 per dose level. The comparison of the results showed that the EYTEX™ System classified 4 liquids and 1 powder at the same level of irritancy as their UJMOT classification; 2 liquids and 4 powders at 1 to 2 levels more irritating; and another 1 liquid and 3 powders at 1 to 5 levels less irritating. The coefficient of correlation (r) for the scores of EDF and the 24-hour UJMOT were r=0.56 for all compounds, r=0.73 for liquids, and r=0.29 for powders. The r for EYTEX™ and the UJMOT classification were r=0.32 for all compounds, r=0.56 for liquids, and r=0.11 for powders. The r values for EDF scores and the pH value were r=0.45 for all compounds, r=0.6 for liquids and r=0.29 for powders. The low r values suggested that a pH value and an EYTEX™ assay result may not be accurate predictors of the irritancy of a test material in many instances.
EVALUATION OF SIX IN VITRO TOXICITY ASSAYS; COMPARISON WITH IN VIVO OCULAR AND DERMAL IRRITATION POTENTIAL OF PROTOTYPE COSMETIC FORMULATIONS.

There has been an increasing interest in the potential of in vitro toxicity assays to serve as accurate and inexpensive non-animal predictive models of in vivo ocular and dermal irritation potential for many types of products and raw materials. In this study sixteen different cosmetic formulations including oil-in-water emulsion systems, hydroalcoholic products, and cleansers were evaluated using the EYTEX™ assay, the choriocapillaris membrane vascular assay (CAMVA), Microtox™, the Tetrahymena motility assay, and the TESTSKIN™ Living Dermal Equivalent (LDE™) cytotoxicity assay. These in vitro analyses were compared to historical in vivo ocular and dermal irritation data for these same test materials. Rank-order analyses and regression modeling indicate that CAMVA, the Tetrahymena motility assay, the LDE™ cytotoxicity assay, and the Microtox™ assay all exhibited good correlation with corresponding in vivo data. In contrast, neither the EYTEX™ nor the TESTSKIN™ assays exhibited good correlation with the in vivo data.

RELEASE OF PGE 2 FROM CORNEAL FIBROBLASTS INDUCED BY EXPOSURE TO OCULAR IRRITANTS.
T A Ellick, D K Wilcox & D Roberts. Miami Valley Laboratories, The Procter & Gamble Co., Cincinnati, OH. Sponsor: G P Daston

To aid in developing alternatives to ocular irritancy testing, the ability of ten proprietary mixtures (shampoos, household cleansers, laundry detergents and dishwashing liquids) to induce release of the eicosanoid PGE 2 was tested on a corneal fibroblast cell line, SIRC. PGE 2 is known to play a role in the ocular response to irritation and inflammation. The in vivo maximum average irritancy scores (MAS) for these materials in the Lov Volume Eye Test ranged from 0 - 44. Cultures of SIRC cells, 2 to 3 layers thick, were prepared on permeable culture membranes coated with extracellular matrix. Each test material, in its native undiluted form (10 ul of liquid or 10 ug of solid), was placed directly on the cell layer for times ranging from 30 to 120 seconds. Following removal of the test material the release of PGE 2 was measured over a 30 minute time period. A good correlation (r = 0.80) was observed between PGE 2 release and MAS scores. These results suggest that this model may be useful in the development of in vitro alternatives to ocular irritancy testing.

POTENTIAL CYTOTOXICITY OF SURFACANTS EVALUATED WITH PRIMARY CULTURES OF RABBIT CORNEAL EPITHELIAL CELLS. R L Grant, C Yao, and D Acosta. College of Pharmacy, Univ. of Texas at Austin, Austin, TX.

The Draize eye test has been criticized as inhumane with poor accuracy and reproducibility among different laboratories. This investigation was undertaken to develop cytotoxicity assays using primary cultures of rabbit corneal epithelial cells as an in vitro method for assessing ocular toxicity of xenobiotics. Leakage of the cytosolic enzyme lactate dehydrogenase (LDH) into the medium, morphological alterations, neutral red uptake, and (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction were measured at 1 hr and 24 hrs. The surfactants, benzalkonium chloride (BzCl), sodium dodecyl sulfate (SDS), and Tween 20 (Tw20) were compared and ranked according to irritation potential. EC50 for 24 hr LDH leakage and cytotoxicity potential were: BzCl (27 ug/ml) > SDS (98 ug/ml) > Tw20 (4000 ug/ml). Neutral red and MTT EC50 values were similar to LDH EC50 values. Cytotoxicity was also evaluated at 72 hrs after exposure by measuring aldolase and LDH specific activity and protein content as endpoints. EC50 values obtained from all 72 hr tests were similar to EC50 values at 24 hr LDH leakage. There was greater recovery of LDH activity when compared to aldolase activity and protein content at comparable concentrations of surfactants. We conclude that cytotoxicity assays using primary cultures of rabbit corneal epithelial cells ranked irritation potential that corresponded to in vivo irritation rankings: BzCl > SDS >> Tw20. (Supported by a grant from Johnson & Johnson.)

SERUM-FREE CULTURE OF PRIMARY RABBIT CONJUNCTIVAL EPITHELIAL CELLS AS AN IN VITRO TOXICITY TEST SYSTEM. C Yao, R L Grant and D Acosta. Dept. of Pharmacology and Toxicology, University of Texas, Austin, TX.

Rabbit conjunctiva, cornea and iris have been used to evaluate and rank the ocular irritation potential of chemicals in the Draize eye irritancy test primarily by subjective criteria. In order to develop a more objective and biochemically a primary culture method for rabbit conjunctival epithelial cells has been developed in our laboratory as a potential in vitro method for ocular toxicity testing of xenobiotics. Conjunctival epithelial cells were dispersed by Dispase II, followed by trypsin treatment. Cells were cultured in serum-free medium of 1:1 ratio of Dulbecco’s Modified Eagle Medium (DMEM) and F-12 nutrient mixture plus various concentrations of growth factors. At a plating density of 85,000 cells/cm², cells grew to confluency in 4-5 days. Conjunctival cells showed a positive antikeraatin stain which demonstrated that they were epithelial in origin. These cells also showed a positive periodic-acid-Schiff (PAS) stain which is consistent with their mucin-secreting function in vivo. Three surfactants, benzalkonium chloride (B2), sodium dodecyl sulfate (SDS), and Tween 20 (T-20) at concentrations 2-20, 10-50 and 200-1500 µg/ml, respectively, were evaluated for their potential cytotoxicity. Cell injury was assessed by lactate dehydrogenase leakage (cell membrane integrity), up-take of neutral red (lysosomal membrane activity), and the reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide, MTT (mitochondrial metabolic activity). Results indicated that the potential for causing cell injury was in the order of B2 > SDS >> T-20. (Supported by a grant from Johnson & Johnson.)
COMPARISON OF PRIMARY CULTURES OF CORNEAL AND CONJUNCTIVAL EPITHELIAL CELLS IN THE EVALUATION OF POTENTIAL CYTOTOXICITY OF SURFACTANTS. D M Gabaldon, C Yao, R L Grant and D Acosta, Dept. of Pharmacology and Toxicology, University of Texas, Austin, TX.

Ninety percent of the Draize score is based on the contribution of rabbit conjunctival and corneal responses to ocular toxicants. With the importance of these two tissues to the Draize test as a rationale, we have recently developed primary cultures of conjunctival and corneal epithelial cells as in vitro model systems for ocular toxicity testing of xenobiotics. This investigation was initiated to compare the response sensitivity of these two cell types to surfactant-induced cytotoxicity. Benzalkonium chloride (BZ), sodium dodecyl sulfate (SDS), and Tween 20 (T-20) at concentrations 1-20, 10-60 and 250-2000 μg/ml, respectively, were evaluated for their potential cytotoxicity. Cell injury was assessed by lactate dehydrogenase leakage, LDH (cell membrane integrity), uptake of neutral red, NR (lysosomal membrane activity), and the reduction of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide, MTT (mitochondrial metabolic activity). LDH, NR and MTT results indicated that the potential for causing cell injury was in the order of BZ > SDS > T-20 for both cell types, which is consistent with the in vivo ranking as severe, moderate and mild, respectively. A comparison of 24 hour LDH leakage and MTT results showed that conjunctival epithelial cells were more sensitive than corneal epithelial cells in assessing surfactant cytotoxicity in vitro (calculated 24 hour LDH EC50 values for BZ, SDS, and T-20 were 4, 40 and 531 for conjunctival epithelial cells, and 7, 47, and 1000 μg/ml for corneal epithelial cells, respectively). (Supported by a grant from Johnson & Johnson.)

IN VITRO EVALUATION OF OCULAR IRRITANTS USING CORNEAL PROTEIN PROFILES. T E Burell1, J M Sinn1, and C L Alden2. 1Dept. of Veterinary Biosciences, University of Illinois, Urbana, IL and 2Procter & Gamble Company, Cincinnati, OH.

Ocular irritancy tests are often limited by factors such as the discomfort experienced by experimental animals and the difficulties associated with standardization and extrapolation of test results. We have developed a semi-quantitative in vitro technique, based on tissue isoelectric focusing of rabbit and human corneal specimens, which will provide new information for the evaluation of irritant-tissue interactions and the extrapolation of ocular irritancy test results. The in vitro exposure of corneal sections to acetic acid (1% and 4%) and ammonium hydroxide (0.25% and 2%) solutions resulted in biochemically selective modifications of tissue protein profiles which were evaluated by scanning densitometry. Direct comparison of the rabbit and human corneal protein changes induced by in vitro irritant action revealed a similar acid/base effect on a common rabbit and human corneal protein band (pI≈8.1).

A DOSE-RESPONSE STUDY ON THE TOXICITY OF THE VESICANT ARSENICAL, LEWISITE, IN THE ISOLATED PERFUSED PORCINE SKIN FLAP. J R King and N A Monteiro-Riviere, Cutaneous Pharmacology & Toxicology Center, NC State University, Raleigh, NC.

Lewiste (L) is a toxic arsenical and a potent cutaneous vesicant. The isolated perfused porcine skin flap (IPPSF) has shown utility in toxicity studies of vesicant compounds. The objective of this study was to evaluate IPPSF responses after topical exposure to 6 l concentrations ranging from 0.07 to 5.0 mg/ml. Parameters examined included measures of biochemical viability, vascular resistance (VR) and pathology. The 5.0 mg/ml dose was in the “most affected” position for all parameters in which differences were noted. This dose resulted in the lowest glucose utilization, the highest LDH release, the most severe histological and ultrastructural lesions, and significant changes in non-specific esterase and alkaline phosphatase staining. All doses resulted in elevations of VR. All doses resulted in gross vesication which is unique among in vitro systems and many in vivo models of vesicant toxicity. The lower doses did not demonstrate a linear dose-response suggesting either a lack of parameter sensitivity, a non-linear dose-response curve or doses that were below the linear portion of the dose-response curve. The IPPSF appears to be a relevant and humane alternative to in vivo studies of cutaneous vesicants and offers the unique ability to document the vascular effects of these compounds in a viable, full-thickness skin system. (Supported by USAMRDC DAMD17-87-C-7139)
IN VITRO EVALUATION OF BIS-2-CHLOROETHYL SULFIDE INDUCED VESICATION IN PERFUSED PORCINE SKIN. N.A. Monteiro-Riviere, J R King and T O Manning, Cutaneous Pharmacology and Toxicology Center, North Carolina State University, Raleigh, NC, 27606.

Sulfur mustard (bis-2-chloroethyl sulfide; HD) is a potent cutaneous vesicant. High doses of HD cause severe bullous-type vesicles in human skin. The objective of this study is to evaluate and characterize HD toxicity in the isolated perfused porcine skin flap (IPPSF). Twenty-eight IPPSF's were treated with 0.2, 0.5, 1.25, 2.5, 5.0, 10.0 mg/ml of HD and controls (n=6/dose). Biochemical, physiological and morphological endpoints were used to define a dose response profile. HD decreased glucose utilization at all doses throughout the 8 hr perfusion period and increased skin flap vascular resistance. Crossly, blisters were observed at the higher doses. Light microscopy (LM) demonstrated focal basement membrane separation and basal cell pyknosis at the higher doses. Transmission electron microscopy (TEM) was consistent with these findings. For all parameters, higher doses resulted in a more severe response with morphological changes being most closely correlated to dose. Enzyme histochemistry did not show an HD effect. In conclusion, LM and TEM on the IPPSF produced lesions similar to those seen in humans upon exposure to HD and should be a useful in vitro model for vesicant research. [Supported by USAMRDC DAMD17-87-C-7139]


It has been proposed that sulfur mustard (HD) may indirectly activate poly(ADP-ribos) polymerase (PARP) by alkylating cellular DNA. Activation of PARP results in the depletion of cellular NAD, which initiates a series of biochemical processes that have been proposed to culminate in blister formation. Preventing PARP activation and NAD depletion should inhibit blister formation. Niacinamide is both an inhibitor of PARP and a precursor for NAD synthesis. The present study was undertaken to determine whether niacinamide can protect against HD-induced microvesication in cutaneously exposed hairless guinea pigs. Each site was exposed to HD for 8 min by means of a vapor cup. Niacinamide (750 mg/kg, ip) given as a 30 min pretreatment inhibited microvesicle formation by 50% after HD application. However, niacinamide given 2 hr after HD application did not reduce microvesicle formation. There was no benefit when niacinamide was given as both a pretreatment and treatment when compared to niacinamide given only as a pretreatment. The reduction in microvesication 24 hr after HD did not correlate with skin NAD content. Niacinamide did not reduce the degree of erythema and edema. Ballooning degeneration of basal epidermal cells was present in some niacinamide pretreated HD exposure sites. These results suggest that niacinamide reduces the incidence of microvesicle formation due to HD, but does not reduce the degree of erythema or edema. Maintenance of skin NAD content may not be solely responsible for inhibiting microvesicle formation and inhibition of PARP may be of greater importance.

1095 A MULTI-PHASED SCREEN FOR THE EVALUATION OF TOPICAL SKIN PROTECTANTS AGAINST VARIOUS CHEMICAL AGENTS. D W Hobson, R S Snider, and G S Dill, Battelle Memorial Institute, Columbus, OH. Sponsor: C T Olson

A multi-phased screen involving both in vivo and in vitro tests was developed and used to evaluate the efficacy of topical skin protectants (TSP) against dermal exposure to sulfur mustard (HD), a sulfur mustard/Lewisite mixture (HL), pinocerol methylphosphonophosphate-fluoride (soman or GD), thioenyl soman (TGD) and 0-ethyl S-2-diisopropylaminoethyl (VX). TSP efficacy was assessed in vitro by dosing 0.5 μL of either GD, TGD, or VX onto a 0.1 mm thick application of each TSP sandwiched between two layers of Silastic in a penetration cell and using eel acetylcholinesterase (ACHE) inhibition in 5-min fractions of the receptor fluid as an endpoint to evaluate efficacy. Assessment in vivo involved the application of chemical agents onto a 0.1 mm thickness of TSP spread on the dorsum of rabbits. ACHE inhibition in lysed red blood cells sampled daily until 24 hours after dosing was used as an endpoint. Efficacy against HD was assessed using the areas of dermal lesions from 1 μL doses applied at multiple sites on rabbits. Fourteen TSPs were ranked from best to worst for each of the evaluations performed. Polyethylene glycol (540 daltons) was included in all tests as a standard for quality control. [Supported by USAMRDC DAMD17-89-C-9050]

1096 SUBCHRONIC PERCUTANEOUS TOXICITY STUDIES OF NICLOSAMIDE (SCHISTOSOME TOPICAL ANTIPERNENT LOTION) IN GUINEA PIGS AND RABBITS. BA Myers, H Chuang, and GM Wolfe. Hazleton Washington, Vienna, VA, and Walter Reed Army Institute of Research, Washington DC.

The subchronic percutaneous toxicity of niclosamide (schistosome topical antiperenent lotion) was evaluated in two studies, one with albino guinea pigs and the other with New Zealand White rabbits. A solution of 1% niclosamide was applied daily to 10 animals/year/group for 13 weeks and the volume was varied to achieve dose levels of 4.0, 12.0, and 40.0 mg/kg/day in the guinea pig study and 3.33, 10.0, and 33.3 mg/kg/day in the rabbit study. In both studies, a concurrent control group was dosed with placebo topical ointment. Slight to well-defined erythema was observed in all groups of compound-treated guinea pigs and rabbits. In general, the incidence and severity of erythema was greatest in the high-dose groups of both studies. In rabbits, a slight increase in the severity of epidermal irritation compared with respective control groups was observed microscopically in 33.3 mg/kg-dosed males and females in 10.00 mg/kg-dosed males. Microscopic findings in rabbits were characterized as minimal-to-slight hyperkeratosis, with occasional animals having minimal-to-slight dermal mononuclear infiltrates. No other findings of toxicological significance were noted in either study.
Two studies evaluated skin photoactivated responses to dermally applied niclosamide (schistosome topical antipenetrant lotion). In a primary dermal photo-activated irritation study, 6 New Zealand white rabbits per group received a single application of 0.3 ml of 1.0% niclosamide lotion or 0.3 ml of placebo topical lotion for a 2-hour occluded exposure. This exposure was followed by irradiation with 10J/cm² of 320-400 nm UVA light. There was no evidence of photo-activated primary dermal irritation of niclosamide lotion or placebo topical lotion in study animals. A photoreactive contact dermatitis study of niclosamide lotion and placebo topical lotion was conducted with albino guinea pigs. Test groups and primary challenge control groups were treated with either 0.3 ml of a 1.0% niclosamide lotion or 0.3 ml of placebo topical lotion. Two positive control groups received a suspension of musk ambrette in acetone. Photoactivation was delivered by 10J/cm² of 320-400 nm UVA light. In this study, niclosamide was shown to be a photoallergen and to a lesser degree a contact sensitizer. The placebo topical lotion was shown not to be a photoallergen or a contact sensitizer.

**Diazolidinyl urea is non-sensitizing based on predictive patch testing.**

A clinical repeated insult patch test is a medically recognized and valid indicator of a substance's potential to induce allergic contact sensitization in humans. Germall II (Diazolidinyl urea) was tested under occlusive patching on a panel of 109 subjects. It has been suggested, based on diagnostic patch testing using very high concentrations, that Germall II might be a mild sensitizer. The much more stringent and definitive predictive patch testing sequence of this presentation showed no incidences of contact sensitization. This repeated insult patch test was conducted according to a randomized protocol consisting of nine induction patches followed by a challenge patch. All subjects were treated under occlusive and non-occlusive conditions.

**Evaluation of potential drug phototoxicity using human skin cells in vitro.**

The objective of these investigations was to develop a standardized, sensitive, and quantitative, in vitro assay to predict the phototoxic potential of drugs and other chemical compounds. Cultured normal human keratinocytes and skin fibroblasts were used in these studies. The target cells were subject to phototoxic chemicals in vivo. Multiple endpoints of cell damage and cellular function were examined, using different experimental conditions that were believed to be capable of influencing the sensitivity of the test system. The phototoxic potential of a new drug or chemical was determined by measuring the decrease of neutral red uptake. In a preliminary investigation study with 26 substances of different phototoxic potential, there was a good correlation of the results from this in vitro system to be published in vivo phototoxicity data.
EVALUATION OF CALCIUM MAGNESIUM ACETATE AND ROAD SALT FOR CONTACT HYPERSENSITIVITY POTENTIAL AND DERMAL IRRITATION IN HUMANS. J R Cusick1, V A DeFr2, G H Buteau1, L B Ault2, N Caldwell2, W Lazer2. 1Chevron Environmental Health Center, Inc., Richmond, CA and 2Hill Top Research, Inc., Winnipeg, Manitoba, Canada.

Calcium magnesium acetate (CMA) and Road Salt are both deficing agents to which workers may be dermally exposed. A commercial formulation of CMA (CHEVRON Ice-B-Gon® Deicer) and Road Salt, each at 10% and 50% w/w in distilled water, were tested in a human repeat insult patch test to evaluate the contact hypersensitivity potential of these materials and to evaluate dermal irritation following single or multiple applications.

Neither CMA nor Road Salt produced contact hypersensitivity in any panelists. Following the first application, moderate acute irritation was observed only at one skin site exposed to 30% Road Salt. Repeated exposure to CMA or Road Salt produced mild to moderate irritation. The highest incidence of moderate irritation was observed with 30% Road Salt. Thus, neither material is expected to cause significant dermal effects in exposed workers. CMA is expected to cause dermal irritation equivalent to or less than that caused by Road Salt.


The most extensively studied species for assessing potential contact sensitisers is the guinea pig. Recently developed alternative methods include several mouse models. We have compared two epicutaneous assays, the local lymph node assay (LLNA) and the vitamin A-enhanced ear swelling assay (VAESA); one measures cell proliferation, the other an inflammatory response after re-exposure. Five chemicals were tested on CBA mice according to the standard protocol established for each assay using similar concentrations. Cinnamaldehyde and hydroxyacetone were strongly positive in both systems, resulting in a 27-fold and 18-fold increase in isotope incorporation in the LLNA respectively, and ear swelling was increased with a significance of p<0.001 as analysed by the non-parametric Mann-Whitney test in the VAESA. However, nonanoic acid, methyl salicylate and sodium laurel sulphate which are nonsensitisers gave rise to >3-fold increase in the LLNA, whereas they were all negative in the VAESA. A chemical is regarded as a potential sensitizer in the LLNA if at least one concentration of the chemical results in a 3-fold increase in isotope incorporation compared with control values. It thus appears that the LLNA is a very sensitive assay but positive results using irritant chemicals need to be verified with an assay system that monitors both induction and elicitation. (Supported in part by the UK Department of Trade and Industry.)

CORRELATION OF DERMAL IRRITATION IN HUMANS AND RABBITS WITH THREE IN VITRO CYTOTOXICITY ASSAYS USING SIX PETROCHEMICALS. C W Johnson, N S Hatoum and J K Yermanoff. IIT Research Institute and Amoco Corporation, Chicago, IL.

Development and validation of in vitro methods for dermal irritation testing is a continuing process aimed at reducing the use of live animals in toxicity testing. Three in vitro assays were used to assess the cytotoxicity, and thus dermal irritation potential, of six petrochemicals consisting of three lube oil additives, diesel fuel, transmission fluid and a polybutene. Petrochemicals are generally insoluble in aqueous media; therefore, this limitation must be overcome when selecting in vitro assays. The in vitro assays were all conducted using normal human epidermal keratinocytes and consisted of the Agarose Diffusion Method, Neutral Red Biosay and Total Cellular Protein Assay. Results of the in vitro assays were compared with the results of in vivo dermal irritancy studies in rabbits using the Draize method and in humans using the Repeated Patch Test. Comparison of the results from each of the different methods showed a positive correlation between the in vivo dermal irritation score in both rabbits and humans and all the in vitro tests for two lube oil additives, in that all the in vitro assays showed these materials to be cytotoxic, while the in vivo assays showed them to be irritants. Diesel fuel was identified as a dermal irritant in both rabbits and humans, however, it was noncytotoxic in the agarose test and relatively nontoxic in the neutral red assay. Transmission fluid, the polybutene and the third lube oil additive were all noncytotoxic to rats and nontoxic to humans and were nontoxic in the agarose test and relatively nontoxic in the neutral red and cellular protein assays. Thus, the in vitro methods used in this study were generally capable of predicting the in vivo irritation, or lack of irritation, for 5 of the 6 products tested, and therefore appear to be useful as screens which can be used to evaluate the dermal irritation potential of insoluble petrochemicals. However, it must be kept in mind that the insolubility of these materials makes evaluations using in vitro systems difficult.


Cultured human skin cells appear to be promising in vitro models for skin irritancy testing. We evaluated the effects of chemical irritants and product formulations on human epidermal keratinocytes (NHEK), keratinocyte-dermal fibroblast (NHEK/DF) co-cultures, and full thickness living skin equivalents (LSB). Cell viability (in NHEK cultures), measured as incorporation of the vital dye neutral red, was reduced in a dose-dependent manner in response to chemical irritants. The IC50 values correlated with irritation in human patch tests with these materials. NHEK/DF cultures were treated with 10 prototype surfacants, and were evaluated for cell viability (MTT dye incorporation), cytotoxicity (release of the enzymes LDH and MNG), metabolism (glucose utilization), and inflammatory mediator (prostaglandin E2) release. There was close agreement in the dose-response characteristics among the endpoints tested, and between the in vitro responses and human patch test scores. Using the same endpoints described for NHEK/DF cultures, LSB responded to an array of product formulations. These results demonstrate the potential utility of human skin cell cultures and cell viability, cytotoxicity, and inflammatory mediator endpoints for in vitro assessments of skin irritancy.
Previous studies of skin irritation in man quantified skin reaction by subjective estimation of erythema and edema, and objectively using reflectance spectroscopy and laser Doppler flowmetry. In some reports erythema determined visually and objectively highly correlated with the degree of skin irritancy, but some authors have reported difficulties in correlating the results of laser Doppler flowmetry to irritancy. Therefore, we examined skin irritancy using an improved analysis of in vivo reflectance spectroscopic data. The improved analysis technique uses a priori obtained values of the absorbance of skin chromophores. The method calculates the relative amount of oxygenized and deoxygenized haemoglobin and melanin in erythema and pigmentation. Skin irritancy was induced by four compounds with pKa's in the range from 3.8 to 9.5. Although skin irritation is an extremely complex biologic phenomenon involving solution properties, percutaneous absorption and the biological drug response, high pKa was predictive (p<0.01) for acute skin irritancy in man using the improved analysis of reflectance spectroscopy.

Employment of in vitro assays for dermal irritancy could result in significant reduction of animal usage and cost savings in premarketing testing. We have previously shown that surfactants induce the release of arachidonic acid (AA) from prelabeled mammalian cells in culture and this release relates positively to animal skin irritancy. We studied the effect of 27 finished products (FP) on AA release. C3H10T1/2 cells were cultured; prelabeled with [3H]AA, and treated with selected dilutions of FP, with media alone as control. An Irritant Dilution 200 (ID200) was determined for each product by combining a number of assays and generating a best fit line. ID200 represents the dilution of FP necessary to induce release of [3H]AA twice control values. When the ID200 for each of the 27 FP was compared to the animal irritancy score for each product (PDII - Primary Dermal Irritancy Index), the correlation using both Pearson and Spearman-Rho tests was significant at p<0.0314 and p<0.025, respectively. When insoluble agents and those of extreme pH were excluded the correlation between ID200 and PDII improved to p<0.0001 and p<0.001, N = 19 FP. These data suggest that ID200 determination holds promise as a non-animal assay for dermal irritancy testing.

To investigate the feasibility of using fewer than 6 animals for dermal irritation testing, we conducted a retrospective evaluation of data from 124 studies performed within the last 10 years using standard methodology. For each study, an irritation classification was made from dermal scores calculated using irritancy data available for 5, 4 or 3 rabbits randomly selected from the original 6 rabbit pool. The original 6 rabbit analyses resulted in classification of 49 nonirritants, 75 negligible, 66 mild, 24 moderate and 10 severe. Agreement with the 6 rabbit classification was obtained for 5, 4 and 3 rabbit groups, respectively, as follows: for negligible irritants- 95%, 85%, 69%; mild irritants- 85%, 80%, 76%; moderate irritant- 85%, 88%, 71%; severe irritants- 100%, 90%, 90%. Differences in the dermal irritation scores between 6 and 5, 4 and 3 rabbit groups were small, with an average no larger than 0.3 (Range 0-2.0). When disagreements in classification occurred, the test material was most often classified less severe. Using 90% or greater as acceptable criteria for reducing the number of test animals, our results suggest that the use of less than 6 rabbits would not be suitable for evaluating dermal irritation based solely on a qualitative classification. However, the use of 3 rabbits would be adequate to evaluate dermal irritancy based on quantitative dermal scores.
1111 TRANSDERMAL IONTOPHORESIS OF PYRIDOSTIGMINE BROMIDE IN VIVO IN PIGS AND IN ISOLATED PERFUSED PORCINE SKIN. J E Riviere, B H Sage and J L Parmentier, Cutaneous Pharmacology and Toxicology Center, NC State University, Raleigh NC and Becon Dickinson Research Center, Research Triangle Park, NC.

Pyridostigmine bromide (PB) is a quaternary ammonium acetylcholinesterase (AChE) inhibitor which has many therapeutic uses. Certain applications would benefit from a delivery strategy producing sustained PB blood concentrations while avoiding untoward GI side effects. Because of PB's charged nature, iontophoresis would appear to be an ideal strategy. The purpose of these studies was to demonstrate its feasibility using pig skin, an accepted animal model for man. Iontophoresis was performed using constant current (0.9 mAmp DC) delivered to 4.5 cm² electrodes. 10% PB was placed in the active (+) electrode. PB concentrations were determined by HPLC. In vitro experiments (n=4) utilized the isolated perfused porcine skin flap (IPPSF) to monitor transdermal PB flux into the venous effluent. At the end of 4 hrs of current, steady state fluxes were approximately 40 mcg/min which is greater than the estimated amount (35 mcg/min) required to treat a 75 kg man assuming normal pharmacokinetics and a target concentration of 0.1 mcg/ml. PB was then given as above in vivo to weaning swine and AChE activity monitored. Inhibition of AChE could be maintained at 70% normal for the duration of iontophoresis (4 to 12 hrs). These studies demonstrate the feasibility of delivering a constant and systematically effective dose of PB by transdermal iontophoresis.

1110 IN VITRO AND IN VIVO PERCUTANEOUS ABSORPTION/METABOLISM OF HYDROPHOBIC COMPOUNDS. K ME Ng, J Chu, R L Bronaugh, C A Franklin and D A Somers. Environmental Health Directorate, Health Protection Branch, Ottawa, Canada, and *Division of Toxicology, EPA, Washington, DC.

In vitro and in vivo dermal penetration and metabolism of 3 hydrophobic compounds were investigated in hairless guinea pigs. C-14 labelled pyrene (6 µg/cm²), benzo(a)pyrene (BAP, 3 µg/cm²) and diethylnyl phthalate ester (DEHP, 14 µg/cm²) were each applied on discs of guinea pig skin of 200 µm thickness mounted in flow-through diffusion cells. The cells were perfused with Hapes-buffered Hank's balanced salt solution, and perfusates and skin samples were analyzed. The amounts of dermal absorption (total of percutates and skin), expressed as percentage of the applied dose, were 64%, 59% and 47% for pyrene, BAP and DEHP respectively during a 24 hr period. These data were consistent with in vivo results which showed 94%, 73% and 53% of the dosed compounds were absorbed respectively for pyrene, BAP and DEHP. Hydroxyl derivatives of pyrene and BAP were identified in the perfusates. Mono-ethylnyl phthalate was found as the metabolite of DEHP. These data indicate that the in vitro system serves as a good model for the prediction of in vivo results from these compounds. It was also observed that lipophilicity of the compounds affects the absorption and partition of the absorbed compounds between the skin and receptor fluid.

1109 INFLUENCE OF SKIN IRRITATION ON PERCUTANEOUS ABSORPTION. A NANGIA, E CAMEL, B BERNER AND H MAIBACH. Dept. Dermatology, University of California at San Francisco, San Francisco, CA and Ciba Giegy, Ardsley, NY.

The effect of skin irritation on the percutaneous absorption of three model compounds of diverse physico-chemical properties, caffeine, indomethacin and hydrocortisone, was investigated through human cadaver skin. Norephedrine and imipramine, basic drugs with known irritation potential, were employed to induce skin damage to the skin. Treatment with norephedrine resulted in increase in permeation of caffeine and hydrocortisone by 2-4 fold while absorption of indomethacin declined by 8 times. Similar results were obtained with imipramine, however, the amount absorbed were substantially higher. In particular, it promoted the absorption of hydrocortisone by 12 folds and unlike norephedrine, it did not alter the permeation of indomethacin. The highest degree of absorption was seen with poorly absorbed compounds. The magnitude of the alteration of absorption appears to be influenced by the nature and the extent of damage caused by the irritant.

The objective was to determine percutaneous absorption of cadmium as the chloride salt from water and soil into and through human skin. Soil (Yolo County 65-California-57-8) was passed through 10, 20 and 48 mesh sieves. Soil retained by 80 mesh was mixed with radioactive cadmium-109 at 13 ppb. Water solutions of cadmium-109 at 116 ppb were prepared for comparative analysis. Human cadaver skin was dermatomed to 500 microns, and used in glass diffusion cells with human plasma as the receptor fluid (3 ml/hr flow rate) for a 16 hr. skin application time. Cadmium in water (5 μg/cm²) penetrated skin to concentrations of 8.8 ± 0.6% and 12.7 ± 11.7% of applied dose for two human skin sources. Percent doses absorbed into plasma were 0.5 ± 0.2% and 0.6 ± 0.6%, respectively. Cadmium from soil (0.04 g soil/cm²) penetrated skin at concentrations of 0.06 ± 0.02% and 0.13 ± 0.05% for the two human skin sources. Amounts absorbed into plasma were 0.02 ± 0.01% and 0.07 ± 0.03%. Most of the non-absorbed cadmium was recovered in the soap and water skin surface wash. Cadmium from water (2.5 μg/cm²) and from soil (0.02 g soil/cm²) was applied to two additional human skin sources at the indicated reduced amounts. Results were similar to the first tests. Cadmium will penetrate human skin and be absorbed into plasma. Absorption from soil is less than absorption from water.

IN VITRO PERCUTANEOUS ABSORPTION OF ANTHRAQUINOIDE DYE FROM TWO GREASE FORMULATIONS THROUGH RAT AND HUMAN SKIN. AI Yang, AJ Krueger, TA Roy and WJ Richman. Mobil Environmental and Health Science Laboratory, Princeton, NJ. Sponsor: CV Kommeren.

Anthraquinone dye is present in relatively small quantities in some formulated greases. A number of compounds in this chemical class were found to be mutagenic and some were carcinogenic in rodents dosed orally. Since human exposure to greases is primarily by skin contact, the present study was conducted to measure the dermal penetration of anthraquinone dye (<15%, w/w) from two representative grease formulations using skin sections from female Sprague-Dawley rats and human cadavers. A 96 hr in vitro percutaneous absorption experiment using rat skin was carried out with two greases formulated with the dye. The greases were fortified with radiolabelled 1,4-bis[3H]-methylenamino)anthraquinone to facilitate measuring the dermal flux of the dye component. The results showed dermal flux rates of 0.08 and 0.2 μg/cm² skin surface/hr for the dye component in these two greases. In addition, a 96 hr in vitro percutaneous absorption experiment was performed using human abdominal skin with the dye-grease formulation showing the higher rat skin permeability. A dermal flux rate of 0.08 μg/cm² skin surface/hr was obtained for human skin in comparison with 0.2 μg/cm² skin surface/hr for rat skin. Following normal skin contact, the systemic absorption of the dye by humans can be estimated to be several orders of magnitude lower than the oral intake of similar anthraquinones used in rodent carcinogenicity studies. The above findings suggest that human skin acts as an effective barrier to the anthraquinone dye in greases. Thus, it is unlikely that the low amount of anthraquinone dye present in greases will become a potential risk to humans through the dermal route under normal conditions of production and use.
2- and 4-CNFB have been used as chemical intermediates in the manufacture of many industrial chemicals. These two chemicals are undergoing in-depth toxicological evaluation by the NTP; their major toxicity is methemoglobin formation. The major route of human exposure is via the skin. This study was undertaken to investigate the effect of dose on the absorption of 2- and 4-CNFB following non-occlusive, protected dermal application at 0.65, 6.5 and 65 mg/kg. At the three doses, 33-40% and 51-62% of the dose of 2- and 4-CNFB, respectively, was absorbed from the skin within 72 hr. The balance of the dose was recovered in the protective device and the organic trap, i.e., that portion unavailable for dermal absorption. The absorbed 14C was excreted in urine (21-28%, 2-CNFB; 43-45%, 4-CNFB) and feces (11-15%, 2-CNFB; 5-12%, 4-CNFB). The extent and rate of dermal absorption and of urinary and fecal excretion of 2-CNFB were linear over the 0.65-65 mg/kg dose range; for 4-CNFB they were linear over the 0.65-6.5 mg/kg dose, and non-linear at the 65 mg/kg dose. Supported by NIEHS contract no. N01-ES-65138.

To further characterize the effects of argon laser exposure to pig skin, the in vivo percutaneous absorptions (PA) of C-14 labeled N,N-diethyl-m-toluamide (DEET) and parathion were determined following irradiation (514 nm, 15-17 seconds at 3.0-3.5 watts/cm²). PA was measured by excretion of radioactivity in the urine. Mean PA of DEET in control pigs was 8±1% of applied dose (1 mg droplet). For parathion, the corresponding value was 9±5%. Laser exposure did not significantly increase PA of either compound. A previous study has shown that in vitro PA of DEET and parathion was not altered by Argon laser exposure, suggesting that the barrier properties of the stratum corneum were not altered. The results of this study confirm the in vitro results and further suggest that the dermal micro-circulation, ablated by Argon laser exposure, is not a critical factor in the PA of DEET and parathion.

Exposure of guinea pigs in vivo to organic solvents, toluene, 1,1,1-trichloroethane and n-butanol, on normal skin was compared to exposure to acutely injured skin. Skin was also exposed to solvents in mixture with surfactants and water. The efficacy of protective means - barrier creams and protective gloves - was studied. The skin on the back was exposed to solvents and the subsequent percutaneous absorption was followed in blood samples withdrawn from a catheter in the carotid artery. The blood concentration of solvent was analyzed with gas chromatography, head space technique. Acute injuries to the skin increased the absorption of butanol markedly whereas absorption of toluene and trichloroethane was reduced. Incorporation of butanol in microemulsions increased absorption when the surfactant used was of anionic type. Barrier creams showed poor protective effect. Protection from gloves varied considerably between different materials. The water solubility of the studied solvents were of major importance to the differences found at acute injuries. When using microemulsions for cleaning applications, care should be taken not to expose skin to mixtures with anionic surfactants and butanol. The use of barrier creams and thin protective gloves should be limited to situations where short exposure times are anticipated. Some of the above findings, although counter intuitive, may influence current occupational practices.

The purpose of this study was to determine the effect of vehicle on the penetration and distribution of [3H]PbTrix-3 into skin layers. Disks of excised guinea pig skin were mounted on diffusion chambers in which the dermal surface was bathed by receptor fluid. The epidermal surfaces were dosed with 320 ng/cm² of PbTrix-3 dissolved in 50 µl of vehicle (water, methanol, or dimethylsulfoxide [DMSO]), and exposed to ambient conditions. The diffusion cells were incubated for various time periods (0.25 - 48 hr) and distribution of radioactivity into skin layers and receptor fluid determined. Regression models were fitted to the results for each compartment (skin surface, epidermis, dermis, receptor fluid) and each treatment (vehicles). There was a significant relationship between accumulation of radioactivity in 3 separate compartments (epidermis, dermis and receptor fluid) and time when the vehicle was water or DMSO. In vitro skin penetration by PbTrix-3 during 48 hr of exposure (calculated by summing radioactivity recovered in the dermis and receptor fluid) was 6.9, 4.7 and 15 for water, methanol, and DMSO, respectively (expressed as % of dose).
THE DERMAL IRRITATION POTENTIAL OF FOUR ANTIMICROBIAL-STEREO CREAM COMBINATIONS. P Selan, R E Squibb, B F Murphy, R J Szet, E Schwartz, and H E Black. Schering Corp., Lafayette, NJ.

The dermal irritation potential of four antimicrobial-steroid cream formulations, tolnaftate 1%, hydrocortisone 0.5%, clotrimazole 1% hydrocortisone 0.5%, clotrimazole 1% betamethasone 0.02%, and clotrimazole 1% gentamicin 0.1% betamethasone 0.005%, was compared in separate studies. All cream formulations were applied to the intact skin of rabbits for 21-25 consecutive days. For comparison, two control groups were used in each study: one dosed with the appropriate vehicle and one nontreated group.

Erythema produced on intact rabbit skin by each of the medicated cream formulations was equivalent or slightly less than that produced by each of the corresponding vehicles. There was no significant difference between the medicated and vehicle creams in the time of onset of erythema or in the incidence of edema, atonia, papules, pustules, desquamation, wrinkling, fissuring, or skin thickness. These findings suggest that tolnaftate, hydrocortisone, clotrimazole, gentamicin, and betamethasone can be used in combination in topical preparations with no increase in the potential for dermal irritation.


Alterations in clinical pathology parameters have been noted in several percutaneous toxicity studies performed recently at BRRC. Seven 9-day repeated-dose percutaneous studies involving 6 test materials in Fischer 344 rats have been reviewed to determine if a correlation exists between clinical pathology alterations and cutaneous irritation. Clinical and histologic evidence of skin irritation and inflammation were compared to hematologic and serum chemistry findings. The degree of irritation observed clinically at the site of application ranged from slight to severe. Frequent alterations associated with marked to severe irritation included increases in total leukocyte count (including increased segmented neutrophils, lymphocytes, and monocytes) and decreases in erythrocytes, hemoglobin concentration, and hematocrit. Severe irritation was generally associated with decreases in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), and alterations in serum enzymes, albumin, glucose and/or Ca++, and Pi. In addition, effects on serum urea nitrogen, urine pH, urinal volume and/or specific gravity were observed in animals treated with two of six of the test materials that produced severe irritation. Increases in leukocytes were considered to be a response of the immune system to local irritation at the site of treatment. Other alterations were considered to be secondary to increased vascular permeability and destruction of tissues associated with inflammation and/or possible toxicity to the kidneys or hematopoietic system.

EFFECTS OF PARAQUAT ON ISOLATED PERFUSED PORCINE SKIN. V Srikrishna and F A Monteiro-Riviere. Cutaneous Pharmacology and Toxicology Center, North Carolina State University, Raleigh, NC.

Paraquat (P), a commonly used herbicide, has been shown to be toxic in field workers. The objective of this study was to test the toxicity of P in the isolated perfused porcine skin flap (IPPSF). Low (3mg), moderate (24mg), and high (200mg) concentrations (n=4) of P were topically applied on the IPPSF and perfused for 6 hr. The dosed area of the skin was sampled for light and transmission electron microscopy (TEM) and enzyme histochemistry. The IPPSF's were also treated topically with C methyl paraquat dichloride (n=4) using the above three concentrations. Hourly perfusate samples were collected for biochemical and physiological parameters in all P treated IPPSF's. Morphology of the treated IPPSF's showed intercellular and intracellular edema by both LM and TEM in all doses, which was similar to previous in vivo experiments. Acid phosphatase and nonspecific esterase increased in the stratum spinosum and stratum granulosum layers in the treated skin compared to the controls. Glucose utilization of all treated flaps were lower when compared to the controls. Vascular resistance showed a different profile in all the (P) treated flaps. Radiocarce studies indicated minimal penetration and no significant absorption. Therefore, P at very high concentrations has a deleterious effect on isolated perfused skin.

(Supported by NIEHS Grant ES 00044)


The cutaneous effects of the thermal decomposition residue from 5 synthetic pyrethroid pesticide formulas were determined in human subjects. Test articles included azodicarbonamido-based fumigation mixtures containing either cyfluthrin, cyphenothrin, permethrin, or tralomethrin. A pesticide-free control mixture was also used. Aerosol particles produced during the thermal decomposition of the formulas at approximately 250°C were collected on filter paper for use in the assays. No definitive irritation responses were seen in any of the subjects to any of the test articles following a single dermal application of 15 mg of residue to 25 adults or following repeated dermal application of 15 mg for 21 consecutive days to the same site on adults.

Subjective cutaneous sensitization, (itch, sting, and burn) was assessed in humans by application of 3 mg of the residues on the earlobe. The residue containing cyfluthrin elicited a mild response in 6/10 subjects and a moderate response in 1/10 at 24 hours following application. Materials containing permethrin or tralomethrin, as well as the control, evoked a mild response from only 1/10 subjects. No subjective sensitization was evident from any of the materials at 48 hours.
THYROID HORMONE RECEPTOR EXPRESSION AND THYROID HORMONE EFFECTS ON PROTEIN SYNTHESIS IN THE NEONATAL RAT CEREBELLUM. T T Sheng, R R Sylvester and R J Bull. Pharmacology/Toxicology Program, Washington State University, Pullman, WA.

Thyroid hormone (TH) is known to have substantial effects on brain development. Alterations in brain architecture that occur during a hypothyroid insult include increased cell death and decreases in the dendritic arborization of specific cell types. While these effects are well documented it is not clear which, if any are the direct result of TH interactions in the cerebellum. We have evaluated the expression of TH receptors during the postnatal period in the rat. This is the time period when much of cerebellar development and maturation is occurring. Receptor expression was determined using 48 base oligonucleotide probes to the various mRNAs which are encoded from the TH receptor gene (c-erbA). Probes were hybridized to Northern blots containing mRNA from the cerebells of pd 1, 6, 12, 21 and adult animals. Results show that the receptor is present at all ages, supportive of a direct role for TH in cerebellar development. To investigate the role of TH on protein expression in the cerebellum we have utilized a pronylthiouracil-induced hypothyroid pd mouse during the weaning period. Protein was isolated from the cerebells of these animals and 2-D PAGE was performed on samples from control and hypothyroid groups. No qualitative differences in protein expression were found using silver staining and spots were compared on a computer software program. We are currently examining quantitative differences in protein expression using radio-labelled amino acids in the same system and visualizing these proteins with autoradiographic techniques. (Supported by NASA Grant No. NAG 3-226).

A micromass cell culture system for rat embryo limb bud (LB) cells was employed to assay the in vitro developmental toxicity of several anti-tubulin agents. The benzimidazoles, methyl-5-thiabendazole (TBZ), thabendazole (TBO), 5,6-dihydro-2-thiabendazole (ABZ) and nocardazole (NCZ) were tested in addition to the classic microtubule inhibitors colchicine and vincristine sulfate. Two parameters for assessing developmental toxicity were measured: 1) differentiation, by alclan blue-staining of extracellular proteoglycans and 2) cytotoxicity, by quantifying neutral red uptake. The IC50 values for cytotoxicity were equivalent to those for differentiation. Thus, the inhibitory effects of these agents were probably due to cytotoxic mechanisms. The relative potencies of the benzimidazoles in the micromass system (NCZ-MBZ-ABZ-TBZ) mirrored their activity in an assay for inhibition of in vitro tubulin polymerization. Vincristine and colchicine also exhibit high affinities for mammalian tubulin and were potent inhibitors of chondrogenesis. Immunofluorescent staining of day 1 cultures with a monoclonal antibody to β-tubulin revealed that, with the exception of TBZ, these agents elicited mitotic (metaphase) arrest. Many anti-tubulin agents are teratogenic in rats and their in vitro developmental toxicity may reflect perturbation of microtubular structure or function. Although these agents did not inhibit cell differentiation in the absence of cell death, they should be considered potential developmental toxicants since they are toxic at nanomolar concentrations. NIH ES-03157, ES-97052.

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During cardiac development, β-adrenergic receptors are transiently coupled to enzymes that transduce neuronal stimulation into trophic responses. Because thyroid status is known to regulate adrenergic function in the adult, and because the thyroid is a common target for environmental alterations (stress or chemical exposure), we examined the effects of hyperthyroidism on β-receptor binding and receptor-mediated stimulation of adenylate cyclase and ornithine decarboxylase (ODC). Hyperthyroidism was induced by administration of triiodothyronine (T3, 0.1 μg/g postnatal days 1-5), and resulted in an initial enhancement of receptor binding followed by prolonged deficits into young adulthood; this is consistent with the view that thyroid hormone accelerates adrenergic development but eventually compromises function. During either the period of enhanced binding or subsequent deficits, hyperthyroidism increased basal, isoproterenol-stimulated and forskolin-stimulated adenylate cyclase activity; in contrast, β-agonist stimulation of ODC was uncoupled during the phase of increased receptor binding. The differing patterns of thyroid hormone's effects on β-receptors, receptor-mediated stimulation of adenylate cyclase, and receptor-mediated stimulation of ODC, suggest that individual components in the adrenergic signal transduction cascade are independently regulated. Specifically, measurements of receptor binding alone are inadequate to screen for adverse effects on development. (Supported by EPA CR813769).

EFFECTS OF TRANSFORMING GROWTH FACTOR β ON LB AND CNS CELL DIFFERENTIATION IN VITRO: DEPENDENCY ON CONCENTRATION, CELL TYPE AND CELL GROWTH FACTORS. P. Fassetman, D. Lafslamme. Dept. of Env. Hld., Univ. of WA, Seattle, WA.

The aim of these studies was to characterize the effects of transforming growth factor β (TGF) on cell differentiation. Growth factors have been shown to be important regulators of cellular growth and differentiation in vitro and alteration of these factors has been proposed as a mechanism for developmental toxicity. The differentiating micromass rat embryo midbrain (CNS) and limb bud (LB) primary culture systems were used and activated TGF was added 2 hours after plating on day 0. Differentiation, cell growth and cytotoxicity were evaluated on day 5 of culture. Biochemical assays employed to evaluate differentiation were [3H]-glycine incorporation into sulfated proteoglycans (LB). Differentiation was also evaluated using image analysis of stained cultures. Cytotoxicity was monitored using the neutral red uptake assay. LB cultures exposed to TGF showed dose-dependent decreases in differentiation as measured by image analysis of stained foci and [35S]-sulfate incorporation (ED50 = 2 and 3 ng/ml respectively). Flow cytometry revealed that TGF reduced the population of LB cells in G1 but did not alter the percentage of these cells that were labelled with chondroitin sulfate antibody. CNS cultures exposed to TGF showed no changes in differentiative endpoints. However, when the CNS cultures were pre-treated with mitomycin C prior to addition of TGF showed a dose-dependent increase in differentiative markers. No changes were observed in protein or cytotoxicity. Thus TGF seems to selectively inhibit the differentiation of LB cultures, but differentially affects CNS cultures depending on cell growth. NIH ES-03157.
A rat embryo limb (LB) cell culture system was used to determine the relative developmental toxicity of three model N-nitrosourea compounds (NNO), and to compare their effects with those observed previously in rat embryo post-implantation culture system (WEC). Public health concern for NNO compounds stems from their in vivo developmental toxicity and ubiquitous human exposure. Toxicity of methylazoxymethanol (MNU), a direct acting methylating agent; and ethynitrosourea (ENU) and ethyleneiminoethylsulfonate (EMS), direct acting ethylating agent, was tested in LB cells. Previous studies using rat whole embryos indicate that MNU and ENU are much more potent developmental toxicants than EMS (50-100x). In LB cells, cytotoxicity (neutral red assay) and cell growth (cell number, protein content) were monitored. Differentiation was assessed by 35S uptake and alcian blue staining of proteoglycans. Relative potencies of these three NNO compounds, as assessed in LB cells, are the same as those observed in rat whole embryos (MNU→ENU→EMS). However, effective concentrations for cytotoxicity and inhibition of differentiation (LC50 and IC50 values, respectively) by MNU and ENU were at least 10 fold higher than comparable values previously reported for WEC (LC50 and MC50 values). In contrast, effective concentrations for EMS were lower in LB cells than were observed in rat whole embryos. These observations suggest that mechanisms responsible for developmental toxicity in rat whole embryos may be different from those in LB cells. NIH ES-03157 and ES-07032.

Phenylthoin (P) teratogenicity may require maternal and/or embryonic enzymatic bioactivation to an embryonic reactive intermediate. To assess the teratologic relevance of maternal drug metabolism in embryo culture, hepatocytes were chosen to provide a more physiological balance of phase I and II pathways. Gestational day 9.5 (morning of plug = day 0) murine embryos were co-cultured for 24 hr with day 12E maternal murine hepatocytes and radiolabeled P (5.5-4.4 nM/P: 1.7 Gbq/mole; 0.93 µg/ml). In the absence of hepatocytes, covalent binding of P was 40% higher in live embryos than in fixed controls (0.71 vs. 0.51 pmol/mg protein) (p < 0.05). Live hepatocytes, when compared to fixed hepatocytes, did not increase the covalent binding of P to fixed embryos, nor to co-culture medium proteins. Furthermore, with a therapeutic concentration of P (20 µg/ml), addition of hepatocytes to the embryo culture medium reduced P embryopathy, as determined by embryonic crown-lump length and yolk sac diameter, without affecting somite number. Compared to in vivo P metabolism, the ratio of para-hydroxy-phenylthoin to P dihydrodiol (DHD) was 3:1 in both in vivo and in vitro (hepatocyte) samples. Glucuronidation of P-HPPH was somewhat greater in vivo (82%) than in vitro (64%), while DHD was 100% glucuronidated both in vivo and in vitro. Although a deficit that in vivo and in vitro metabolism of P were comparable. The covalent binding data suggest that, in mouse, the P reactive intermediate produced in maternal hepatocytes is detoxified in situ and does not escape the liver, and that P teratogenicity is a consequence of embryonic bioactivation. (Support: Medical Research Council of Canada)
GLUTATHIONE SULFIDE REDUCTASE (GSGRd) AND DIAMIDE-INDUCED EMBRYOTOXICITY IN RAT CONCEPTUSES DURING ORGANGENESIS. R Hiranruengchok and C Harris. Toxicology Program, University of Michigan, Ann Arbor, MI.

GSGRd is known to be an important enzyme in maintaining normal intracellular levels of reduced glutathione (GSH). The important protective role of this activity in tissues of the developing conceptus has not been clearly established. We show that cultured rat embryos have GSGRd activity of 1.83 (nmol of NADPH oxidized/min/mg protein) and 1.48 on days 10 and 11 of gestation, respectively. Visceral yolk sac (VYS) activities were somewhat higher at 7.06 and 13.87 also on days 10 and 11, respectively. Addition of the GSGRd inhibitor 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) on day 10 resulted in a dose of 50µM produced no effects on viability, yet oppositely inhibited GSGRd by greater than 95% in both the embryo and the VYS within 60 min. The GSH oxidant, diamide (50µM) produced no significant alterations from controls in viability, growth or GSH/GSGC in cultured day 10 conceptuses. Inhibition of GSGRd with BCNU (50µM) 2 hr prior to addition of diamide (50µM), however, resulted in decreased viability (40% of controls) and an increase in abnormal axial rotation defects from 0% to 8%. The BCNU-diamide combination also resulted in a 50% reduction in intracellular GSH in whole conceptus by 60 min. These changes were not seen with either diamide or BCNU alone. Alterations in GSH/GSGC levels due to diamide alone were seen only when diamide concentrations were raised to 500µM. This treatment resulted in considerable embryotoxicity when conceptuses were evaluated at day 11. These data show that GSGRd is important in protection against deleterious effects of embryotoxic agents such as diamide, which can oxidize GSH and require intracellular reduction to maintain normal thiol status. (Supported by ES-05235-01).

EFFECTS OF L-BUTHIONINE-S-R-SULFOXIMINE (BSO) ON PROTEOLYSIS IN THE RAT VISCERAL YOLK SAC (VYS) DURING ORGANGENESIS. J Ambrosio and C Harris. Toxicology Program, University of Michigan, Ann Arbor, MI.

In order to evaluate possible effects of glutathione (GSH) alterations by embryotoxic agents, we have developed a fluorometric assay for measuring lysosomal proteolysis in the VYS of cultured rat conceptuses. Viable day 11 rat conceptuses are incubated in the presence of the coumarin analog Z-Phe-Arg-7-amido-4-methylcoumarin (Z-Phe-Arg-7AMC), which has been found to be an excellent substrate for cathepsins B and L. The substrate is readily taken up and cleaved to the highly fluorescent product, 7-amido-4-methylcoumarin (7AMC) by VYS lysosomal cathepsins. Addition of leupeptin to the media inhibited product formation by greater than 90%, while the serine protease inhibitor phenylmethylsulfonyl fluoride (100µM) was ineffective, confirming cathepsin activity. 7AMC is not retained within VYS or embryonic tissues, but is released into the culture media, where it can be easily monitored. The assay shows a reproducible, linear increase in 7AMC production over time. The mean amount of 7AMC liberated was 47±2.02 nmol/mg protein (n=23) in controls after 120 minutes of exposure. Previous studies using the inhibitor of glutathione biosynthesis, BSO, led us to suspect a possible regulatory role for GSH in proteolysis. No significant changes in proteolytic activity were observed following 23 hr pre-exposure with 1mM BSO or 4 hr pre-exposure to 5mM BSO even though significant reductions in GSH are evident in the VYS. When BSO (5mM) is added together with 10mM Z-Phe-Arg-7AMC, however, a significant increase in the amount of product liberated (7.2±0.04 nmol/mg protein, n=11) results. These data point to the possibility that GSH depletion per se does not influence lysosomal proteolysis but that Z-Phe-Arg-7AMC may interact directly with BSO, thereby increasing activity. (Supported by ES05235-01).

DEVELOPMENTAL TOXICITY OF DESBROMOLEPTOPHOS IN CHICKENS: ENZYME INHIBITION, MALFORMATIONS AND FUNCTIONAL DEFICITS. B Magnus Francis, W Parage-Ellaway, and JS Duffy. Institute for Environmental Studies, University of Illinois, Urbana IL.

Previous studies suggested that exposure of embryonic chicks to organophosphorus esters can result in long term functional deficits in exposed chicks. We evaluated the relationship of inhibition of acetylcholinesterase (AChE) and of neuropathy target enzyme (NTE) to teratogenicity and functional deficits, using the organophosphorus ester desbromoleptophas (Q-methyl Q-2,5-dichlorophenyl phenylphosphonothionate; DBL). Chicks were exposed to DBL on day 3 or day 15 of incubation or 10 days post-hatching. DBL induced prolonged inhibition of AChE and NTE when administered either early or late in incubation. DBL induced structural malformations if injected before organogenesis; post-hatching paresis if administered after organogenesis, and delayed deficits of gait if administered after hatching. The post-hatching paresis and abnormal gait are not determined solely by either AChE inhibition or NTE inhibition, since they occur in the absence of the latter and are not invariably seen in the presence of the former.

DEVELOPMENT OF ESTERASE ACTIVITIES IN THE CHICK EMERGENT AND CHICKN. W Parage-Ellaway, VA Regional College of Vet. Med., Blacksburg, VA.

The embryonic chick provides a model for developmental biology and toxicology. Although numerous studies detail the structural development of chick embryos, few describe embryonic levels of enzyme synthesis and their changes during development. In this study, the development of esterase activity in chick embryos was measured from day 9 of incubation until 46 days after hatching. Brain acetylcholinesterase (AChE) activity was detected on day 9 of incubation at 0.364 nmol/minute/gram tissue, AChE activity increased with age of the embryos. The liver cholinesterases (ChE) and carboxylesterase (Cae) activities during incubation were not different from activities after the chicks had hatched. Plasma ChE and Cae activities did not change with age after hatching. Brain neuropathy target esterase (NTE) activity was not detected on day 9 of incubation and was very low (6.12 nmol/15 min/mg protein) the next day, but increased rapidly with increasing age. This study demonstrates that chick embryos have developed esterase activities in the brain and liver by day 10 of incubation. Thus, the insensitivity of chick embryos and young chicks to organophosphorus ester-induced delayed neurotoxicity is not due to absence of NTE. In addition, these results provide baseline data for evaluating the response of embryonic and immature chicks to teratogens and neurotoxicants.
1139 DEXAMETHASONE POTENTIATES THE TERATOGENIC ACTION OF ALL-TRANS RETINOIC ACID IN CD-1 MICE.
C. G. Rousseaux, Department of Veterinary Pathology, University of Saskatchewan, Saskatoon, SK. CANADA.

All-trans retinoic acid (ATRA) is a teratogen in mice. A 2 x 2 factorial design experiment was undertaken using ATRA and dexamethasone (DEX), to determine whether ATRA interacted with DEX. Forty timed pregnant mice (bred 08:00-09:00) were divided into four groups. ATRA was given orally (200mg/Kg) on day 10.5 (plug date day 0) in 10ml/Kg corn oil. DEX was diluted in sterile saline and given ip (2.5mg/Kg) on days 10, 11, 12, and 13 at a volume of 10ml/kg. Maternal weight, feed consumption, implantations, and number of fetuses did not differ among treatment groups; however, less female pups were present in the left uterine horn following ATRA treatment and less males were found in the right uterine horn following DEX administration (DEX P < 0.001; ATRA P < 0.05). The sex, crown rump length and weight did not differ among groups. Severe skeletal malformations were seen in pups from dams treated with ATRA. Significant dam treatment related differences occurred for major (ATRA P < 0.0001; DEX P < 0.005; interaction P < 0.005), minor (ATRA P < 0.001; DEX P < 0.005; interaction P < 0.01), and variation type defects (ATRA P < 0.0001; DEX P < 0.01; interaction P < 0.0001). In was concluded that ATRA and DEX were synergistic in their combined action. (Support: NSERC OGP0036697).

1140 meso-2,3-DIMETHYLENEDIOXYSTEROIC ACID (DMA) ALLEVIATION OF ACETALDEHYDE-INDUCED INTRACRANIAL PRESSURE EFFECTIVENESS WITH TIME.
M A Bouque, V Piana, J L Domingo and J Corbella, Laboratory of Toxicology and Biochemistry, School of Medicine, University of Barcelona, Spain.

The diethyl compound meso-2,3-dimethoxybenzaldehyde acid (DMA) is an antidote for the treatment of experimental and human poisoning by a number of heavy metals. DMA has also been reported to reduce the lethality of animals poisoned with ammonium more effectively than BAL. Recently, we demonstrated that c. c. administration to pregnant mice of 80, 160 or 320 mg/kg/dose given at 0, 24, 48 and 72 h after l.p. administration of 12 mg/kg of sodium arsenite (NaAsO2) on day 10 of gestation significantly reduced the embryolethality and morphological alterations provoked by arsenite. Based on these findings, the effect of increasing the time interval between acute arsenite exposure and DMA therapy was investigated in the present study. On day 10 of gestation, DMA (c. c. 220 mg/kg) was administered to pregnant SD rat mice at 0, 24, 48, and 72 h after a l.p. dose of NaAsO2. DMA administration at 0, 24, 48, and 72 h after injection of NaAsO2 significantly reduced the embryolethality provoked by NaAsO2. However, DMA given at later times did not protect against the embryolethality of NaAsO2. Maternal and skeletal abnormalities caused by NaAsO2 were also significantly reduced when DMA was given during the first hour after NaAsO2 administration. According to these results, a delay between arsenite intoxication and DMA treatment should be avoided to have a practical beneficial effect on the arsenite-exposed conceptus. (Supported by HSFCT, Spain, Grant FIS/FCT-3002).
Interspecies diversity in metabolic conversion of xenobiotics limits the use of mammalian surrogates in toxicological testing. Studies were conducted to augment the metabolic capacity of developing shrimp (0-48 hr post-hatch at 25°C) by culturing nauplii in rodent plasma. After 24 hr incubations (0-24 hr and 24-48 hr) in 200 μl of rat plasma [heat or 50% in physiological saline (PS)] naupliar viability and growth (body length - BL) were not altered (<4% lethality; BL in PS versus plasma were similar). 2-Methoxyethanol (ME), a glycol ether, is teratogenic in mammals after oxidation to methoxycetic acid (MAA). ME (500 ppm in PS) had no affect on naupliar viability or growth whereas 3 ppm MAA in PS produced 50% lethality. Nauplii were exposed for 24 hr to neat or PS-diluted plasma collected from mated female rats 2 hr after oral administration of ME (250 mg/kg). Lethality averaged 38% in neat plasma and decreased to an average of 19% after plasma dilution. BL decreased in a dose-dependent manner (50% plasma, 880 μm; 100% plasma, 820 μm). Culture in plasma facilitated direct exposure of nauplii to products of mammalian metabolism as well as an assessment of metabolite toxicity to the developing brine shrimp. Funded by CAAT, The Johns Hopkins University.

This study concerns application of the Hydra attenuata developmental toxicity assay for testing aqueous samples. First, we modified the assay for testing aqueous samples and those modifications are described. Because water quality has a major impact on aquatic toxicity testing, we examined parameters such as pH, conductivity and hardness for their effects on the hydra assay. Adult hydra thrived morphologically unaffected in media maintained between pH 5.5 and 9.5 and the artificial "embryo" developed normally at pH 6.25 to 8.25. For water hardness, the minimal effective concentration (MAC) was 1000 mg/L (as CaCO₃) in adults, and 625 mg/L in the artificial embryos. Salinity in excess of 5 ppt killed both adults and embryos, hence the assay may not be applicable to marine or highly saline samples. Finally, we tested "grab" samples from rivers in Maryland, Pennsylvania and Delaware as well as several industrial wastewaters. With these samples the assay functioned normally; thus, we believe it can test the developmental, and perhaps the acute, toxicity of natural waters and wastewaters.
1145 EVALUATION OF THE DEVELOPMENTAL TOXICITY OF OCHRATOXIN A AND CYCLO-PHIAZONIC ACID IN POSTIMPLANTATION RAT EMBRYOS. M Small, A Reine and T D Phillips. Cooperative Research Center, Prairie View A&M University, College Station, TX.

Ochratoxin A (OA) and cyclopiazonic acid (CPA) are secondary fungal metabolites produced by species of *Penicillium* and *Aspergillus* and have been found to occur together in corn. OA and CPA have been reported to be toxic to several animal species. This study was designed to evaluate the developmental toxicity of OA and CPA (singly and in combination) utilizing extracorporeally maintained postimplantation whole rat embryos in culture (WECC). Explanted rat embryos were exposed to OA (0.025-0.25 mM) and CPA (0.01 mM) on day 10 of gestation and cultured in rat serum for 45 hr. In the WECC assay, OA induced a dose-dependent reduction in embryonic and protein content, somite number count, crown-rump length, and yolk sac diameter. Embryos treated with CPA alone were not significantly different from the controls (p>0.05). In the presence of CPA, there was a significant enhancement of the developmental toxicity of OA in rat embryos (Supported by TAES H6215).

1146 ALTERATION IN PROTEIN SYNTHESIS INDUCED BY OCHRATOXIN A IN POSTIMPLANTATION RAT WHOLE EMBRYO CULTURE. E Smith, C Brathwaite, M Small, A Reine and T D Phillips. Cooperative Research Center, Prairie View A&M University, College Station, TX.

The mycotoxin, ochratoxin A (OA), is a secondary metabolite produced by species of the genera *Aspergillus* and *Penicillium*. The potential intoxication of livestock and man through the ingestion of contaminated grains and other foods makes OA a significant food chemical. Morphological studies suggest protein synthesis may be affected by OA. Preliminary studies to characterize the major proteins induced or repressed in the whole embryo were carried out by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was performed on in vitro whole embryos exposed for 45 hr in gassed rat serum containing 0.00-0.25 mM OA. Densitometer analysis of the silver stained gels was also performed to determine the relative change in the quantity of the synthesized protein. Following OA treatment, a remarkable reduction in the synthesis of a protein band (M, = 45K) was observed on silver stained SDS-PAGE. This band exhibited a dose-dependent response to OA treatment. Densitometer analysis of the silver stained gel showed six protein bands (M, = 45-14K) with repressed synthesis and two protein bands (M, = 40-30K) with enhanced synthesis. Reduction in synthesis of these proteins with an increase in OA may represent a decline in cytoskeletal activity as observed by a reduction of somites. The identification and characterization of these proteins are under investigation (Supported by TAES H6215).

1147 COMPARISON OF THE DEVELOPMENTAL TOXICITIES OF COMMON PENICILLIUM MYCOTOXINS USING THE HYDRA BIOASSAY. K Mayura, W S Conover, B A Clement and T D Phillips. Department of Veterinary Anatomy and Public Health, Texas A&M University, College Station, TX.

Penicillium elaborated mycotoxins are well recognized as contaminants of many foods. These toxins, including mycophenolic acid (MPA), roquefortine (ROQ), penicillic acid (PA) and patulin (PAT) are reported to be toxic to several mammalian species. Studies were undertaken to prescreen these hazardous chemicals for developmental toxicity utilizing a fresh water coelenterate, *Hydra americana* (HA). The assay was performed in a series of three phases. Phase I consisted of exposing only the adult hydra to whole-log concentrations of the individual toxins. The minimal effective concentrations (MACs) of the toxins were determined in the adult hydra and regenerating artificial hydra embryos (AHE) by bracketing the toxic endpoint to the nearest tenth (1/10) of a log in Phase II. The results (from Phase II) were confirmed in Phase III. Findings from the HA assay indicated that the MACs of MPA, ROQ, PA and PAT which resulted in toxic endpoints were equal to: 40, 30, 0.7 and 15.0 mg/l (in the adult hydra) and 30, 20, 0.7 and 15.0 mg/l (in the AHE), respectively. PAT produced the highest toxicity in the hydra. The A/D ratios (developmental hazard indices) of the toxins were in the range of 1.0 to 1.5, suggesting that these chemicals are coeffective developmental toxins (Supported by TAES Project H6215 and USAID Project 02-5036-2).

1148 CADMIUM-INDUCED LIMB REDUCTION DEFECTS IN CD-1 MICE INVESTIGATED WITH A SUBMERGED LIMB BUD CULTURE TECHNIQUE. L.G. MacNab and C G Rousseaux. Taxology Programme, University of Saskatchewan, Saskatoon, SK.

Offspring of mice exposed to cadmium (Cd) on the ninth day of gestation (E9) exhibit a post- to preaxial sequence of ectrodactyly. Submerged limb bud culture was used to follow these defects in *vitro*. On E9, mice received 3 i.p. injections of either CdCl2 (3 mg/kg/injection) or saline. On E12 forelimb buds were excised and stained for cartilage immediately (day 0) or submerged in B6a media with (25% FCS) in a roller bottle and cultured for 3 days prior to staining (day 3). An image analysis system measured total limb bud, autopod, and cartilaginous bone anlagen areas. Cadmium treatment reduced limb bud and autopod areas in both day 0 and day 3 limbs (p<.0001). In day 3 limbs, long bone and paw bone areas were decreased by 23% and 45% respectively leading to increased soft tissue to bone anlagen ratios (p<.0001). The frequency of missing paw elements increased in a pre-to postaxial fashion with those remaining having diminished areas (p<.0001 for most elements). Syndactyly, primarily between metacarpals 2-3 and proximal phalanges 2-3, occurred in 28% of Cd exposed limbs. This study demonstrates that marked changes in limb development have already occurred 3 days after Cd administration (day 0) and that these changes can be followed and further monitored using this limb bud culture system to yield defects similar to those observed in *vitro*.

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THE EFFECT OF WATER SOURCE ON THE DEVELOPMENT OF MURINE LIMBS IN CULTURE. G Thompson and C G Rousseaux. Department of Veterinary Pathology, University of Saskatchewan, Saskatoon, SK, CANADA.

Submerged murine limb bud culture was used to determine the effect of different water sources on limb development. Morphometric, morphologic and biochemical endpoints. Forelimbs (317) were removed from day 12.0 CO-1 fetuses (day 0 plug date), and placed in BC0, organ culture media containing 20% fetal calf serum. The water source for making up the media consisted of double distilled deionized (control), water from a saline canal, a slough draining recent carboufan spraying, a lake with many dead fish and tap water (treatments). Limbs were cultured for 3 days then divided for morphologic and morphometric analysis following staining, and total and total nucleic acid measurement. Treatment effects were seen for total limb bud area (P ≤ 0.0001), humerus, radius, ulnar, metacarpal and phalangeal areas (P ≤ 0.0001). Differences were also seen in the number of carpals, metacarpals and phalangeal bones (P ≤ 0.0001). Limb weights, total protein and nucleic acids differed (P < 0.0001), but protein/mg and nucleic acids/mg tissue did not. It was concluded that water source can severely effect the growth and differentiation of limbs in culture. (Support: NSERC OGP0036697).

EFFECT OF SINGLE AND REPEATED ADMINISTRATION OF GARLIC OIL ON BIOTRANSFORMATION ENZYMES IN RATS. R R Dalvi and P S Dalvi. Toxicology Laboratory, Sch. of Vet. Med., Tuskegee University, Tuskegee, AL.

The purpose of this study was to examine the effect of a single dose and a repeated administration of garlic oil (diallyl sulfide) on phase I and phase II biotransformation enzymes in rats. Male Sprague-Dawley rats were treated with a single dose of garlic oil (500 mg/kg, i.p.) and sacrificed 24 hr later. A significant depression was observed with hepatic cytochrome P-450, aminopyrine N-demethylase and aniline hydroxylase while microsomal protein content, cytochrome b5, NADPH-cytochrome c reductase, benzphetamine N-demethylase and glutathione S-transferase remained unaffected. On the other hand, administration of the compound at 50 mg/kg/d for 5 days resulted in a significant stimulation of cytochrome P-450, aminopyrine N-demethylase and benzphetamine N-demethylase activities with no effect on the other parameters of the biotransformation enzymes. These results suggest that the effect of garlic oil on biotransformation enzymes is dose-dependent. (Supported by NIH Grant #5 G12 RR03059-02).

JOINT ACTION OF DEVELOPMENTAL TOXICANTS EVALUATION OF BINARY MIXTURES. D A Dawson and T S Wilke. University of Tennessee, College of Veterinary Medicine, Knoxville, TN. Sponsor: D L Frazier.

The Frog Embryo Teratogenesis Assay (FETAX) was used to determine the joint action for 25 binary mixtures of developmental toxicants. Analysis of joint toxic action is based on the concepts of similarity and interaction. Chemicals are similar or dissimilar depending on whether the primary site of action in the organism is the same or not. The chemicals may also be interactive or noninteractive, depending on whether the biological activity of one compound is affected by a second or not. Generally, noninteractive chemicals which have the same mode of action show strictly additive toxicity when combined, whereas chemicals that exert toxicity via a different mode of action have less-than-additive rates of toxicity. Potentiation and antagonism are joint actions signifying interaction. Normally an interactive joint action cannot be predicted without testing. In this study, a variety of developmental toxicants have been tested in combination using X. laevis embryos. For each combination, each compound was tested alone and with the second compound at three mixture ratios (3:1, 1:1, 1:3) based on the concentration required to cause 50% of the embryos to become malformed. Generally, combinations composed of chemicals which had the same putative mode of action showed strictly additive rates of malformation, while combinations containing chemicals with different modes of action had less-than-additive rates of malformation. Incidences of potentiation and antagonism were also noted. The results are not directly applicable for mammalian hazard assessment but may provide a base for hazard assessment when metabolism and pharmacokinetic factors are considered.

IN VITRO METABOLISM OF 14C-DINITROTOLUENE BY HUMAN AND RAT LIVER MICROSOMES AND LIVER SLICES. DE Chapman, Sr Michelear and G Pask. Department of Pharmacology, Mayo Clinic and Foundation, Rochester, MN.

2,6-Dinitrotoluene metabolites produced under aerobic conditions by liver microsomes from seven human subjects included: 2,6-dinitrobenzylalcohol; 2,6-dinitrobenzaldehyde; and relatively minor amounts of 2-amino-6-nitrotoluene. Hepatic microsomes from male, Fischer F344 rats did not produce measurable amounts of 2-amino-6-nitrotoluene under aerobic conditions. 2,6-Dinitrobenzylalcohol was the major metabolite produced by rat and human liver microsomes. Rates of 2,6-dinitrobenzaldehyde formation by rat and human liver microsomes, in pmol/min/mg protein, were 136.6 ± 5.0 (± SD, n = 3) and 264.2 ± 80.8 (± SD, n = 7), respectively. The major metabolite produced by human liver microsomes under anaerobic conditions (nitrogen atmosphere) was 2-amino-6-nitrotoluene. The rates of 2-amino-6-nitrotoluene formation under anaerobic conditions by human liver microsomes and 2,6-dinitrobenzaldehyde formation under aerobic conditions were comparable. 2,6-Dinitrotoluene metabolites produced by human liver slices included: 2,6-diaminotoluene; 2,6-dinitrobenzylalcohol, 2-amino-6-nitrotoluene; and 2,6-dinitrobenzaldehyde. Rat liver slices did not produce 2,6-diaminotoluene. These results suggest that human liver is capable of the reductive metabolism of 2,6-dinitrotoluene. Supported by ES 55110.
DIFFERENTIAL EFFECT OF CYCLOSPORIN ON CYTOCHROME P-450 AND HEME METABOLISM IN RAT KIDNEY AND TESTES. B A Krueger, G M Traksel and M D Maine, Environ, Health Sciences Center and Dept. of Biophysics, Univ. of Rochester, Rochester, NY.

The dose-limiting factor of the immunosuppressive drug cyclosporin (CsA) is nephrotoxicity. Other side-effects have been associated with CsA treatment, including reduced testosterone levels and infertility in male rats. Hemoproteins are essential for cellular functions such as oxidoreduction. This study was undertaken to examine the effects of CsA treatment on renal and testicular heme metabolic activities.

Male rats received s.c. injections of CsA (25 mg/kg) for 6 days. Total renal heme oxygenase activity was significantly reduced by CsA treatment (72-74% of controls). It is noteworthy that CsA is the only agent identified to date which down regulates heme oxygenase. Cytochrome P-450 levels of kidney microsomes, however, were elevated, although benzo(A)-pyrene hydroxylase activity was unaffected. Biliverdin reductase activities were also unchanged in CsA animals.

In the testes, the most prevalent form of heme oxygenase is HO-2 which is refractory to chemical treatment. Total testicular heme oxygenase activity was unaltered by CsA. Testes microsomal cytochrome P-450 levels were decreased significantly (66% of controls). Cytochrome P-450 levels in testes mitochondria were also reduced by CsA, but not as dramatically as in microsomes. Additionally, the activity of cytochrome c reductase, which is required by cytochrome P-450 for metabolism was found to be lower in CsA animals. Results of this study offer a plausible explanation for reduced testosterone levels seen in animals undergoing CsA treatment. Supported by ES03968 and ES07026.

METABOLISM OF PHENYLHYDROQUINONE BY PROSTAGLANDIN (H) SYNTHASE: POSSIBLE IMPLICATIONS IN O-PHENYL-PHENOL CARCINOGENESIS.

P Kolachana, V V Subrahmanyan, D A Eastmond and M T Smith School of Public Health, University of California, Berkeley, CA.

O-Phenylphenol (OPP) and its sodium salt (sodium ortho-phenol) are broad spectrum fungicides and antibacterial agents. Both are urinary bladder and renal carcinogens in the Fischer 344 rat. OPP is converted by mixed function oxidases in the liver to phenylhydroquinone (PHQ). Since appreciable amounts of prostaglandin (H) synthase (PGS) are found in rat bladder and kidney-medullary papilla, the target sites of OPP- and NaOPP-induced tumors, we hypothesized that a secondary PGS-mediated activation of PHQ to phenylbenzoquinone (PBQ) may occur in the bladder and kidneys. We have studied the metabolism of PHQ by PGS in the presence of arachidonic acid and hydrogen peroxide as cofactors. These studies showed that PHQ is indeed metabolized to a product having identical spectral and electrochemical properties to PBQ. The disappearance of PHQ with time was stoichiometric to the formation of PBQ. Less than 10% of PHQ was converted to PBQ in the absence of enzyme indicating that autooxidation may play only a minor role in the conversion of PHQ to PBQ. Similar results were obtained when PGS was replaced with either myeloperoxidase or horseradish peroxidase and hydrogen peroxide as cofactor. These studies suggest that the peroxidative metabolism of PHQ by PGS to the reactive PBQ could play an important role in OPP-induced urinary bladder and kidney carcinogenesis. Supported by Health Effects Component of the U.C Toxic Substances Program.

TARGET ORGAN ACTIVATION OF PARATHION IN SITU AND IN PARTIALLY HEPATECTOMIZED RATS. J R Snyder and J F Chambers, Dept. of Biol. Sci., Miss. State Univ., Miss. State, MS.

Phosphorothionate insecticides, such as parathion, are activated to their oxon metabolites by a cytochrome P-450-mediated desulfuration reaction. The strong affinity of the oxon for acetylcholinesterase (AChE) in the brain. To test the ability of the brain to activate parathion in situ, the ascending aorta was ligated to remove the liver from circulation in anesthetized rats. Parathion was injected into the jugular vein and animals were kept under anesthesia for 15 minutes. Inhibition of brain AChE activity was observed in experimental animals treated with a high dose (48 mg/kg) of parathion. In other studies, male rats which were partially (70%) heptatectomized and injected in the tail vein with 1.5 mg/kg parathion, had a significant amount of brain AChE inhibition after 30 min. Liver and plasma acetylcholinesterase of heptatectomized animals were not lowered. Sham operated animals had high levels of liver and plasma acetylcholinesterase inhibition after 30 min. These results suggest that the brain is capable of parathion desulfuration activity in vitro. (Supported by ES04394 and ES00190).

METABOLISM OF 2-AMINO-1-METHYL-6-PHENYLIMIDAZO[4,5-b]PYRIDINE (PhIP) IN C57BL/6N MICE. M H Bucaradi, M Roer and J F Felton, Lawrence Livermore Natl. Lab, Livermore, CA.

The metabolism of PhIP, a heterocyclic amine carcinogen detected in cooked meats was investigated in C57BL/6N mice administered 0.1, 1.0 and 10 mg/kg 14C-PhIP (i.p.). In 3-MC pretreated mice, urinary and fecal excretion over 24 hr accounted for 16% and 42-56% of the dose, respectively. At all doses, the major urinary metabolite (5% of the dose) was identified as the sulfuric conjugate of 4-OH-PhIP. In noninduced control mice, excretion of urinary PhIP was accounted for 13% of the dose at 10 mg/kg PhIP. Excretion of unchanged PhIP and a glucuronide conjugate of N-OH-PhIP also was higher (4-fold) in control mice. The N-OH-glucuronide metabolite, which was mutagenic toward Salmonella only in the presence of S9, was detected in the urine of mice treated with N-OH-PhIP, a direct acting mutagenic microsomal metabolite of PhIP. Covalent binding in liver was linear over the dose range. At 10 mg/kg, adduct formation in liver was nearly 2-fold higher in induced vs. control mice. In control mice, binding was at least 2-fold higher in control kidneys. These data confirm previous in vitro studies showing cytochrome P450 involvement in PhIP metabolism and activation, but further indicate the importance of other enzymatic pathways. (Performed under the auspices of the U.S. DOE by LLNL under contract W-7405-ENG-48 and supported by Interagency Agreement #2227-01-ES-70158 between the NHES and the DOE.)
In vivo studies in the rat have shown that the urinary tract analgesic, PAP (2,6-diamino-3-phenylxylpyridine), is metabolized by hydroxylation of the phenyl and pyridyl rings. Primary rat hepatocytes were used in vitro in order to determine some of the factors affecting metabolism. Preliminary studies showed that PAP up to 0.4 mM had no cytotoxic effect, as measured by LDH leakage, after 48 h incubation. However, when metabolism was measured in the range 0.02 to 0.32 mM PAP it was found that it tended to peak around 0.05 mM and decreased at 0.32 mM, indicating a toxic effect. In hepatocytes from control rats, SKF-525A, and to a lesser extent metyrapone (MP), inhibited metabolism of 0.02 mM PAP by both pathways. Phenobarbital (PB), β-naphthflavone (NBF), 5-pregnen-3β-ol-20-one-16α-carbinolone (PCN) and Arochlor 1254 (PCB) pretreatment of rats markedly increased the metabolism of PAP. After these pretreatments the effects of inhibitors changed. Neither SKF-525A, MP nor α-naphthflavone inhibited PAP metabolism after induction by NBF or PCB, but SKF-525A did inhibit after PB or PCN induction. The inhibition produced by SKF-525A after PB or PCN induction was only seen with the phenyl 4'-OH-PAP metabolite and not the pyridyl 5'-OH-PAP metabolite. The failure to inhibit in so many cases is unusual and raises the possibility of non-microsomal metabolism.

Bladder cancer has long been associated with exposure to aromatic amines. Dog, a non-acetylator species, is the animal model for assessing aromatic amine induced bladder cancer. Dog liver slices were incubated with 50 μM 3H benzidine in alpha MEM. After 4 h, only 15% of benzidine remained unmetabolized. HPLC elution profiles were similar to that observed in dog bile and urine (Carcinogenesis 11: 139, 1990). A peak corresponding to the previously identified 3-hydroxybenzidine was observed. Another peak corresponded to a metabolite made during the incubation of dog liver microsomes with UDP-glucuronic acid. HPLC profiles indicated that DNA adducts observed in dog liver were distinct from those observed in rat and rabbit but similar to synthetic N-(deoxyguanosin-8-yl)-benzidine. Only a small amount of NADPH-dependent metabolism was observed with dog liver microsomes. The rate of metabolism was 2.5 and 3 pmol/mg/min in aqueous and protein bound fractions, respectively. Metabolism was partially prevented by 0.2 mM DPEA (60%). Thus, the lack of acetylation in dog offers the opportunity to directly assess metabolism of primary aromatic amines, i.e., benzidine, by alternative pathways.
The marked difference in drug and steroid metabolism between male and female rats arises largely from sex-dependent, post-pubertal changes in liver microsomal P450 enzyme levels and activity. The present study was designed to test the hypothesis that thyroid hormones regulate the levels of liver microsomal P450c (II1C1) and P450a (IIA1) in mature male rats. Changes in P450 levels were determined by Western immunoblotting with polyclonal antibodies of known specificity, and by HPLC analysis of testosterone 2β-hydroxylation (II1C1) and 7α-hydroxylation (IIA1). Thyroidectomy (sodium 125I iodide) caused a marked decrease in the levels and activity of II1C1 and an increase in the levels and activity of IIA1. Compared with females, mature male rats express high levels of II1C1 and low levels of IIA1. Therefore, thyroidectomy effectively caused a demasculinization of these forms of P450. Replacement therapy with triiodothyronine (T3) was more effective than replacement with thyroxine (T4) in restoring normal levels of both forms of P450. Treatment of euthyroid (normal) rats with T3 and T4 also caused a demasculinization of II1C1 and IIA1. The fact that the effects of hyperthyroidism were similar to those of hypothryoidism on these P450 enzymes may be ascribed to a down-regulation of hepatic thyroid hormone receptor number with elevated levels of thyroid hormone. The effects of thyroidectomy on II1C1 and IIA1 paralleled the effects of thyroidectomy. However, thyroid hormone replacement therapy in thyroidectomized rats was unable to restore normal levels of these P450 enzymes. The results of these experiments indicate that thyroid hormones, particularly T3, serve an important function in regulating the levels of II1C1 and IIA1 in mature male rats. However, the effects of thyroid hormones on liver P450 enzymes are dependent on the pituitary gland.

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The generation of carbon monoxide during the microsomal metabolism of 2,4,5-trifluoroaniline: a property of dexamethasone induced microsomes. Z Li and M R Franklin, School of Pharmacy, Zhejiang Medical Univ, Hangzhou, PRC and Dept. Pharmacology and Toxicology, Univ. of Utah, Salt Lake City, UT, USA.

Aerobic incubation of 2,4,5-trifluoroaniline with NADPH fortified microsomes from dexamethasone pretreated male rats resulted in the formation of carbon monoxide. Carbon monoxide was detected from the absorbance change (432-425nm) of hemoglobin (2.5μM) present in the incubation medium. The Km and Vmax for carbon monoxide generation were 25μM and 3.8 nmol/min respectively. 2,4,5-Trifluoroaniline was determined using C18 reverse phase HPLC, eluting with a mobile phase of 50% acetonitrile containing 50mM phosphate buffer (pH 7.4) and 25mL diethylamine, and detected by absorbance at 280nm.

2-Chloro-5-methylaniline was used as an internal standard and 1,2,4-trifluorobenzene in methanol was added during the microsomal precipitation to displace the 2,4,5-trifluoroaniline from the biological matrix and give 100% recovery. The ratio of 2,4,5-trifluoroaniline disappearance correlated with carbon monoxide generation in a 1:1 ratio and there was negligible loss of cytochrome P450 (<3%). The presence of EDTA did not affect carbon monoxide generation. No carbon monoxide was generated if NADPH was substituted for NADPH, or if dithionite was added to the incubation medium. Substituting 1,3,4-trifluorobenzene for 2,4,5-trifluoroaniline in the incubation medium did not produce any carbon monoxide, nor did it inhibit the metabolism of 2,4,5-trifluoroaniline if added concurrently. Microsomes prepared from naive rats or rats pretreated with S-nitrosothiols failed to generate carbon monoxide, and microsomes from phenobarbital pretreated rats catalyzed the reaction at 25% of the rate seen with dexamethasone induced microsomes. The reaction appears to be a property of cytochrome P450IIA1 isozymes.

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The effects of several nitrogen and sulfur heterocycles, which are present in tobacco, tobacco smoke and in a variety of foods, on cytochrome P450 have been examined. Thiazole, pyrazine and pyridazine administration (200 mg/kg, i.p., 3d) to rats was found to elevate hepatic P450IIE1 levels; a 6-fold increase with no significant increase in total P450 content. Whereas pyrimidine and triazole caused a 2-fold induction of IIE1 as shown by p-nitrophenol hydroxylase activity, SDS-PAGE and immunoblot analyses confirmed the time-dependent increase in IIE1 levels following the treatment of rats with thiazole, pyrazine and pyridazine for 1 and 3 days, respectively. 5% and Northern analyses revealed a 4-fold reduction in IIE1 poly(A) mRNA at 48 h following thiazole treatment and at 24 h following pyrazine and pyridazine treatment, respectively. IIE1 mRNA content appeared to increase gradually up to 50% of the initial IIE1 mRNA level in untreated animals at 48 and 72 h posttreatment with pyrazine and pyridazine. These results show that thiazole, pyrazine and pyridazine induce IIE1 in vivo and that induction is associated with a rapid decrease in IIE1 mRNA. These results suggest that an efficient utilization of existing IIE1 mRNA is involved in the induction of IIE1 following treatment of animals with these agents. Supported by NIH grant ES03556.

Reconstitution of Rat Cytochrome P450 IIIA1 (P450dp) Requires Phospholipids with Unsaturated Fatty Acids. D. Eberhart and A. Parkinson, University of Kansas Medical Center, Kansas City, KS.

The cytochrome P450 IIIA1 family of enzymes characteristically has poor catalytic activity when reconstituted with NADPH-cytochrome P450 reductase and diacyrlphosphatidycholine (DLPC). Our laboratory and others have recently reported the successful reconstitution of several rat P450 IIIA enzymes with a microsomal lipid extract (MLE) in place of DLPC. This prompted us to examine the lipid requirement of P450 IIIA enzymes in more detail. Several natural and synthetic lipids were tested for their ability to support testosterone oxidation by purified P450 IIIA1. Of fourteen natural membrane lipids tested, only phosphatidycholine (PC) and phosphatidylserine (PS) supported rates of testosterone 6β-hydroxylation comparable to those obtained with MLE. Higher turnover rates were obtained in the presence of MLE, PC and PS when the nonionic detergent, Emulgen 911, was included in the reconstitution system. Twelve synthetic PC's containing fatty acids of differing chain length and varying degrees of saturation were also tested for their ability to support the catalytic activity of reconstituted P450 IIIA1. Testosterone 6β-hydroxylase activity was barely detectable when P450 IIIA1 was reconstituted with PCs containing saturated fatty acids regardless of chain length. In contrast, good catalytic activity was obtained with PCs containing unsaturated fatty acids such as dienophosphatidycholine (18:1). When these same synthetic lipids were used to reconstitute purified rat liver cytochromes P450 IIA1 (P450a), IIA2 (P450m), IIB1 (P450b), and IIC1 (P450d), optimal turnover rates were obtained with either dicacyrlphosphatidycholine (10:0) or DLPC (12:0). These data establish that P450 IIIA1 has a specific requirement for phospholipids containing unsaturated fatty acids which explains why IIIA1 can be reconstituted with MLE but not DLPC. Supported by GM 37044, ES 00166 and ES 07079.

Induction and Suppression of Rat Hepatic Microsomal Cytochrome P450 Isozymes by Chronic Ethanol Treatment in a System Utilizing Total Enteral Nutrition. MJ Ronis, CK Lumpkin, M ElDeib, R Hakkak, C Valentine, MG Ingelmas-Sandberg, PB Thomas & TM Badger. Department of Pediatrics, University of Arkansas for Medical Science, Little Rock, AR.

Rat aversion to ethanol containing diets leads to slower weight gains in ethanol-treated animals and the necessity for pair feeding. Feeding ethanol directly into the stomach as part of a total enteral nutritive (TEN) system allows for an examination of direct ethanol effects in the absence of drug/starvation synergism. In the current study, the effects of chronic ethanol treatment at 35% total calories for 35 days was examined in male Sprague-Dawley rats. Changes in the activities in the hepatic microsomal monooxygenase system were studied using P450 isoform specific substrates. P450 approprotein levels were measured by Western blot analysis and P450 IIC11 mRNA levels were determined by Northern blot analysis using a specific oligonucleotide probe. The TEN diet alone suppressed P450 IIE1-dependent activities and expression, while chronic ethanol treatment induced this P450 isoform 10-15 fold as has been previously reported. However, no significant increase was observed in total P450 content over TEN controls, and in addition to P450 IIE1 induction, the male specific testosterone 16α-, 2α- and 6β-hydroxylase activities were suppressed. This reflected suppression of P450 IIC1 and one or more members of the P450 IIIA family. In addition, ethanol-treated males had plasma growth hormone patterns more reminiscent of female rats than male rats. These data suggest that the demasculinization of the hepatic monooxygenase system described here after chronic ethanol treatment may be an indirect endocrine effect of ethanol acting at the level of the hypothalamic-pituitary axis. Supported by AA08645 and AA08213.
AN AUTOMATED PROCEDURE FOR DETERMINATION OF THE STATUS OF CYTOCHROME P-450 ACTIVITY USING ETHOXY- AND PENTOXY- ANALOGUES OF PHENOAZONE. S J Glass, K K Schmiegel, and R B L van Lier. Toxicology Division, Lilly Research Laboratories, Greenfield, IN.

Metabolism of prototype substrates to determine the activity of cytochrome P-450 monoxygenases has been used in preference to rigorous biochemical separation techniques in order to increase sample throughput. Significant shortcomings with some of the classical substrates that are used for these assays are lack of specificity for a particular isozyme or subclass of P-450 and the time needed to perform these multiple assays. To alleviate these shortcomings, several alkyl derivatives of phenoxazone have been used to assess P-450 isozyme activities; however, metabolism to the fluorescent product, resorufin, occurs so rapidly that the reaction must be conducted within the fluorometer cuvette, thus limiting the number of samples that can be assayed. A new procedure using a Fluorokan II microtiter plate reader (Flow Laboratories, McLean, VA) has been developed to repeatedly scan a microtiter plate in which 96 separate simultaneous reactions can be conducted. Using the new method, a 17-25 fold induction in 7-ethoxyresorufin O-deethylase and a 4-6 fold induction in 7-pentoxyresorufin O-deethylase was observed in rats after daily treatment for 2 weeks with a model compound known to cause induction. Both assays may be conducted using the same method since resorufin is the end product using either substrate, and reproducibility of both procedures compares favorably with the cuvette methods. The new method significantly enhances the capability to assay and report the results for a large number of samples, and may be used to easily assess the spectrum of P-450 isozymes induced by other drugs undergoing toxicologic evaluation.

METHACRYLONITRILE: IN VIVO METABOLISM IN RATS, MICE AND GERBILS. M Y H Paronetto, R G Diaz, D Ybarra and J Deleon. Department of Biology, University of Texas Pan American, Edinburg, TX.

Methacrylonitrile (MeAN), an industrial monomer used in the production of plastic products, is a potent neurotoxin, causes depletion of glutathione and is metabolized to cyanide in rats. We have studied species differences in the metabolism of MeAN to cyanide in three mammalian species including male Sprague-Dawley rats, Swiss white mice and Mongolian gerbils. The studies included both time course and dose response (0.25, 0.5 and 1 LD50) experiments. Following 1 LD50 doses of MeAN (200 mg/Kg, 17 mg/Kg and 4 mg/Kg respectively), the signs of toxicity were that of central nervous system disturbances including depression, convulsions and respiratory failure. Cyanide concentrations in blood, liver, kidney and brain, after MeAN administration, were dose dependent in all species. Maximum blood cyanide concentrations were observed 1 hour after dosing in mice and gerbils but at 3 hours in rats. Blood cyanide concentrations increased after phenobarbital treatment (-180% of control) or fasting (-220% of control) and decreased after colchicine chloride treatment (-70% of control) in rats. Cyanide concentrations in liver homogenates showed that gerbils metabolized significantly greater quantity of MeAN to cyanide than either rats or mice. These data indicate that there are species differences in MeAN metabolism in these 3 mammalian species which appear to be related to its toxicity and suggest that MeAN is metabolized to cyanide via a mixed function oxidase system.

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KINETICS OF HEXAMETHYLPHOSPHORAMIDE (HPMA) METABOLISM IN RAT NASAL MUCOSAE. B A Treila, and M S Bogdanoff. Haskell Laboratory for Toxicology and Industrial Medicine, E I du Pont de Nemours & Co, Newark, DE.

HPMA is an aprotic polar solvent used in synthetic organic chemistry and in limited applications in the polymer industry. HPMA is a potent nasal carcinogen inducing tumors of the respiratory (RES) but not olfactory (OLF) epithelium of rats at concentrations of 50 ppb. Studies of HPMA metabolism have shown that HPMA undergoes cytochrome P-450-mediated N-demethylation reactions to yield formaldehyde, another nasal carcinogen. The kinetics of the N-demethylation reaction have not been thoroughly studied in isolated microsomal preparations of rat RES and OLF mucosa. The purpose of these studies was to generate kinetic parameters for HPMA N-demethylation in nasal RES and OLF mucosae. V_max values for male and female rats were 3 to 10 times larger in microsomes from OLF than in microsomes from RES. V_max/K_m was approximately 8 times greater in OLF than RES of both male and female rats. Thus, OLF catalyzes the demethylation of HPMA more readily than RES suggesting that regional differences in the demethylation of HPMA by the nasal cavity alone do not correlate with regions of tumor formation. Other factors, such as water solubility, tissue susceptibility, and airflow patterns, may also be important in the regional specificity of HPMA-induced nasal tumors.


The metabolism of the pyrrolizidine alkaloid monocrotaline (MCT) was examined using isolated perfused rat liver and 14C-MCT. Hepatic tissue was perfused in a recirculatory fashion for 90 min, and the distribution of metabolites between the bile and perfusate were measured. Monocrotaline acid (MCA) was found to be the major acidic metabolite of 14C-MCT with trace amounts of (+) 1-formyl-7-hydroxy-6,7-dihydro-5H-pyrrolizine, (+) 6,7-dihydro-7-hydroxy-1-hydroxy-methyl-5H-pyrrolizine (DHP), and 1-hydroxymethyl-7-oxo-6,7-dihydro-5H-pyrrolizine being identified in the perfusates using GC/MS. Retronecine was not present at detectable levels in the perfusion medium. A large portion of the 14C recovered from both the bile and perfusate was not extractable, under acidic or basic conditions, into organic solvents. Using Fast Atom Bombardment (FAB) MS/MS, a portion of this material was identified as glutathione conjugated DHP. In addition, this non-extractable material retained a portion of the radioactivity which was equivalent to the acidic fraction. Given these findings and the absence of retronecine, the major pathway for the metabolism of MCT could potentially involve the production of MCT pyrrole which subsequently reacts with cellular nucleophiles producing MCA in addition to highly water soluble conjugated pyrroles.
1173 EXTENT AND IMPLICATIONS OF INTERSPECIES DIFFERENCES IN THE INTESTINAL HYDROLYSIS OF GLUCURONIDE CONJUGATES. E M Kenyon and E J Calabrese. School of Public Health, Univ. of Massachusetts, Amherst, MA.

On the basis of in vitro data, large interspecies differences have been projected in beta-glucuronidase activity in the intestine of man and rabbits relative to rats and mice. High beta-glucuronidase activity has the potential to result in amplification of the toxicity of compounds excreted into the bile as glucuronide conjugates since the hydrolyzed aglycone would be available for reabsorption and enterohepatic circulation. In these studies, the capacity of the gut microbiota from the proximal and distal small intestine and cecum of mice, rats and rabbits to hydrolyze these glucuronide conjugates was evaluated under anaerobic culture conditions closely resembling the in vivo gut environment. Hydrolysis of glucuronide conjugates in the proximal and distal small intestine was uniformly low in all species relative to the cecum. There were species and substrate specific differences in the hydrolysis of glucuronide conjugates in the cecum which could have important implications for animal extrapolation.


Inhibition of neuropathy target esterase (NTE) and its dealkylation ("aging") is important in the genesis of organophosphate (OP)-induced delayed neurotoxicity (OPIDN). An in vitro assay for aging was devised; OPs were treated with a microsomal fraction from phenobarbital-induced chick embryo liver, incubated with embryo brain, and treated with the reactivator RF to determine aging. This approach, and in vivo testing of hens, were used to study technical, analytical and chiral isomers L(+), D(-), of methamidophos (MT), 35 mg/kg of technical MT averaged 22% aged NTE in vivo, higher than the 6% aged NTE produced by analytical MT. GC-MS and TLC revealed two contaminants in technical MT, O,O,S-trimethyl phosphorothioate and O,O-dimethyl methylphosphoramidothioate. L(-) and D(+)-isomers of MT (5 mM) in vitro produced 6% and 4% aged NTE, respectively, similar to analytical MT in vitro. Although total NTE inhibition (aged plus reactivated NTE) was higher for D(+)-MT, L(-)-MT produced slightly more aged NTE both in vivo and in vitro. The possibility that the contaminants in technical MT contribute to its OPIDN potential is under study.


Generally, young chickens (<50 days of age) are reported to be resistant to OPIDN. The present study examined whether PMSF posttreatment could alter the development of OPIDN in young chickens (35, 49 or 70 days of age; n=5/group). We found that the severity of disopropylphosphorofluoridate (DFF, 2 mg/kg)-induced ataxia increased with increasing age. Moreover, chickens treated with DFF followed four hours later by PMSF (90 mg/kg) showed more extensive motor deficits and cervical spinal cord lesions than those treated with DFF alone. Relatively similar degrees of ataxia were evident in PMSF posttreated animals regardless of age. We conclude that 1) young chickens (<50 days of age) are not entirely resistant to OPIDN and 2) PMSF posttreatment potentiates both the clinical and neuropathological signs characteristic of this syndrome.

1176 CHLORPYRIFOS DAILY DOSING FOR 20 DAYS IN HENS WITH MONITORING OF BRAIN, PLASMA, AND LYMPHOCYTE ESTERASES. RJ Richardson, TB Morris, US Kayali, and JC Randall. Toxicology Program, The University of Michigan, Ann Arbor, MI.

Chlorpyrifos (CPS) was evaluated for clinical signs of delayed neuropathy (OPIDN) as well as inhibition of brain and lymphocyte neurotoxic esterase (NTE and L-NTE, respectively), brain acetylcholinesterase (AChE), and plasma butyrylcholinesterase (BuChE) during daily dosing (10 mg/kg/day po in corn oil, 2 ml/kg/day) in hens for 20 days with further observation for 4 weeks. Esterases were assayed on days 1 (corn oil control only), 5, 11, 16, 21, and 49. During the treatment period, AChE and BuChE activities were reduced to 30-40% and 20-50% of control, respectively. At 4 weeks after the end of dosing, AChE and BuChE had recovered to 86 and 133% of control, respectively. NTE and L-NTE activities during the treatment period were 82-98% and 83-114% of control, respectively. Weight loss in treated hens during the treatment period was largely regained in surviving hens by the end of the study. None of the hens developed clinical signs of OPIDN as evaluated by clinical inspection and the ability to perch on a wooden rod. The results indicate that daily dosing of CPS sufficient to significantly depress body weight, AChE and BuChE, resulted in NTE and L-NTE inhibitions less than 20% and no signs of OPIDN. (Supported by NIH ST32ES07062, NIAAA PS0A07378, a grant from The American Diabetes Association, and a gift from The Dow Chemical Company).

Organophosphorus ester-induced delayed neuropathy (OPIDN) has been reported in rats and hens, but sequential pathological studies exist only for the latter. We performed studies of the temporal development of bilateral lesions elicited by 30 mg/kg of mipafox (ip) in the medulla and cervical spinal cord of male Long-Evans rats (>60 days of age) and White Leghorn hens (2-18 months of age). This dosage inhibited brain neurotoxic estimates 4 hours after administration by 90±2% in rats and 89±11% in hens (n=4). In rats, lesions were seen in distal levels of the fasciculus gracilis, and consisted of numbers of swollen, vacuolated myelinated axons, restricted to medullary levels of the tract. These varied in intensity between animals and were seen in 2/3 on day 7, 3/3 on day 14, 3/4 on day 21 and 3/7 on day 28. Occasional swollen fibers were seen in this region in some controls as well. Hens had extensive regions of myelinated fiber degeneration consisting of swollen, debris-laden axons progressing to Wallerian-like degeneration in distal (rostral) levels of spino-cerebellar tracts and fasciculus gracilis. These were seen in 0/4 on day 7, and 4/4 on days 14, 21 and 28. The prominent central nervous system lesions of OPIDN in hens evolved in a stereotypical fashion, becoming most florid by day 21. In contrast, rats had a more erratic evolutionary course of lesion development, which did not approach the hen in severity. (Supported by USEPA 68D80098)

COMPARATIVE STUDIES OF ORGANOPHOSPHORUS ESTER-INDUCED DELAYED NEUROPATHY (OPIDN) IN HENS AND RATS. B. S. Jortner, M. Ehrich, L. G. Shell, S. Padilla*, Virginia Tech, Blacksburg, VA and *U.S. E.P.A. Research Triangle Park, NC.

Recent studies have suggested the rat may, along with the chicken, be an appropriate model for OPIDN. We report comparative 21-day studies of OPIDN in these species, using White Leghorn hens and male Long-Evans rats. The following compounds and single dosages (mg/kg) were used; tri-ortho- toyl phosphate (po); hens - 50, 90, 500; rats-300, 600, 1000; mipafox (ip) - hens - 3, 6, 30; rats - 3, 10, 30; phenyl saligenin phosphate (ip) - hens - 0.1, 0.2, 2.5; rats - 5, 8.5, 24; DFP (sc) - hens - 0.25, 0.4, 1; rats - 1, 2.3. In both species, these elicited dose-related depression in activity of brain neuro-toxic esterase (n4/ group), exceeding 80% at highest OP dosage levels. Clinical signs and bilateral CNS myelinated fiber degeneration were more prominent in hens than rats. Hens had progressive ataxia and weakness, beginning in week 2 with all 4 compounds, while rats, except for weight loss, had inconsistent deficits. Lesions on day 21 (n > 5/group) were extensive in hens, evolving from axonopathy to Wallerian-like degeneration in distal regions of several long fiber tracts. Rats had relatively small numbers of altered fibers, mainly swollen, vacuolated axons in rostral fasciculus gracilis. These studies are in accord with others indicating interspecies differences in clinical and pathological responses to neuropathic organophosphates. (Supported by USEPA 68D80098)

THE UTILITY OF AN ACUTE DOSING REGIMEN TO DETERMINE ORGANOPHOSPHATE NEUROTOXICITY. L. Linderer, P. E. Kinkead, J. J. Yang, M. L. Barh, and C. R. Mackerer, Naval Medical Research Institute, Wright Patterson AFB, OH and Mobil Environmental and Health Science Laboratory, Princeton, NJ.

In 1959, human paralysis was reported after the ingestion of lubricating oil containing TCP (tricresyl phosphate, mixed isomers). This study was done to determine whether current lubricants containing TCP with ortho isomer content controlled would be similarly neurotoxic. Adult hens were given 5 daily oral doses of 420, 360, 300 or 240 mg/kg generic jet engine oil containing 3% TCP or 3% TOCP. The equivalent doses of phosphate ester (PE) are 12.6, 10.8, 9, and 7.2 mg/kg. Larger amounts of TOCP in corn oil (90, 75, or 60 mg/kg TOCP) were used as a positive control.

Ataxia was observed in all birds in the positive control group. None of the birds given the engine oils became ataxic. Brain NTE was inhibited by 80% or more in the positive controls, up to 78% by engine oil with TOCP, and up to 38% by engine oil with TCP. These numbers suggest that larger doses and/or longer treatment might reach the threshold for neuropathy. In fact, preliminary results from a subchronic study in which hens were dosed 5 days/wk for 10 wks with the engine oil containing 3% TCP showed this material to cause ataxia. This confirms that acute studies which are effective in identifying neurotoxic PEs when tested undiluted, may not be adequate to evaluate the neurotoxic potential of formulated products with low levels of PEs.

PURIFICATION OF Ca2+/CALMODULIN-DEPENDENT PROTEIN KINASE II FROM ADULT HEN BRAINS AND ITS INTERACTION WITH DIISOPROPYLPHOSPHOROFULORIDATE. R. P. Gupta, D. M. Lapadula, M. B. Abou-Danta. Duke Univ Med Ctr, Dept of Pharm, Durham, NC.

A calcium/calmodulin-dependent protein kinase II was purified from adult hen brains and its interaction with Diisopropylphosphorofluoridate (DFP) was investigated. Microtubule associated protein (MAP-2) was used to assay kinase II activity during purification. The specific activity was about 58 nmol/min/mg protein and the purification 525-fold. Two columns, phosphocellulose and calmodulin-bound Sepharose 4B, were used for the purification of enzyme. Among the substrates used (MAP-2, histone II, histone VIII, myelin basic protein, phosvitin, tubulin, phosphorylase b, glycogen synthase, myosin light chains, and diphosphorylated casein), histone II exhibited the maximum activity of the enzyme and MAP-2 was phosphorylated at a slightly lower rate. The phosphorylation of tubulin was about 10% of MAP-2. DFP at 0.5 μM concentration inhibited MAP-2 phosphorylation by about 25%, and had no effect on autophosphorylation of the enzyme. Ca2+/calmodulin dependent protein kinase II inhibition by DFP was partially competitive with respect to ATP, and not affected by varying concentrations of calcium and calmodulin. (Supported in part by Grants ESO 2717 and ESO 5154).
Chicks were known to be resistant to OPIDP. D1-n-butyl dichlorvos (DDCVP, 1mg/kg sc) or diisopropyl fluorophosphor (DFP, 1.5mg/kg sc) when given to chicks inhibited NTE above the threshold for initiation (70%) but did not cause OPIDP as in hens. 30 and 40-day-old chicks treated with higher DDCVP doses (5mg/kg sc or 1mg/kg sc x 5 daily) developed OPIDP. OPIDP severity was higher in 40-d chicks than in 30-d chicks. Recovery was complete ranging from 9 to 40 d after onset, being slower in older chicks. 76-d chicks were sensitive to DFP (1.5mg/kg sc) but recovered completely. Protection from OPIDP was achieved by PMSF (30mg/kg sc) given 24 h before DDCVP (5mg/kg sc). OPIDP was promoted in 40-d chicks with PMSF (300mg/kg sc) given 24h after DDCVP or DFP (5 and 1.5mg/kg sc, respectively). The clinical picture was characterized by flaccidity and not paresis. In conclusion, single high doses of OPs cause OPIDP in chicks but in contrast to hens, the clinical picture is different, less severe and reversible.

ACRYLAMIDE ALTERS ELEMENTAL AND WATER CONTENT OF RAT TIBIAL AXONS AND SCHWANN CELLS. R M LoPachin, C M Castiglia and A J Sauermann. Dept. Anesthesiology, SUNY Stony Brook, Stony Brook, NY.

ACR-induced nerve damage might involve changes in axonal elements and water. X-ray microanalysis was used to measure Na, K, etc. and % water in frozen sections of treated and control rat tibial nerves. After 15 days of oral ACR exposure (2.8 mM, drinking water), only axoplasmic K and Cl levels exhibited changes. Mean data (mmol/kg dry wt ± SEM) increased only modestly (e.g. large axons: KCON=1586±29 vs Kacr=1810±68), yet corresponding variance and range increased substantially. Axonal water content showed a similar rise in variance and range. At 22 days, the magnitude of these changes increased while axoplasmic and Schwann cell Na levels also rose. At 60 days, element and water content of "surviving" axons returned toward normal. Degenerating axons, observed at all time points, displayed a complete loss of elemental regulation. Injection of ACR (50 mg/kg/day, i.p.) for 10 days produced effects similar to those at 22 days of oral dosing. These selective temporal changes in axons and glial cells might be mechanistically involved in ACR neurotoxicity.

These studies were undertaken to determine whether variations in the dose rate of exposure alter qualitatively and/or quantitatively the neurotoxicity of acrylamide (ACR). Dose-response functions were determined in adult male LE rats (N=5-12) for 1-, 10-, and 30-day exposures to ACR. Endpoints included tests of motor function (motor activity, grip strength), acoustic startle response, and axonal transport and were usually assessed prior to dosing, and 2 hr, 2, 7, 14 and 28 days post-exposure. ACR was administered ip in 1.0 ml/kg saline in the following ranges of dose rates: 1-day (0-150 mg/kg); 10-day (0-30 mg/kg/day); and 30-day (0-20 mg/kg/day). Behavioral results were consistent with previous findings that ACR induced a reversible decrement in motor function. Tests of sensorimotor reactivity indicated ACR did not impair auditory function. A novel approach to monitoring retrograde axonal transport of phospholipids failed to detect any effects of ACR. The data for motor impairment indicate that the effects of ACR depend more on the dose rate of administration than on the total dose administered. Preliminary analyses also indicate the rate of recovery of motor function is directly related to dosing duration.

IN VITRO COVALENT LYSINE ADDUCT FORMATION BY CARBON DISULFIDE IN NATIVE AND CYSTEINE-BLOCKED BOVINE SERUM ALBUMIN. A. P. DeCaprio and J. H. Frohke. Wadsworth Center for Laboratories and Research, NY State Dept. of Health, Albany, NY.

Carbon disulfide (CS₂) is one of a class of chemical neurotoxicants producing axonal neurofilament accumulations. We are currently exploring specific lysine adduction as a common mechanistic step in these neuropathies. The present study examines aspects of covalent binding of CS₂ using bovine serum albumin (BSA) as a model protein. Incubation of BSA in a physiological buffer with CS₂ resulted in an exposure- and time-related appearance of a chromophore (λₘₐₓ = 434 nm), UV absorption bands (λₘₐₓ = 250 and 291 nm), decreased lysine content, and increased net negative charge. No acid-stable lysine adduct could be detected by amino acid analysis. After removal of unreacted CS₂ and continued incubation, these changes were partially reversed, and the presence of an acid-stable lysine adduct was indicated. In addition, SDS-PAGE revealed a new protein band migrating slightly faster than the native BSA consistent with either a conformationally altered or intramolecularly-crosslinked species. Intramolecular crosslinking was not seen. Identical results were obtained with cysteine-blocked BSA, indicating the lack of participation of free thiols. These findings are consistent with proposed mechanisms of CS₂-lysine binding involving formation of a labile dithiocarbamate followed by conversion to the isothiocyanate and other products. (Supported by NIH ES-05172).

EFFECT OF ACRYLAMIDE ON NEURITE EXTENSION IN PC12 CELLS. J. Knoth Anderson, D. M. Lapadula, M. Friedman, and M. B. Abou-Donia. Duke Univ Med Ctr, Durham, NC and American Cyanamid Co., Wayne, NJ.

PC12 cells (rat pheochromocytoma cells) differentiate from a chromaffin-like cell to a neuronal phenotype in response to nerve growth factor (NGF). The most notable feature is the extension of neurites from the cell within 24 to 48 hrs. Acrylamide potentiated this NGF response by increasing the rate of neurite production within 48 hrs. The length of the neurite extensions increased with increasing concentrations of acrylamide with a maximum effect at about 1 mM acrylamide. Incubation of the cells for 48 hrs with acrylamide alone did not induce any substantial neurite outgrowth, however, the cells did flatten out and very short projections were evident. In contrast, when the cells were primed with NGF for 7 days and then replated in the presence of acrylamide alone, a significant percentage of cells exhibited neurite outgrowth. These results suggest that acrylamide might be operating at a posttranscriptional level. One dimensional gel electrophoresis indicated that acrylamide decreased the phosphorylation of a few proteins following incubation with 1 mM acrylamide. This suggests that one mechanism by which acrylamide effects neurite extension in PC12 cells is by altering the phosphorylation events associated with NGF induced neuronal differentiation. (Supported in part by NIH Grant No. ES0 5071).

DEVELOPMENT OF A MODEL CELL CULTURE SYSTEM FOR THE STUDY OF EARLY EFFECTS OF NEUROPATHY-INDUCING ORGANOPHOSPHATES (OPs). A. F. Nostrand and W. F. Ehrlich. VA Tech, Blacksburg, VA.

The earliest biochemical events associated with the interaction of OPs with neural cells are difficult to study in animals. A model cell culture system could facilitate examination of the events taking place in the first minutes after OP exposure. The human neuroblastoma cell line, SY-SY, was chosen for its neurotoxic esterase (NTE) and acetylcholinesterase activity. The neuropathic OP mipaflox was chosen for its ability to inhibit NTE and for its minimal effect on viability of differentiated SY-SY cells in vitro. NTE inhibition and aging in differentiated SY-SY cells after exposure to 5 x 10⁻⁹ M mipaflox was examined. NTE was inhibited by 28% of control activity at 2 minutes, by 67% at 5 minutes and by 81% at 10 minutes. Aged NTE, as determined by sample reactivation with isonitrosocacetophenone (INAP), was 0% at 2 minutes, 15% at 5 minutes and 73% at 10 minutes. NTE activity of SY-SY cells at a concentration of 2 x 10⁷ cells/ml was comparable to that of brain tissue from untreated chickens. NTE inhibition in brain tissue after mipaflox treatment was 39%, 58%, and 85% at 2, 5, and 10 minutes, respectively. At these times, 0%, 33%, and 59% of control NTE activity was aged. In summary, the SY-SY cell line shows promise as a model system in which to study the early effects of neuropathy inducing OPs on neural cells.
HIGHLY DIFFERENTIATED THREE-DIMENSIONAL CHICK EMBRYO BRAIN CELL REAGGREGATES WERE USED IN AN IN VITRO MODEL TO STUDY ORGANOPHOSPHATE (OP) INDEuced DELAYED NEUROTOXICITY (OPIDN). 10-DAY-CHICK EMBRYO BRAINS WERE DISSOCIATED, REAGGREGATED IN CHEMICALLY DEFINED MEDIA, AND THE CULTURES WERE TREATED FOR 20 MINUTES ON DAY 7 WITH EITHER THE NON-NEUROPATHIC PARAOXON (PO, 10^{-6}M) OR THE NEUROPATHIC DIOXYL FLUOROPHOSPHATE (DFP, 10^{-4}M) AND SAMPLED FOR UP TO 14 DAYS. ENZYME ASSAYS INCLUDE ACETYLCHOLESTERASE (ACH-E), NEUROPATHY TARGET ESTERASE (NTE), OXIDE REACTIVITY OF OP-INDUCED ACHE WITH 2-PAM AND "PROTECTION" OF NTE WITH PHENYLMETHYLISULFONYL FLUORIDE (PMSF). PO AND DFP INHIBITED >99% OF ACHE ACTIVITY, WHICH RECOVERED AFTER 2 DAYS OR WITH 2-PAM TREATMENT. DFP BUT NOT PO INHIBITED NTE BY AT LEAST 95%. THE REAGGREGATES ALSO WERE EVALUATED ULTRASTRUCTURALLY. THE RESULTS DEMONSTRATED BIOCHEMICAL DIFFERENCES IN VITRO BETWEEN THE NEUROPATHIC DFP AND THE NON-NEUROPATHIC PO SIMILAR TO THOSE FOUND IN VIVO. THE POSSIBILITY THAT ULTRASTRUCTURAL DAMAGE AND ENZYME ACTIVITY DIFFERENCES BETWEEN DFP AND PO IN VITRO WILL PREDICT OPIDN IN VIVO IS UNDER STUDY.

CHARACTERIZATION OF MUSCARINIC RECEPTORS OF INSECT BRAIN. E A Abdallah, M E Eldebrani and A T Eldebrani. Dept. Pharmacol. Exp. Ther., Univ. of Maryland Sch. of Medicine, Baltimore MD.

With the identification of at least five muscarinic receptor subtypes in mammalian brains, it is now possible to characterize insect brain receptors utilizing specific pharmacologic agents. Very little specific binding of [H]AF-DX (M_3 selective) or [H]pirenzepine (P2) (M_4 selective) to honeybee head membranes was detected but binding of the nonselective muscarinic antagonist [H]quinuclidyl benzilate had B_max of 108 fmol/mg and K_d of 0.47 ± 0.13 nM. In cockroach and housefly heads the K_d values were 0.13 and 0.17 nM and B_max 64.9 and 69.3 fmol/mg, respectively. In all insects, [H]QNB binding was inhibited best by atropine (K_i values 1-5 nM) followed by 4-DAMP (M_4 selective) and the least by AF-DX 116 (K_i 1-6 µM). It is suggested that the majority of insect brain muscarinic receptors are of the M_3 subtype (supported in part by NIH grant No. ES02594).

INTERACTIONS BETWEEN SYNTHETIC FOOD COLOURS AND NEUROCHEMICAL RECEPTORS IN THE GUINEA PIG ILEUM. A P Hutchinson, B Carrick, K Miller & S Nicklin. BIBRA, Carshalton UK. Sponsor: D Gangolli

The potential of food additives to elicit adverse reactions in susceptible individuals remains an issue of public concern. It has been suggested that food colours may elicit adverse effects via a direct interaction with specific receptors within the gut. In order to test this hypothesis we investigated the ability of a number of food colours to trigger intestinal hyperactivity. Organ bath studies revealed that guinea-pig ileum was highly sensitive to FD&C Yellow No.5 (Tartrazine). Intestinal contraction occurred in a dose-dependent fashion down to a minimum effective dose of 1µg/ml. All other colours examined proved negative. Further studies investigating the biological activity of tartrazine metabolites revealed that sulphanilic acid was active whereas 1-p-sulphophenyl-3-carboxy-4-amino-5-oxo pyrazole (SCAF) proved significantly more active than the parent compound. Blocking studies showed that tartrazine contraction was inhibited by atropine but not by hexamethonium or any of the other blocking agents tested. The fact that atropine was the only effective agent within the panel of chemical antagonists studied implies that tartrazine acts either directly or indirectly upon the muscarinic acetylcholine (ACh) receptor associated with parasympathetic innervation. (Supported by the UK Ministry of Agriculture Fisheries and Food.)


Toxicological studies of TCDD in animals have revealed adverse effects of reduced food intake and progressive weight loss. Animals exposed to TCDD typically waste away in a starvation-like manner with death occurring from two to six weeks after administration of a single oral dose. The TCDD induced anorexia may be due to a specific effect on regulatory systems associated with the control of food intake. It is known that endogenous opiate peptides (EOP) play a physiological role in appetite and regulation of food intake. Our laboratory has shown that exposure of rats to TCDD results in decreased hypothalamic concentrations of the EOP, endorphin (E). The decrease in hypothalamic E concentrations suggests a possible mechanism involving opiate peptide receptors for the altered feeding behavior. Thus, it is important to determine whether TCDD alters the receptor for E (mu receptor). This study was performed to assess whether brain mu receptors are associated with TCDD induced anorexia and body weight decline. Male Sprague-Dawley rats (180-200g) were given a single, oral dose of TCDD (50 µg/kg). Rats were sacrificed three days after treatment, cerebri were removed and processed. Mu receptors in brain homogenates were measured by homologous displacement binding studies using a ligand specific for the mu receptor, [H]DAMGO (N,N-diacyl-D-Ala<sub>2</sub>[Leu<sub>5</sub>]enkephalin (DAMO)). The K_d and B_max were determined by Scatchard analysis. Brain mu receptor number was found to be decreased 30% by TCDD without a change in the K_d. These results indicate that TCDD causes early perturbations in brain mu receptor number which may contribute to the mechanism by which TCDD exposure leads to anorexia and progressive weight loss. (Supported in part by T32 ES-07062).
Whereas the mechanisms of mercury intoxication have been studied extensively, little is known about its primary target site through which a variety of symptoms of poisoning are produced. We previously studied the actions of methylmercury on neuromuscular transmission and neuronal sodium and potassium channels. Recently we have started working on the GABA-activated ion channels and found that mercury modulates their activity in a striking manner. Whole-cell patch clamp techniques were applied to the rat dorsal root ganglion neurons maintained in primary culture. The application of GABA generated inward currents which reversed their polarity at the equilibrium potential for chloride, indicating that the currents were carried by chloride ions. The chloride currents were desensitized to lower levels depending on the concentration of GABA. The GABA-induced peak chloride current was greatly enhanced by mercuric chloride, reaching about 130% and 200% of control by 1 and 10 μM HgCl₂, respectively. This would cause a suppression of the synaptic activity. At 10 μM, HgCl₂ also generated slow inward currents, which appear to be due to opening of non-selective cation channels. On the contrary, methylmercury also induced slow inward currents but suppressed the GABA-induced current only at a high concentration (100 μM). It is concluded that the GABA-chloride channel complex is an important target site of mercury.

Methylmercury (MeHg), the most neurotoxic form of mercury, produces severe neurological symptoms. Histological studies following MeHg exposure have demonstrated extensive damage in the striatum. Presently, there is no satisfactory explanation for this selective toxicity. MeHg has high lipid solubility and affinity for sulphydryl groups, thus it is thought that neuronal membranes may be a primary site of action. Neurotransmitter release, a membrane associated process, may be adversely affected during MeHg exposure. The present study investigates the effects of MeHg on dopamine (DA) release. In vitro superfusion system is being used to expose striatal slices to Kreb's buffer containing MeHg (10 μM to 100 μM). Nonselective and pargyline are added to the Kreb's buffer to block DA reuptake and monoamine oxidase respectively. Endogenous DA levels are measured by high pressure liquid chromatography using electrochemical detection. The effects of MeHg on spontaneous, K⁺ and glutamate (GLU) evoked release are being studied. 100 μM MeHg appeared to increase spontaneous and 35 mM K⁺ evoked DA release. 10 μM MeHg had no significant effect. We are currently investigating the effects of intermediate concentrations of MeHg on spontaneous, K⁺ and GLU-evoked release. (Supported by NSERC)

Activation of dentate gyrus granule cells leads to a period of depressed excitability normally mediated by recurrent inhibition. Administration of the pyrethroid, deltamethrin, intensifies this response many fold. To determine the role of GABA_A-mediated mechanisms in the action of deltamethrin, we have looked at its antagonism by the specific GABA_A antagonists, bicuculline and picrotoxin. Neither reduced the duration of the inhibition produced by deltamethrin. The only effect observed was a reduction in intensity of inhibition during the period of 10-40 msecs after activation of the granule cells. The GABA_A antagonists were able to antagonize the enhancement of inhibition produced by pentobarbital, diazepam, and SKF 100330A, a GABA uptake blocker. These results suggest that the prolongation of inhibition produced by deltamethrin in dentate granule cells does not result simply from an extension of GABA_A-mediated inhibition, but rather derives from a different source.

PCP (angel dust), a widely used drug of abuse and its related analogs inhibit the reuptake and enhance the release of dopamine. We designed this experiment to evaluate whether prenatal exposure to PCP produces alterations in vivo in glutamate-evoked release of [³H]-dopamine in vitro. Fetal rats from pregnant, Wistar, Sprague-Dawley, or F344 dams were injected at embryonic day 15 with either 5, 10, or 20 mg/kg PCP daily on gestational days 8 through 20. On postnatal days (PNDs) 8, 21, 45, or 60 pups were sacrificed and brain regions were dissected for neurochemical analysis. Glutamate-evoked tritium releases from striatal slices labeled with [³H]-DA was determined by superfusion techniques. [³H]-DA release was barely detectable in striatal slices at PND 8 without 5 μM nomifensine and 5 μM sulfuride. However, with 5 μM nomifensine and 5 μM sulfuride present, 40 μM glutamate-evoked release was 50% greater in PCP treated pats at PND 8, 21, 45, and 60. There were no significant changes in the in vivo levels of DA or its metabolites in striatum. These data indicate that prenatal exposure to PCP produces selective changes in glutamate-evoked DA release without affecting in vivo levels.

Organotins have a wide application as heat stabilizers for PVC polymers, biocides and catalysts. There is increasing evidence that organotins are potent neurotoxicants causing severe brain edema, myelin degeneration and reduction of neurotransmitter levels. Recent studies indicated that these organotins affect Ca\textsuperscript{2+}-regulated events in brain causing neuronal dysfunction. Since protein kinase C (PKC), a Ca\textsuperscript{2+}-dependent enzyme plays a vital role in mediating the actions of hormones, neurotransmitters, antigens and growth factors, an attempt was made to study the in vitro effect of three organotins - tributyltin bromide (TBBT), triethyltin bromide (TET) and trimethyltin chloride (TMT) on PKC activity of rat brain soluble fraction. All the three organotins inhibited PKC activity in a concentration dependent manner, TBBT being more potent. The order of potency of these three organotin compounds was TBBT > TET > TMT. A 50% inhibition of PKC activity was observed with 50 and 100 \textmu M of TBBT, TET and TMT respectively. These results suggest that organotins may be exerting neurotoxicity by modulating the PKC activity which is known to be involved in the cellular signal transduction pathways. (Supported by NIH/MBRS grant # RR08047)

1199 INTRACELLULAR CALCIUM TRANSIENTS IN CARDIAC MYOCYTES EXPOSED TO CARBON TETRACHLORIDE. M. Torrance and M. Breitenstein. Cellular Toxicology, ETB, DBBS, CIC, NIGSH, Cincinnati, OH.

Carbon tetrachloride (CCI\textsubscript{4}) sensitizes the heart to arrhythmia and has been reported to elevate intracellular ionic calcium ([Ca\textsuperscript{2+}]) in hepatocytes. Because myocardial contraction is dependent of rhythmic [Ca\textsuperscript{2+}] transient, we investigated changes in [Ca\textsuperscript{2+}] induced by CCI\textsubscript{4} in cardiac myocytes. Isolated cardiac myocytes from neonatal rats were loaded during a 10-20 min perfusion with 1 \textmu M fura-2/AM in buffer containing 2 \textmu M CaCl\textsubscript{2} at a pH of 7.2. Fura-2 was excited at 340 and 380 nm with a spectrofluorometer (PTI) equipped with light chopper operating at 60 Hz. Emitted fluorescence was filtered with a 515 PM filter. Changes in [Ca\textsuperscript{2+}] were assessed by changes in the ratio of the 340/380 nm emission intensities. Cells were exposed to CCI\textsubscript{4} by a single pass perfusion in a Dvorak-Stockel chamber. Below 1 \textmu M, CCI\textsubscript{4} slowed the rate of contraction of spontaneously beating myocytes, increased diastolic [Ca\textsuperscript{2+}], and decreased peak systolic [Ca\textsuperscript{2+}],. Above 1 \textmu M, CCI\textsubscript{4} stopped contractions. Upon washout of CCI\textsubscript{4}, rate of contraction recovered as did systolic [Ca\textsuperscript{2+}], but diastolic [Ca\textsuperscript{2+}], remained elevated for at least 30 min. In quiescent myocytes, CCI\textsubscript{4} below 1 \textmu M caused only a transient (~30 sec) increase in [Ca\textsuperscript{2+}], despite continued exposure. Above 1 \textmu M CCI\textsubscript{4}, the increase in [Ca\textsuperscript{2+}], was maintained during exposure, and returned to initial levels upon washout of CCI\textsubscript{4}.


Triorganotins have been reported to affect hem metabolism as well as cardiovascular system. Our recent studies indicated that these organotins inhibit cardiac sarcoplasmic reticulum Ca\textsuperscript{2+}-transport and cAMP-stimulated phosphorylation of specific proteins involved in Ca\textsuperscript{2+} transport suggesting their interference with cardiac adenergic function. In the present study, attempts were made to study the effect of three organotins, tributyltin bromide (TBBT), triethyltin bromide (TET) and trimethyltin chloride (TMT), on rat cardiac ATPases and catecholamine binding, since these phenomena can regulate cardiac function. Cardiac membrane fraction was prepared from heart ventricles of male Sprague-Dawley rats. All the three organotins inhibited cardiac Na\textsuperscript{+}, K\textsuperscript{+}-ATPase, H\textsuperscript{+}-ouabain binding and oligomycin sensitive (OS) Mg\textsuperscript{2+} ATPase in a concentration dependent manner. OS Mg\textsuperscript{2+}-ATPase was more sensitive to these organotins when compared to Na\textsuperscript{+}, K\textsuperscript{+}-ATPase. TET and TMT, but not TBBT inhibited [H]-norepinephrine and [H]-dopamine binding to cardiac membranes in a concentration dependent manner, the effect being more with TET. These results suggest that triorganotins inhibit sodium pump activity and ATP synthesis and thus to some extent reduce other cellular processes such as catecholamine uptake, affecting cardiac function. (Supported by MIRT-NHLBI grant # T32 HL 07653)


We have previously reported that cyanide initiates release of dopamine and norepinephrine from rat phenochromocytoma (PC12) cells. The present study was initiated to observe the effect of cyanide on catecholamine metabolism in a neuronal cell model and mouse brain. On incubation of PC12 cells with cyanide (1-10 mM) for 30 min at 37°C, an unidentified metabolite was detected which eluted as a peak between dopamine and Dopac in HPLC-EC analysis. Formation of the compound was rapid, dose-dependent and blocked by pretreatment with pargyline. The compound was formed rapidly when cyanide was added to a mixture of MAO enzyme and dopamine. It was concluded that a deaminated metabolite of dopamine was an intermediate, possibly 3,4-dihydroxyphenyl acetaldehyde (DOPAL) and that cyanide reacted non-enzymatically with DOPAL to form a cyanohydrin. The cyanohydrin of Dopac, 2-hydroxy-3-(3,4-dihydroxyphenyl) propionitrile (THPN) was synthesized by the reaction of 3,4-dimethoxybenzaldehyde with methyl chloroacetae using sodium methoxide as the base. The synthesized THPN coeluted in HPLC-EC analysis with the unknown and it was concluded the metabolite was THPN. The generation of THPN in mouse brain was examined by incubation of fresh mouse brain slices with 1 mM cyanide, 0.9±0.08 ng of THPN was generated per 100 mg of the tissue over 30 min. Furthermore 5 min after injection of 8 \mu l of 30 mM (15.6 \mu g) cyanide into the brain lateral ventricles of mice, THPN was detected in the brain tissue. The cyanohydrin adduct of Dopac may contribute to the toxic syndrome produced by cyanide and presently the biological significance of THPN formation is being investigated. (Supported in part by NIH grant ES4140).
ANF, a peptide hormone present in the brain, has been postulated to be a neurotransmitter involved in central regulation of blood pressure and fluid volume. Exposure to cadmium (Cd) is associated with cardiovascular toxicity, including hypertension which may have a CNS component. Previously, we have shown that hypothalamic ANF levels are decreased in Cd treated rats. The aim of this study was to determine if Cd treatment disrupts the neurotransmitter action of ANF by measuring ANF mediated inhibition of K+ stimulated [3H]norepinephrine ([3H]NE) release from rat hypothalamus. Male rats were treated with CdCl2 0.5 mg/kg twice a day for 7 days and then maintained for an additional 30 days. On day 38, hypothalamic slices were prepared and loaded with [3H]NE. Basal [3H]NE release was estimated in 5 mM K+ and stimulated release in 27.5 mM K+ containing buffer over a 10 min incubation. ANF (10^-5M) significantly decreased [3H]NE release (29.0% of control). 8-Bromo cGMP (10^-4M) did not alter [3H]NE release, but in the presence of ANF (10^-5M) significantly decreased [3H]NE release (32.9% of control). In Cd treated animals ANF (10^-5M), both alone and with 8-Bromo cGMP, did not alter [3H]NE release. The effect of ANF was significantly different in control and Cd treated animals. In treated animals Cd was not detected in select brain regions by atomic absorption spectrometry. When hypothalamic slices were incubated directly with Cd (500 uM), decreased release of [3H]NE was observed (49.4% of control). Furthermore, ANF and/or 8-Bromo cGMP treatment did not alter release of [3H]NE in the presence of Cd in the incubation media. It is concluded that ANF neurotransmission is highly sensitive to Cd. The mechanism underlying these observations is not clear, but may involve alterations of the ANF receptor and its transduction system in the brain.

Receptor-activated hydrolysis of phosphatidylinositol biphosphate yields two second messengers, diacylglycerol (DG) and inositol 1,4,5-trisphosphate (IP3). DG activates protein kinase C and IP3 releases Ca2+ from intracellular stores. IP3 mediated signalling activity is terminated rapidly by a variety of phosphatases and kinases. A slight delay in the termination can be of serious consequence to a cell. The organochlorine compounds are known neurotoxicants and interfere with Ca2+- homeostasis. The present study was carried out to understand how chlordecone (CD) and dieldrin (DI) affect IP3 metabolism. The rat brain microsomal and soluble cytosolic fractions were incubated (30 seconds) with 50 uM of CD and DI. The metabolites formed were separated on HPLC. CD and DI significantly inhibited inositol 1,3,4,5-tetrakisphosphate (IP4) formation in the microsomal and cytosolic fractions. However, CD was more potent in inhibiting IP3 formation. CD and DI slightly elevated 1,4-bisphosphate formation in the cytosolic fraction whereas, no significant change was observed in the microsomes. The results suggest that neurotoxicity associated with organochlorine compounds may partially be due to the inhibition of 1,4,5-trisphosphate 3-kinase activity. (Supported by NIH/MBRS grant # RR 08110)

Previous studies in our laboratory indicate cyanide produces a significant alteration of neuronal calcium handling mechanisms in PC12 cells. The objective of this study was to measure cyanide-induced alterations of cytosolic calcium levels in primary cultures of hippocampal neurons. Cytosolic free Ca2+ levels were measured with microfluorescence dye Fura-2. A concentration dependent rise in Ca2+ occurred following the exposure to 0.5 mM-5 mM NaCN. Within seconds of exposure to 2 mM NaCN, Ca2+ levels were elevated two-fold over basal values. Increased Ca2+ levels following cyanide exposure were blocked by pretreatment with the NMDA antagonist, 2-amino-5-phosphonovalerate (APV). Ca2+ elevation could also be blocked by removing Ca2+ from the external medium. These results indicate that in hippocampal neurons cytosolic calcium accumulation by cyclase originates from the extracellular compartment. Furthermore, the NMDA receptor ionophore appears to be a significant route for calcium entry following cyanide exposure. Thus excitotoxic mechanisms may contribute to the altered neuronal homeostasis and injury associated with cyanide toxicity. (Supported in part by NIH Grant ES4140)

Inositol 1,4,5-trisphosphate (IP3) and diacylglycerol, the hydrolysis products of membranes-bound phosphoinositides are involved in the receptor mediated signal transduction. IP3 mobilizes Ca2+ from intracellular stores, whereas diacylglycerol activates protein kinase C (PKC) which in turn regulates a variety of enzymes and Ca2+-dependent processes. Any modulation in the activity of PKC and/or Ca2+-homeostasis can cause impaired cellular function. Mirex, the polycyclic organochlorine pesticide, is known to interfere in Ca2+-regulated pathways in the cell. The present study was carried out to understand the interaction of mirex and its derivatives (reduction products of mirex), with IP3 receptor and PKC activity in rat brain. IP3 receptor binding was determined using rat brain membranes and PKC activity was determined in the soluble fraction. Mirex and its derivatives at the concentration of 50 uM showed increase in PKC activity, but showed no significant effect on IP3 receptor binding. The data suggest that mirex and its derivatives may potentially modulate PKC activity in rat brain which may result in neuronal dysfunction.
Aluminum (Al) is believed to exert a primary role in the neurotoxicity associated with dialysis encephalopathy and has been suggested to be involved in a number of other neurological disorders, including Alzheimer's disease. Al, complexed with fluoride to form AlF₄⁻, can activate the GTP-binding (G) proteins of the adenylate cyclase system. Since an involvement of G proteins with phosphoinositide (PtdIns) metabolism has also been suggested, in this study we investigated the interaction of Al ions, alone and with fluoride, with this system. In rat cerebral cortical membranes AlCl₃ elicited a biphasic response: concentrations up to 1 mM stimulated [³H]inositol phosphates ([³H]InsPs) accumulation (EC₅₀ = 358 ± 70 μM), while higher concentrations were less effective. In the presence of NaF (5 mM) the dose-response curve for AlCl₃ was shifted to the left and the maximal stimulation elicited by 1 mM AlCl₃ was increased by 10-fold. Both Na⁺ and the stable GTP analogue GTP(S) also stimulated PtdIns metabolism. The stimulatory effect of Al was not additive to that of GTP(S). Differently from membranes, in cortical slices Al ions did not stimulate accumulation of [³H]InsPs, but inhibited carbamol-stimulated PtdIns metabolism. These preliminary results suggest that the interaction of Al ions with G-proteins and muscarinic receptor-stimulated PtdIns metabolism might represent a relevant target for Al in the nervous system. (Supported in part by a grant from Fondazione Clinica del Lavoro, Pavia, Italy).

Inositol 1,4,5-trisphosphate (IP₃) is widely recognized as a second messenger linking receptor-stimulated breakdown of polyphosphoinositides (PI) with the generation of intracellular Ca²⁺ signals. IP₃ generated by the receptor stimulation is rapidly metabolized and any slight modulation in IP₃ metabolism would affect physiological events in the cell by several folds. Amiodarone (AM), an antiarrhythmic drug is associated with neurologic and respiratory side effects. Since, AM and its major metabolite desethylamiodarone (DEA) are known to interfere with Ca²⁺-regulated cellular processes, the present study was initiated to understand how these drugs affect IP₃ metabolism. The rat brain microsomal and soluble cytosolic fractions were incubated with [³H]IP₃ (100 nM) in the presence and absence of 30 μM AM and DEA. The reactions were stopped at various time intervals ranging from 5 to 45 seconds. The various [³H]-inositol phosphates formed were separated on HPLC. Both AM and DEA inhibited IP₃ metabolism. DEA increased inositol 1,4-bisphosphate in the brain microsomes. AM and DEA inhibited inositol 1,3,4,5-tetrakisphosphate formation both in the microsomal and soluble fractions. However, DEA was more potent. These data suggest that some of the side effects associated with AM therapy may be due to the interference of AM and DEA with PI turnover and Ca²⁺-homeostasis.

The effects of a single and repeated doses of LiCl on cerebral inositol and inositol monophosphates and Li⁺ turnover, were studied in male Han/Wistar rats. There was a remarkable, 36-80 fold elevation of brain Li⁺ as the single dose of LiCl increased 4 to 6-fold. The accumulation of brain Li⁺ was slow during repeated administration. Brain inositol was markedly decreased after high single doses of LiCl whereas cerebral inositol-1-phosphate (InsIP) accumulation was more prominent with the lower doses. Brain InsIP was only transiently increased at 1 and 7 d whereas inositol remained at control levels throughout the 14 d observation period. Brain lithium did not correlate with changes in PI turnover either after a single or after repeated doses. Lithium probably caused the transient decrease in brain inositol by inhibiting several enzymes in the PI cycle, in addition to inositol monophosphatas. A slow dampening down of PI turnover by lithium, possible via an inhibitory action on G-protein coupling, can explain the lack of permanent effects of lithium on brain inositol and InsIP.

Malaxson-induced convulsions and alterations on cerebral inositol and inositol-1-phosphate levels in rats at different ages. M.-R. Hirvonen and K. Savolainen, NaHl Publ Hlth Inst Dept Env Hyg Toxicol, P.O.B. 95, SF-70701 Kuopio, Finland.

Malaxson-induced (MD) alterations in brain regional inositol and inositol-1-phosphate (InsIP), products of phosphoinositide (PI) metabolism, were measured in male Han/Wistar rats at the ages of 10 weeks or 18 months and in female nonpregnant and pregnant rats as well as their offspring on the 18th day of pregnancy. The offspring were taken out by cesarean section. The rats were followed for 1 or 4 h for tonic-clonic convulsions. In young male rats, malaxson (39.2 mg/kg i.p.) caused convulsions in 60 % of the exposed rats. In aged rats, the same effect was obtained by 8.7 mg/kg and in female rats by 8.2 mg/kg. In all groups of control rats the levels of brain inositol and InsIP were similar. Decrease in inositol and increase in InsIP by MD both had a cholinergic and a seizuresogenic component. Only small elevation of InsIP were seen in the offspring exposed to MD in utero. Thus, age, female gender, and pregnancy remarkably increased MD-toxicity and associated PI-turnover.
Protein kinase C (PKC) and inositol 1,4,5-triphosphate (IP$_3$) are involved in the receptor-mediated signal transduction. PKC regulates a variety of enzymes and Ca$^{2+}$-dependent processes whereas IP$_3$ mobilizes calcium from intracellular stores. Any modulation in the regulation of receptor-mediated inositol phospholipid metabolism by external chemicals can cause impaired cellular function leading to cell death. Organophosphates are known neurotoxants due to their ability to inhibit primarily the cholinesterase activity. The present study was initiated to understand the interaction of organophosphates such as ethyl parathion (EP), methyl parathion (MP), and malathion (M) with PKC and IP$_3$ receptor binding. The concentrations of these compounds used were in the range of 1 to 120 μM. All the three compounds decreased the PKC activity in a concentration-dependent manner and the order of potency was EP > MP > M. Whereas, MP and EP increased the IP$_3$ binding which was also concentration-dependent. However, M did not show any significant change in IP$_3$ receptor binding. These data suggest that the organophosphates may potentially modulate phospholipid-derived second messenger system in brain which may result in neuronal dysfunction. (Supported by NIH Grant No. AI26357).

The nicotinamide analog 3-acetylpiperidine (3-AP) is incorporated into NAD/NADP and inhibits nicotinamide-dependent pathways. Previous data indicate that 3-AP causes degeneration of dorsal root ganglion (DRG) neurons by 24 hrs. This study examined the time-course of 3-AP-induced injury in specific neuronal populations of the DRG. Female Sprague-Dawley rats (180-220 gms) administered 3-AP (65 mg/kg, ip) were sacrificed after 4, 12, 24 hrs and 3, 7, and 30 days. DRG were prepared for light- and electron microscopy. Degenerative changes consisting of peripheral cytoplasmic clearing and perineurial aggregation of organelles appeared in small neurons between 4 and 12 hrs. By 24 hr, follicular degeneration of small neurons, activation of satellite cells, and neuronophagia were noted. Cell debris and macrophage infiltration were prominent at 3 days. At 7 and 30 days, remaining neuronal cell bodies appeared unaltered, but glial scars were noted. Glial scars were somewhat less pronounced at 30 days. Large neurons remained essentially unremarkable throughout the study. The targeting of specific neuronal populations by 3-AP may be useful in elucidating mechanisms of pathogenesis in DRG. (Supported by NS-23325 and ES-04976.)

The sensitivity of mitotic and differentiated (diff) N2AB-1 murine neuroblastoma and C6 glialoma cells to MPP+ (1-methyl-4-phenylpyridinium iodide) and rotenone was examined. N2AB-1 cells can be differentiated by cAMP-induced changes in the neuronal phenotype while C6 glialoma cells represent the glial support cells of the brain. Both MPP+ and rotenone are thought to act by inhibiting ox-phos at NADH complex I. Rotenone is lipophilic and should have equal access to mitochondria while MPP+ must be actively accumulated within the cell and mitochondria to exert its toxicity. Cell response was determined by morphologic observation and survival over time after drug exposure. Cell survival after MPP+ treatment occurred in a dose response manner, with the sensitivity as follows: mitotic N2AB-1 > C6 glialoma > diff N2AB-1. Rotenone cytotoxicity affected the cells as follows: C6 glialoma > mitotic N2AB-1 > diff N2AB-1. Accumulation of [3H]MPP+ in the three cell types indicated that mitotic N2AB-1 cells accumulated twice as much [3H]MPP+ as the diff N2AB-1 cells and ten times more [3H]MPP+ than the C6 glialoma cells. However, diff N2AB-1 cells released 50-60% more previously stored [3H]MPP+ than did mitotic N2AB-1 cells. While C6 glialoma are most sensitive to rotenone, they are less sensitive to MPP+ due to increased exposure. The diff N2AB-1 cells are less sensitive to both MPP+ and rotenone compared to mitotic N2AB-1 cells due to increased accumulation and decreased sensitivity to inhibition of ox-phos as demonstrated by decreased sensitivity to rotenone. Aided by a Grant-in-Aid of Research from Sigma Xi, the Scientific Research Society, and in part by NIH NS 25778.

MECHANISMS OF RELEASE OF MPP+ FROM ASTROCYTES.

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The bioactivation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to 1-methyl-4-phenylpyridinium ion (MPP+) is an essential step leading to neurotoxicity. It occurs via the formation of a dihydroxyphenylalanine intermediate (MDP+) catalyzed by monoamine oxidase (MAO) type B. Although dopaminergic neurons are a primary target for MPTP toxicity, they do not appear to have the capacity to oxidize MPTP to MPP+. Since astrocytes exhibit MAO B activity, MPP+ is thought to be generated in these cells. However, the mechanism(s) involved in the release of MPP+ from astrocytes into the extracellular space remains unclear. We attempted to address this question by using primary cultures of mouse astrocytes. Addition of MPTP to these cultures resulted in its conversion to MPP+ and in the accumulation of this toxic metabolite both intra- and extracellularly. MPP+ release was not due to damage of cell membranes since it was observed well before the onset of cytotoxicity. On the other hand, two mechanisms could account for the presence of MPP+ in the culture medium: (a) MPP+ inducers to the neuronally generated directly in the extracellular space from the spontaneous oxidative removal of the MDP+; and (b) intracellularly generated MPP+ was able to cross cell membranes despite its charged chemical structure.
Astrocytes are likely to play an important role in the events leading to neurotoxicity after exposure to the parkinsonism-inducing compound 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). They may be a primary locus for the bioactivation of MPTP via monoamine oxidase (MAO) type B, generating the toxic metabolite 1-methyl-4-phenylpyridinium ion (MPP+). In this study, we assessed the metabolic and toxic consequences of MPTP exposure to primary cultures of mouse astrocytes. All results indicate that this treatment causes an impairment of mitochondrial function ultimately leading to astrocyte death. MPTP addition was followed by a rapid consumption of glucose and a parallel increase in the levels of lactic acid in the incubation medium. Subsequently, intracellular ATP levels began to decline and cytotoxicity was measured after complete ATP loss. Both ATP depletion and astrocyte death occurred more rapidly when astrocytes were incubated in glucose-free medium. Since all these toxic events were related to the intracellular accumulation of MPP+, and were prevented by preincubation of astrocytes with MAO inhibitors, we conclude that impairment of mitochondrial electron flow by MPP+ is a primary cause of toxicity in astrocyte cultures exposed to MPTP.

The use of in vitro techniques for evaluating neurotoxicants is becoming increasingly important in light of the need to evaluate numerous chemicals with decreased animal use. One of the primary needs for in vitro analyses is early, rapid detection of specific damage, but validation of such techniques is crucial. We have evaluated the use of the supravital dye rhodamine 123 (R123) to detect mitochondrial morphological and functional changes in neuroblastoma cells exposed to neurotoxicants. The neuroblastoma cells (SK-N-SH human neuroblastoma, SY-SY subclone, and PC-12 pheochromocytoma) were evaluated with exposure to the neurotoxicants, lead, cadmium, mepofox and carbaryl. The following parameters were evaluated: morphology, spectrophotometry, and viability (trypan blue dye exclusion and cell number). The results show that both lead and cadmium affected mitochondria distribution and intensity of staining. Carbaryl had an early effect on mitochondrial staining, but this effect disappeared in the surviving cells after 72 hours. This technique is indicative of mitochondrial damage. Ultrastructural assessments are currently underway to validate these results. Funded by NIH Biomedical Research Support Grant.

As chemicals may not interact additively, it is important to assess nonlinear effects and their dependence on measured parameters. Solvent mixes are important as they are ubiquitous and abused at high concentrations. Toluene (TOL) was reported (Takeuchi et al., '81) to prevent the peripheral damage of n-hexane (HEX). To see if the central neuropathy caused by HEX (Rebert et al., 1987) was protected by TOL and if there was protection by HEX from the ototoxicity caused by TOL, the combined solvents were studied in four groups of 12 rats (1200 ppm TOL; 4000 ppm H; 1200 ppm TOL+4000 ppm H=14 hr/day for 9 weeks). The ventral caudal nerve action potential (AP) and brainstem auditory evoked response (BAER) were measured. TOL and HEX alone produced ototoxicity and neuropathy, respectively. TOL countered hexane's effects on the AP, but the solvents additively decreased the size of BAERs. In terms of waveshape, TOL was somewhat protective. Thus, the protective effect of TOL against HEX-induced peripheral neuropathy was confirmed but the central interactions were mixed. These results also indicate that the type of interaction observed depends on the neuronal subsystem examined.

The subchronic neurotoxicity of commercial hexane (CH) was evaluated in rats. The CH tested contained a mixture of primarily six-carbon hydrocarbons in the following liquid volume percent: n-hexane (53%), 3-methylpentane (18%), methylcyclopentane (14%), 2-methylpentane (12%), cyclohexane (3%), 2,3-Dimethylbutane (1%). All Sprague-Dawley rats (12/sex/group) were placed in chambers and exposed to CH vapor for 6 hours/day, 5 days/week, for 13 weeks at target concentrations of 0, 900, 3000, and 9000 ppm. Animals were evaluated in a functional observatory battery (FOB) pre-study and post-exposure on treatment days 1, 7, 14, 35, 63 and 91. Motor activity (MA) testing was performed prior to treatment and on days 34, 62 and 90. At the termination of the study, whole-body perfusion was performed and six animals/sex from the air control and 9000 ppm treatment groups were selected for neuropathological evaluation. No treatment-related mortality, body weight change, alteration in food consumption or other clinical findings was observed for rats of either sex. Muzza cranial/periportal staining was more frequent in CH treated groups. The FOB assessments revealed no treatment-related findings of neurotoxic effect in either male or female animals. No difference could be observed between control and CH treated groups in MA at any timepoint tested. Neuropathologic evaluation of central and peripheral nervous system structures revealed no damage resulting from CH exposure. This study in which rodents were exposed to a hydrocarbon mixture containing 53% n-hexane, a proven neurotoxicant, failed to demonstrate hazard at a level of seventy-five percent of the lower explosive limit.


Timed-pregnant Sprague-Dawley rats (25/group) and CD-1 mice (30/group) were exposed to commercial hexane (CH) vapor for six hours/day on gestational days (gd) 6 through 15 at target concentrations of 0, 900, 3000, or 9000 ppm. Maternal clinical signs, body weights, food and water consumption were measured. At scheduled sacrifice, maternal liver, kidney and gravid uterine weight were taken. Ovarian corpora lutea of pregnancy were counted and all uterine implantation sites were identified. All live fetuses were examined externally and approximately 50% per litter were examined for craniofacial or skeletal malformations and variations. Maternal toxicity observed in rats at 9000 ppm included a significant reduction in body weight gain and food consumption. At 9000 ppm rats and mice exhibited treatment-related color changes in the lung at necropsy. Gestational parameters including number of viable and non-viable implantations per litter and sex ratio were unaffected by CH exposure for both species. Rat and mouse fetal body weights per litter were equivalent across exposure groups. There were no treatment-related increases in the incidence of individual external, visceral or skeletal malformations and variations at any exposure level for rats. There were treatment-related increases in the incidence of two minor individual skeletal variations at 9000 ppm for mice. In conclusion, exposure to CH vapor during organogenesis in rats resulted in maternal toxicity at 9000 ppm with no apparent developmental toxicity at any exposure level. Exposure to CH vapor during organogenesis in mice resulted in maternal effects at 9000 ppm accompanied by slight developmental toxicity in the absence of malformations at 9000 ppm.


Commercial hexane (CH) was tested for mutagenic activity using both the Salmonella/mammalian-microsome assay and the Chinese hamster ovary (CHO) cells Hypoxanthine-Guanine Phosphoribosyl Transferase (HGPR) assay. The CH chosen as test article represented an average of that available in the marketplace. It contained a mixture of six-carbon hydrocarbons in the following liquid volume percent: n-hexane (53%), 3-methylpentane (18%), methylcyclopentane (14%), 2-methylpentane (12%), cyclohexane (3%), 2,3-dimethylbutane (1%) and several minor components (<1%). The Salmonella assay was performed using five standard tester strains exposed to CH vapors for seven hours over a dose range of 600 to 9,000 ppm in the presence and absence of microsomal enzymes (S-9). No toxicity or mutagenic responses were observed in the various tester strains at any dose level. To assess mutagenic potential in a mammalian system, CHO cells were exposed for five hours to liquid CH at five concentrations ranging from 0.01 µl/ml to 0.13 µl/ml. At the three highest concentrations, toxicity was evident in the form of significantly reduced cloning efficiency. Nonetheless, mutation frequency was not significantly increased above control for any of the CH-treated groups, in either the presence or absence of metabolic activation. Therefore, it is concluded that CH is not mutagenic in the Salmonella or CHO/HGPR assays.

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**TOXICOLOGY OF DIETHYLENE GLYCOL BUTYL ETHER:**


Diethylene glycol butyl ether [DGBE, CAS 112-24-5] is a glycol ether solvent used in many consumer products including hard surface cleaners; its acetate ester [DGBA, CAS 124-17-4] is used as a solvent in paints. CMA estimated that typical consumer exposure to DGBE was about 0.06 mg/kg/day of use by the dermal route and to DGBA was about 0.006 mg/kg/day of use by inhalation. EPA estimated dermal exposure to DGBE could be as high as 1 mg/kg/day. DGBA was rapidly metabolized to DGBE in vitro, and systemic toxicity was presumed to be similar to DGBE. Despite a significant data base on DGBE indicating no toxic hazard at anticipated exposure levels, and a lack of genotoxicity, EPA required further toxicology studies in a 1988 TSCA Final Test Rule. The results of these studies, sponsored by the manufacturing companies under the auspices of CMA, are presented in the following three abstracts. They confirm that DGBA and DGBE are not acutely or subchronically toxic to male and female rats [DGBE and by implication DGBA] are not systemic, reproductive or neurotoxicants by the dermal route in rats at the highest dose tested - 2g/kg day. Skin irritation was observed on repeated exposure to high concentrations of DGBE under occlusion. There is little likelihood of systemic toxicity of DGBE or DGBA in normal use in consumer products.

**TOXICOLOGY OF DIETHYLENE GLYCOL BUTYL ETHER:**


The subchronic and reproductive toxicity of DGBE was determined by the dermal route in SD rats by a novel combined protocol. DGBE was administered dermally at 10% or 30% w/w eq. solutions, or undiluted for 13 wk under occlusion, for 6 hr/day, five days/week, at the maximum attainable volume of 2 ml/kg. Male and female rats were examined using a functional observational battery (FOB) pre- and 24 hr after the initiation of the first exposure, and prior to treatment on study days 7, 14, 35, 62 and 91. Motor activity was determined pre-study and prior to treatment on study days 34, 62 and 90. At the completion of treatment, 6 control and top dose group animals were perfused for neuropathology. There was no mortality, and the body weights and food intakes were unaffected. Five females in the top dose group showed scab formation at the treatment site during the study. There were no other treatment-related clinical findings. The FOB and motor activity tests revealed no findings indicative of a neurotoxic effect, and no neuropathological changes. Evaluation of non-neural tissues revealed mild degeneration of the renal tubular epithelium in 2 males in the top dose group; this was not considered to be related to treatment because no such effect was observed in the concurrent definitive subchronic study also using SD rats. No neurotoxicity or other systemic toxicity was seen at the highest dose tested - 2 g/kg/day.

Absorption and elimination of dermally applied doses of 14C-diethyleglycol butyl ether (14C-DGBE) and 14C-diethyleglycol butyl ether acetate (14C-DGBA) were determined in Sprague-Dawley rats. The materials were applied under occlusion for 24 hr at dose levels of 0.2 and 2.0 g/kg (undiluted) and as a 10% aq sol (0.2 g/kg DGRE). Preliminary washing efficiency studies with soap and water recovered greater than 80% of each chemical. In all biotransformation and excretion balance studies with 14C-DGEE, female rats excreted a larger proportion of the applied dose than did male rats. Similar results were obtained with the low dose of DGRE applied neat or as a 10% aq sol, suggesting that the low dose represents a saturating dose. The total recovered 14C for all studies ranged from 80% to 89%. Urinary excretion accounted for the majority of recovered 14C. 2-(2-Butoxyethoxy)acetic acid was the major urinary metabolite identified. The glucuronide of DGRE was present at levels of from 5.2% to 8.2% of the urinary 14C. The dermal absorption rates for 14C-DGBE and 14C-DGBA were estimated to be: 1.58 (DGRE, male), 1.26 (DGRE, female), 0.73 (DGRE, male), 1.46 (DGRE, female), expressed as mg/cm²/hr.

α-XYLENE ALTERS RAT PULMONARY GLUTATHIONE UTILIZATION AND SYNTHESIS. G Furman, T Aucoin and R Schatz, Toxicology Program, Northeastern University, Boston, MA.

Previous studies in our laboratory have demonstrated that α-xylene depletes hepatic glutathione (GSH) levels not only through phase II conjugation reactions but also by an inhibition in GSH synthesis. These effects appear to be isomer-specific since neither p- nor m-xylene alters hepatic levels of GSH or cytochrome P450 (CYS), the sulfur-donating precursor of GSH. The present study investigates the effects of α-xylene (1g/kg, ip, 1.3 & 12h) on rat pulmonary GSH metabolism. α-Xylene depleted GSH levels 39% at 1h while CYS was increased 35% at 12h. GSH synthesis was investigated using [35S]methionine. α-Xylene increased the specific activity of [35S]CYS 24% at 1h while that of [35S]GSH was increased 15x at 3h and remained slightly elevated at 12h. The two enzymes responsible for incorporation of CYS into GSH were also studied. α-Xylene increased the activity of GSH synthetase 21% at 1h while δ-glutamylcysteine synthetase activity remained unchanged. α-Xylene also increased GSH-S-transferase activity 12% at 3h. These data suggest that GSH depletion does not result from the inavailability of CYS; furthermore, compensatory GSH synthesis is initiated following its depletion by α-xylene. As in the liver the effect of xylene on lung GSH metabolism appears to be most pronounced after the administration of the α-isomer; pulmonary GSH content remains unchanged 1.3 & 12h after p-xylene (1g/kg, ip) while CYS content is initially decreased 20% at 1h but returns to control levels by 3h. These data suggest that α-xylene depletes rat pulmonary GSH content primarily through conjugation reactions. In contrast to the liver, resynthesis of pulmonary GSH is not impaired by α-xylene. Supported by NIH-NCI #CA47671.

BEZYLXYORESORFURIN-0-DEBENZYLASE ACTIVITY AS A SENSITIVE INDICATOR OF ORGAN SPECIFIC RESPONSES TO PARA-XYLENE. B J Day and G E Carlson. Dept. Pharmacology and Toxicology, School of Pharmacy and Pharmacal Sciences, Purdue Univ., West Lafayette, IN.

p-Xylene increases P450 mediated activities in the liver while decreasing these in the lung. The purpose of this study was to determine if bezylxyoresorfin-0-debenzylase (BKRD) activity would be useful in monitoring these organ specific responses. Seven rats were exposed in a dynamic flow inhalation chamber to 3400 ± 160 ppm p-xylene for 4 hours. Control rats were exposed to room air. Animals were killed 12 hours after termination of the exposure. Bronchoalveolar lavage fluid (BALF) analysis indicated the absence of pulmonary damage. No alterations in serum sorbitol dehydrogenase (an indicator of hepatotoxicity) suggests the lack of liver damage. Hepatic microosomal BKRD activity increased 20-fold with p-xylene exposure whereas the lung activity decreased by 86%. Echoxyresorfin-O-deethylase activity was not altered by p-xylene in the lung but was increased in the liver. Hepatic cytochrome P450 content was increased by 36%. p-Xylene exposure produced no detectable injury in lung or liver but caused profound opposing effects on microsomal BKRD activity. p-Xylene may prove valuable in differentiating whether bioactivation of toxicants occurs in lung or liver. (Supported by NIHES 04362)

INHIBITION OF CYTOCHROME P450 ISOZYMES BY α-XYLENE AND ITS METABOLITE m-TOULUALDEHYDE IN RAT LUNG MICROSONES. J Silverman and R Schatz, Toxicology Program, Northeastern University, Boston, MA.

m-Xylene administration to rats has been shown to decrease total pulmonary cytochrome P450 content. Three isozyymes of cytochrome P450 have been identified in rat lung: P450IA1, P450IB1and P450IVB1. Specific enzyme substrates were used in this study to assess the ability of m-xylene and its metabolite to alter the activities of these P450 isozymes in rat lung. m-Xylene administration (1g/kg, ip, 1h) inhibited the activity of benzoxylxyresorfin-0-dealkylase (BKRD) and 2-aminofluorene-N-hydroxylase (2AF-NH) which are preferentially catalyzed by P450IB1 and P450 IVB1 respectively. Echoxyresorfin-O-dealkylase (EROD) activity which is mediated by P450IA1 was not altered by m-xylene. m-Xylene and m-tolualdehyde were shown to be potent inhibitors of BROD and 2-AF-NH in vitro; while 3-methylbenzyl alcohol and m-toluic acid were ineffective. EROD activity was not altered by in vivo treatment with any of the above agents. Enzyme kinetic studies revealed that m-xylene (in vivo) and in vivo) and m-toluualdehyde (in vitro) exhibit mixed-type inhibition of BROD activity which is not purely competitive or noncompetitive. m-Tolualdehyde administration to rats (500 mg/kg, ip, 1h) did not result in inhibition of pulmonary BROD and EROD activity possibly due to detoxification by hepatic aldehyde dehydrogenase. P450IB1 is responsible for the detoxification of the pulmonary carcinogen benzo(a)pyrene (BaP) in rat lung. Inhibition of this isozyme by m-xylene results in BaP activation or metabolism to toxic, mutagenic species. Supported by NIH-NCI #CA47671.
Previous studies in our laboratory have documented pulmonary microsomal membrane alterations in rats following p-xylene administration by intraperitoneal (ip) (1 g/kg, 1 h) or inhalation (300 ppm, 6 h) exposure. The pattern of pulmonary damage included decreased phospholipid levels, P450 content and membrane-associated enzyme activities with increased conjugated diene (CD) levels. These results suggested lipid peroxidation as a possible primary mechanism of action. In this study, in vivo experiments document the peroxidative process to be an early event with CD levels elevated at times when other membrane parameters remain unaltered. In vitro studies utilizing control pulmonary microsomes demonstrate that the peroxidative events are not produced as a direct effect of p-xylene but are produced by one of its metabolites, p-toluinaldehyde (p-TA). p-TA lethal levels as low as 0.01 mM in vitro stimulate CD formation while p-xylene and its other metabolites, p-methoxybenzyl alcohol and p-toluic acid, fail to stimulate CD formation at this level. In vivo studies utilizing ip (500 mg/kg, 1h) and inhalation (300 ppm, 6 h) p-TA administration fail to show a direct effect of this metabolite on elevating CD levels. However, pulmonary microsomal membrane-associated enzyme activities are decreased in both p-TA exposure situations. These results suggest that p-TA may be the p-xylene metabolite responsible for the pulmonary inhibition of enzyme activities but the peroxidative events noted are not likely the primary mechanism of action of p-xylene. Supported by NIH-NCI #CA47871 and BRSG Grant SOT RR 05830-09.

The present study investigates the time-dependent effect of intraperitoneal o-xylene administration (1g/kg) on hepatic and pulmonary P450 activity. o-Xylene decreased pulmonary cytochrome P450 content by 70% with maximal inhibition occurring at 3h post-dose. Arylhydrocarbon hydroxylase (AHH) activity was inhibited 47% and 60%, respectively, at 1h and 3h. AHH activity remained maximally decreased until 12h. The isozyme-specific MFO activity responsible for 3-OH benzo(a)pyrene formation (AHH activity) was examined using benzyloxyresorufin-O-dealkylation (BROD) as a measure of P450 11B1 activity and ethoxyresorufin-O-deethylation (EROD) as a measure of P450 1A1 activity. Reduced activity for EROD (51% at 6h, 73% at 12h) and BROD (49% at 3h, 50% at 6 h, 31% at 12h) was noted in agreement with the decreased pulmonary AHH activity. In contrast, increased P450 content was noted in liver at 6h (18%) and 12h (21%) with no change in AHH or EROD activity. Conjugated diene (CD) formation, an index of membrane peroxidation, and phospholipid/cholesterol ratio, an index of membrane fluidity, were also examined in lung and liver microsomes. No changes were observed in either parameter except increased CD formation at 12h post-dose (12% in lung and 15% in liver). These results support that o-xylene shows an organ specific inhibition of pulmonary P450 isozymes with maximum inhibition at 3h and a modest increase in P450 content of the liver with no change in AHH activity or O-dealkylation activities. Supported by NIH-NCI #CA47871.

Previous studies in our laboratory have investigated the effect of intraperitoneal (ip) xylene administration on microsomal membrane structure and mixed function oxidase (MFO) activity in rat liver and lung. The three xylene isomers, p-, m- and o-, produced significant decreases in arylhydrocarbon hydroxylase (AHH) activity and cytochrome P450 content in lung but had little effect in liver. The lung appears to be the more sensitive organ. The present study was conducted to determine the effects of xylenses (1 g/kg, ip, 1h) on rat kidney, an organ ranked between liver and lung in P450 content. Administration of p-, m-, or o-xylene decreased renal AHH activity 71, 48 and 47%, respectively. The previous observation that membrane lipids are necessary for optimal MFO function, paired with the lipophilic nature of the xylenses led us to determine if the observed inhibition in AHH activity could be correlated with perturbations in the lipid domain of the membrane. Microsomal phospholipid (PL) content was decreased by 9, 20 and 15% following administration of p-, m- or o-xylene, respectively; microsomal cholesterol content was not affected by xylene administration. Despite the xylene-induced alterations in membranes composition, membrane fluidity was not altered by xylenses; conjugated diene formation also remained unchanged following xylene administration. These data suggest that xylenses can inhibit P450-associated oxidative metabolism in rat kidney, perhaps through a mechanism involving altered microsomal membrane PL composition. Supported by NIH-NCI #CA47871.

Simultaneous exposure of rats to TOL (150 ppm) and XYL (150 ppm) alters the metabolic disposition of both solvents, thus compelling the interpretation of biological monitoring data for exposure (Toxicologist 9: 250, 1989). We have investigated the effect of simultaneous exposure of human volunteers to TOL and XYL on some biological exposure indices: blood (B-TOL, B-XYL), alveolar air (A-TOL, A-XYL), urine (hippuric acid (HA); hippuric acids (MHA)). Five male volunteers were exposed by inhalation for 7 h to TOL (50 ppm) or XYL (40 ppm) or a mixture of both. Simultaneous exposure did not alter the concentration of solvents in alveolar air (ppm) and blood (μg %) at steady-state. Values are single vs mixed exposure (mean ± SD): A-TOL = 9.9 ± 0.8 vs 9.5 ± 0.5; A-XYL = 5.3 ± 0.4 vs 4.8 ± 0.2; B-TOL = 77.1 ± 5.8 vs 76.1 ± 7.7; B-XYL = 67.6 ± 6.4 vs 77.7 ± 6.2. Similarly mixed exposure did not modify the excretion (g/g creatinine) of HA (1.10 ± 0.06 vs 1.11 ± 0.04) nor that of MHA (0.90 ± 0.06 vs 0.87 ± 0.05). In conclusion, our data suggest that coexposure to TOL (50 ppm) and XYL (40 ppm) does not alter the metabolic disposition of either solvent in humans and thus is not likely to distort the interpretation of biological monitoring data for exposure. (Supported by IRSSS, RS-86-46).
The subchronic inhalation toxicity of N,N-dimethylformamide (DMF) in cynomolgus monkeys and mice was investigated to identify target organs, to characterize the concentration-response, and to identify non-exposure-related causes. Absolute and relative liver weights were increased in rats exposed to 50-200 ppm, while absolute liver weights were decreased at 400 and 600 ppm. In mice, absolute liver weights were increased at 200 and 400 ppm, and relative liver weights were increased at 200-800 ppm. Serum enzyme concentrations were increased both in male and female rats exposed at 200 and 400 ppm. Centriobular hepatocellular necrosis (minimal to moderate) was seen in rats of both sexes exposed at 400 and 800 ppm, with lesions more severe in females. Centriobular hepatocellular cytoplasmic (minimal to mild) was found in all groups of DMF-exposed male mice and in female mice exposed at 100-800 ppm. DMF-related effects were seen primarily in the liver of both species, with rats being more severely affected.
2-Chloroethanol (2-CE) and 2-bromoethanol (2-BE), used as industrial solvents, and found in the environment as food as organic contaminants, are acute toxins. 2-CE and 2-BE are known to conjugate in vitro and in vivo with fatty acids and metabolize to potentially toxic halo-aldehydes and acetic acids. Present study was undertaken to study their subchronic toxicity and lipid conjugation in male Sprague-Dawley rats. Rats were given water (control) and sublethal doses of ethanol (positive control for lipid conjugation), 2-CE and 2-BE at 286, 500 and 776 ppm, respectively, via drinking water for 60 days. The water was changed on alternate days and body weight were recorded periodically. Animals were sacrificed on 61st day, blood was withdrawn and organ weights were recorded. Apparently no gross lesions were observed in major organs and tissues at necropsy. However, ethanol, 2-CE and 2-BE significantly reduced the body weight gain of the animals, most being for 2-BE treated animals. Increased organ weight/body weight ratios were observed for liver, kidney, brain and testes of 2-BE treated animals. Serum enzymes, particularly LDH and GOT activities were significantly low in 2-BE treated rats as compared to the ethanol and 2-CE treated and controls. Low serum cholesterol in 2-CE treated animals and high triglycerides in ethanol and 2-BE treated animals as compared to the controls were also found. These changes due to ethanol, 2-CE and 2-BE may be related to metabolic alterations of lipids and dysfunctioning of plasma membrane. Quantitation of lipid conjugates of ethanol, 2-CE and 2-BE and histopathological investigations are in progress. (Supported by ES 04815)


The mechanism for tolerance to arsenic toxicity is not known. Recently we demonstrated that arsenic induces metallothionein (MT), which is a sulfhydryl-rich protein that binds and decreases the toxicity of a number of metals. These studies were designed to examine the role of MT in arsenic toxicity. Zn pretreatment (1000 μmol/kg, sc) markedly increased hepatic MT (150 fold over controls) and also protected against the lethal effects of arsenite (150 μmol/kg, sc). However, no correlation was found between the ability of other known MT inducers (Zn, Cd, arsenite, monomethyl-arsenate and α-hematin) to increase hepatic MT concentrations and to protect against arsenic lethality in mice. To examine the mechanism of Zn protection of As toxicity, the subcellular distribution of arsenite in liver, kidney and small intestine was determined 2 hr after arsenite injection. Zn pretreatment did not increase the amount of As in the cytosol or alter the distribution of As to the various cellular organelles (nuclei, mitochondria, microsomes and cytosol) in either the liver, kidney, or small intestine. There was no marked association of As with MT in the cytosol of these Zn-pretreated mice, as determined by G-75 gel-filtration chromatography. In summary, these data demonstrate that Zn pretreatment protects mice against As toxicity. The mechanism of tolerance to arsenic toxicity produced by Zn does not appear to be due to the sequestration of As by MT, as is thought to be the mechanism for protection against other metals. (Supported by NIH Grant ES-01142)

ENZYMATIC REDUCTION OF ARSENATE TO ARSENITE BY RAT AND RABBIT LIVER. G M Bogdan and H V Aebersold, Departments of Pharmacology & Toxicology and Molecular & Cellular Biology, University of Arizona, Tucson, AZ.

Inorganic arsenic needs to be in the trivalent state, arsenite, before it can be further metabolized by mammalian systems to less toxic, more excretable, methylated forms. The reduction of arsenate, the pentavalent state, to arsenite is enzymatically catalyzed by a liver cytosolic component in both rats and rabbits. Solid phase extraction techniques employing anion exchange resin were used to determine radio-labeled arsenite (As73) converted from arsenate after incubation with partially purified rabbit and rat liver cytosol. This appears to be enzymatically mediated since 1) increasing the concentration of cytosolic proteins increases the levels of arsenite formed; 2) heat pretreatment of liver cytosol (70°C for 30 sec) prevents this conversion; 3) storage of cytosolic preparations at 70°C preserves reducing activity for at least 3 months while activity is lost over several hours at 4°C; 4) reducing agents such as glutathione and β-mercaptoethanol do not reduce arsenate alone but adding them to incubations potentiates the conversion of arsenite to arsenic; and 5) enzyme kinetics are shown to be saturable. The toxicities of arsenate and arsenite differ significantly and the methylation of arsenic by mammalian systems depends on its first being in the trivalent state. Therefore, it is important to first elucidate the mechanism(s) for the conversion of arsenite to arsenic in order to understand the rest of the arsenic detoxification pathway.


The toxic/tetragenic effects of Cd are inhibited by Zn. We recently demonstrated that when non-confluent MDCK cells are exposed to 10μM Cd for 6 h. there is an alteration in the F-actin (but not microtubule) component of the cytoskeleton. To determine if this effect could be blocked by Zn we exposed MDCK cells to Zn (10μM) in the absence or presence of Cd. Cd alone produced an alteration in the actin cytoskeleton. Membrane-associated actin that normally accumulates in the zone of intercellular contact between cells separated from the junctional region. Stress fibers were almost entirely absent. A dense band of filaments formed near the perimeter of cells with a free edge. We could detect no change in microtubules. There was a progressive change in MDCK cell morphology characterized by an accumulation of cytoplasmic elements in the perinuclear region. Peripheral cytoplasm, both at the free edge of non-confluent cells and the junctional region between cells was clear. Exposure to Zn for 6 h. had no effect on cell number or viability and the effect on was reversed by incubation overnight in culture medium. When Zn and Cd were added together in equimolar amounts (10μM) the cells appeared identical to those exposed to Zn alone. The Zn effect could be elicited if Zn were added as much as 3 h. after Cd or if the Zn concentration was reduced to 0.5μM. The results demonstrate that exposure to Zn results in specific alteration in the organization of actin. This change is distinct from that observed after exposure to Cd and is elicited even when Cd is present in excess of Zn. We propose that a possible site of interaction for the protective effect of Zn against the toxic/tetragenic effects of Cd is at the level of the actin cytoskeleton. Supported by M01-156.
1257 PROTECTIVE EFFECT OF ASCORBIC ACID ON CADMIUM HEPATOTOXICITY IN MICE. E Giannopoulos, J Liu, Y P Liu and C D Klages. Univ Kansas Med Ctr, Kansas City, KS

The purpose of this study was to examine the effect of ascorbic acid (vitamin C, VC) on cadmium (Cd) hepatotoxicity in mice. VC pretreatment (1000 mg/kg, ip for 24 h) dramatically decreased Cd (3.7 mg/kg, iv) induced liver injury as indicated by serum activities of sorbitol dehydrogenase and alanine aminotransferase activities as well as by histopathological examination. To examine the mechanism of this protection, the organ distribution of Cd as well as the subcellular distribution of Cd in the liver was determined 2 h after Cd injection (100 mg Cd, 37 µCi/3.7 mg Cd/kg iv). VC did not protect the liver from Cd by decreasing the accumulation of Cd in the liver, as higher amounts of Cd were found in the liver of VC-pretreated mice (64% vs 51% of dosed Cd). However, VC markedly altered the hepatic subcellular distribution of Cd, with much less Cd distributing to nuclei, mitochondria, and microsomes, and more Cd in the cytosol. The increased cytotoxic Cd was bound primarily to a low-molecular-weight protein, metallothionein (MT). VC (100-1000 mg/kg, ip) produced a dose-dependent increase in hepatic MT with a 48-fold increase over controls after a single injection of 1000 mg/kg (270 µg vs 5.6 µg MT/g liver), as determined by the Cd/hemoglobin radioassay. In conclusion, VC pretreatment decreases Cd hepatotoxicity. This protection appears to be due, at least in part, to VC induction of MT, which binds Cd in the cytosol, with a concomitant decrease of Cd in critical cellular organelles. (Supported by NIH Grant ES-01142).

1259 ABSENCE OF CHANGES IN METALLOTHIONEIN RNA LEVELS IN RAT TESTES MADE REFRACTORY TO CADMIUM TOXICITY BY METAL PRETREATMENT. Z Z Wahba, M S Miller, and M P Haakkes. National Cancer Institute, FCRIC, Frederick, MD.

Testicular toxicity and interstitial cell (IC) tumors induced by cadmium (Cd) are prevented by zinc (Zn) or by low dose Cd pretreatments. The mechanism of this tolerance is unknown, though metallothionein (MT) is thought to play a role in tissue resistance to Cd toxicity. Thus, the possible involvement of the testicular MT gene in metal-induced tolerance to Cd toxicity was studied. Rats were pretreated with Zn (1.0 mmol/kg, sc) or low-dose Cd (3.0 µmol/kg, sc) administered 24 h later. RNA was extracted from ICs isolated by collagenase dispersion 24 h after low-dose Cd pretreatment or whole testes 24 h after Zn pretreatment. RNA was analyzed by the slot blot technique using the p2A10 cDNA probe to the MT gene. In ICs, low-dose Cd pretreatment did not alter the levels of MT RNA as compared to control even though pretreated cells were refractory to Cd toxicity. In pretreatment likewise had no effect on MT RNA in whole testes. Such pretreatments did not alter testicular Cd-binding protein capacity. In contrast, RNAs derived from livers of Zn pretreated rats showed marked increases in MT RNA. Hence, the testicular MT gene does not appear to play a major role in the induced tolerance to Cd toxicity and carcinogenesis generated by Zn or low-dose Cd pretreatment.

1258 BUTYRATE INCREASES METALLOTHIONEIN INDUCIBILITY AND RESISTANCE TO CADMIUM CYTOTOXICITY IN ROS 17/2.8 CELLS. D I Thomas, C R Angle, S A Swanson, T C Caffrey. Dept of Pediatrics, Univ of Nebraska Med Ctr, Omaha, NE.

ROS 17/2.8 cells, a cloned rat osteosarcoma cell line, are extremely sensitive to the lethal effects of Cd (~LC50 = 0.3 µM). The susceptibility of these cells to the lethal effects of Cd is associated with the poor inducibility of metallothionein by this metal. Addition of Na butyrate to culture medium increases the synthesis of metallothionein by these cells. Increased synthesis of metallothionein by Na butyrate-treated cells results in increased resistance to Cd (~LC50 = 1 µM). Na butyrate does not protect cells against Cd cytotoxicity by altering uptake of Cd and the protective effect of this agent is reversed completely within 24 h after its removal from medium. Inhibition of DNA synthesis by butyrate is not adequate to alter metallothionein gene expression. Equivalent inhibition of DNA synthesis with hydroxyurea does not alter inducibility of metallothionein by Cd or increase resistance to Cd-induced cytotoxicity. These results support a model in which a butyrate-induced alteration of metallothionein gene expression in ROS 17/2.8 cells is the major factor which modifies resistance to the lethal effects of Cd. Supported by a grant from The Center for Alternatives to Animal Testing.


Ferric ions as FeCl3 but not ferrous iron (FeSO4) protects 3T3 cells from the toxic effects of cadmium. Toxicity was detected using cultured cell assays that measure either total cell protein or the uptake of the supravital dye, neutral red, after 24 hrs of exposure to test agents. Protection from Cd is even more effective when Fe-Hydex, a complex of hydrogenated dextran and ferric iron (Varitech Custom Specialties) is employed. This compound also protected kidney epithelial cells (MDCK) from Cd. Ferric ammonium citrate was also an effective Cd antagonist; citrate ion was not protective. A ferrous chelate, bisglycino-iron (II), obtained from Albion Laboratories, Verona, NJ, is the only ferrous compound tested thus far that antagonized Cd cytotoxicity. Pre-treatment of cells with cycloheximide does not decrease the protection afforded by these iron compounds, suggesting that protein synthesis is not required for protection. Pre-treatment of cells with Fe-Hydex also does not provide protection. Studies on the uptake of Cd in the presence and absence of Fe-Hydex indicate that the compound does not significantly impair the uptake of Cd during the first 8 hours of treatment, although reduced uptake/mg cell protein is observed after 24 hrs. Initiation of Fe-Hydex treatment 8 hrs after the onset of a 24 hr Cd treatment still provided significant protection, supporting the concept that protection is not the result of inhibition of Cd uptake. Supported by NIHES.

The effects of vitamin E on particulate crystalline NiS or water soluble NiCl2 induced chromosomal aberrations were examined in Chinese hamster ovary (CHO) cells. Pretreatment with vitamin E for 24 h prior to exposure to crystalline NiS decreased the chromosomal aberration frequency as well as the cytotoxicity induced by this agent. In contrast, the chromosomal aberration frequency and cytotoxicity induced by NiCl2 were not affected by pretreatment with vitamin E. Crystalline NiS particles were phagocytosed by cells and these phagocytosed particles are slowly dissolved in the cytoplasm. Pretreatment with vitamin E did not affect phagocytosis of crystalline NiS by CHO cells. With soluble nickel salts, nickel ions enter cells slowly and diffusely. The difference in entry of nickel or dissolution of nickel from NiS may explain these differences. These results suggest that a portion of the cytotoxicity and genotoxicity of crystalline NiS particle may be mediated by an oxidative mechanism. Supported by grant ES 04899 and ES 05512.


The effects of cysteine and glutathione (GSH) on inorganic mercury (Hg) toxicity and transport were studied in isolated perfused S1, S2 and S3 segments of the renal proximal tubule of the rabbit. The tubular segments were perfused with an electrolyte solution containing 18.4 µM HgCl2, 500 µM glutamate (substrate) and 80 µM cysteine or 80 µM GSH. All three segments of the proximal tubule were perfused at an average rate of 8 nl/min. for approximately 45 minutes. When S1 segments were perfused with 18.4 µM Hg and 80 µM cysteine, generalized swelling occurred throughout the length of the tubular segments within about 10 minutes. No further changes occurred in this segment. In S2 segments, cellular swelling occurred only in the initial 50-75 µm during the first 10 minutes of perfusion. S3 segments appeared free of any pathology during the 45 minutes of perfusion. S1, S2 and S3 segments of the proximal tubule displayed no signs of cellular injury when perfused with 18.4 µM Hg and 80 µM GSH. The rate of disappearance (k) of Hg from the perfusing solution (fmol min^-1 mm^-2) in all three segments of the proximal tubule was reduced markedly when either 80 µM cysteine or 80 µM GSH was present in the perfusing solution. In addition, the amount of Hg that appeared in the bath (µM), (fmol min^-1 mm^-2) from the perfused segments and the amount of mercury that accumulated in the tubular epithelial cells were reduced when the cysteine or GSH was present in the perfusing solution. These data indicate that 80 µM cysteine and 80µM GSH protect the proximal tubular epithelium from the toxic effects of 18.4 µM Hg, with GSH offering complete protection. The protection may be related to decreased movement of Hg across the luminal membrane of tubular epithelial cells in the 3 segments of the proximal tubule.

1263 SPECIES DIFFERENCES IN RESPONSE OF KIDNEY COPPER, BISMUTH, AND ZINC TO CISPLATIN AMONG F344 RATS, WISTAR RATS, AND CD-1 MICE. R S Dwoskin and J E Rivera, Toxicology Program, North Carolina State University, Raleigh, NC.

There have been conflicting results on cisplatin's (CDDP) affinity for metallothionein (MT) and MT's ability to protect against CDDP-induced kidney toxicity. We evaluated CDDP's effects on kidney platinum, copper, and zinc levels in male F344 rats, male Wistar rats. Some groups were pretreated with bismuth, a kidney MT inducer. CDDP was delivered in 2.2 % saline ip at 7.3 mg CDDP / kg bw for rats and in 2.2 - 2.7 % saline at 15 - 20 mg / kg for mice. With the doses and routes used, bismuth did not protect against CDDP kidney toxicity in the rat, and only partially in the mouse, as measured by serum creatinine and urea. CDDP does, however, alter the concentrations of MT-bound kidney metals, and in markedly different ways for rats then for mice. In the CDDP treated F344 rat and Wistar rat, kidney platinum and bismuth levels remained relatively stable for 7 days, while kidney copper was reduced to 60% of controls by day 4. In the CDDP treated CD-1 mouse, both kidney platinum and bismuth were reduced to 60% of controls by day 4, while kidney copper was lowered less than 10%. Bismuth pretreatment in mice, but not in rats, increased the peak kidney platinum level. There was a moderate increase in kidney zinc at day 4 in all test animals exposed to CDDP. The profound loss of kidney copper in CDDP exposed rats, the concurrent loss of bismuth and platinum in CDDP exposed mice, and the initial increase in kidney platinum levels in bismuth pretreated mice suggest that metal binding proteins do play an important role in the binding and sequestering of CDDP. The different reported outcomes for studies on MT's protective efficacy may be due to differences in the susceptibility of kidney metal-proteins to CDDP attack and the fate of the depleted metals. Kidney metal concentrations should be measured in MT efficacy studies to control for these differences. (Supported by PHS Grant CA42745 and R1).
Phosgene (carbonyl chloride, carbon oxychloride, chloroforormyl chloride) is a highly toxic colorless gas used in industrial processes mainly as a catalyst in the production of isocyanate based polymers, the starting materials for polyurethane resins. Phosgene is lipophilic and the inhaled gas penetrates deeply into the alveolar spaces. Pulmonary immunotoxicity of phosgene was assessed by measuring the effect on metastasis of tumor cells into the lung. L-W male rats, 8-12 weeks of age, were injected with 1.0 X 10^5 PA III cells in the right rear foot pad. L-W rats were then exposed to 1.0 ppm phosgene or to clean filtered air for 4 hr per day, 5 days per week, for 4 consecutive weeks, beginning 1 week after injection of tumor cells. Animals were sacrificed at 2, 3, 4, and 5 weeks after injection of tumor cells. Lungs were placed in Bouin's solution for 24 hours and then rinsed and placed in 70% ethanol until metastatic tumor foci were enumerated. Exposure to 0.5 ppm phosgene significantly decreased the number of metastatic tumor foci in the lungs of L-W rats exposed to phosgene gas. (This abstract does not necessarily reflect EPA policy.)


Anthraquinone dyes are utilized by the army in colored smoke grenades, for marking, signaling, and identification. Fischer-344 male rats, 8-12 weeks of age, were exposed by inhalation to 300 mg per m^3 red dye mixture for 6 hours per day for 5 days. Red dye mixture contained 90.6% solvent red 1 [1-(2-methoxyphenyl)azo]-2-naphthol] and 9.4% disperse red 11 (1,4-diamino-2-methoxy-anthraquinone). Animals were exposed to either clean filtered air or to red dye in stainless steel/glass exposure chambers in a dedicated containment laboratory. NK activity was quantified by a 4 hr 51-chromium release assay. Effector: target cell ratios of 100:1, 50:1, and 25:1 were quantified. Pulmonary NK activity was significantly enhanced in Fischer-344 rats following exposure to red dye mixture. This enhanced pulmonary NK activity may enhance resistance to viral and neoplastic diseases. However, prolonged stimulation of this nonspecific immunological mechanism may have consequences due to the altered regulation of other immune cells. (Supported by US AMRDC. This abstract does not necessarily reflect EPA or Army policy.)


AD is an iodinated drug that is used clinically to treat cardiac arrhythmias. AD can cause pulmonary toxicity, the mechanism of which is unknown. The AM displays striking biochemical and morphological changes that are directly correlated with the accumulation of AD. In the present study, we induced a phospholipidosis in AMs by treating male Fischer 344 rats with CP for 3 days. AMs were collected by pulmonary lavage, placed in cell culture, and using HPLC, the uptake of AD (2.5 uM) was compared to AMs from control rats. At 1 hr and 18 hrs of incubation, uptake of AD was 50% higher in CP-AMs compared to control AMs. To measure intracellular distribution of AD, X-ray microanalysis of freeze-dried samples was performed to quantify the level of iodine associated with lipid amorphous bodies, dense granules, and the nucleus. Iodine levels were slightly lower in amorphous bodies of CP-AMs compared to control AMs. However, amorphous bodies were far more abundant in CP-AMs resulting in the higher levels of AD in the whole CP-AM. Also, in CP-AMs, there was a higher level of iodine at the dark edges of the amorphous bodies compared to their lighter centers. This is indicative that AD was not able to freely penetrate into the deepest part of the preexisting lipid structure. (Supported by NSF grant DCD 8818863.)
Pulmonary exposure to asbestos is known to increase the risk of pulmonary malignancy. The natural killer (NK) cell is one of the most important cells for the surveillance and destruction of malignant cells. We examined the effects of asbestos inhalation on the pulmonary NK cell population of female C57BL/6 mice. Cell numbers were assessed using the mAb NKL1.1 with flow cytometric analysis, and cytotoxic activity evaluated using a 51Cr-release assay. Mice were exposed to chrysotile asbestos 3 hrs/day for 3 days. The animals were then sacrificed at 2, 7, 28, and 56 days. NK cells were isolated via collagenase digestion of the lung and purified by passage over nylon wool columns. The resulting cell suspensions were >70% lymphocytes with viabilities >85% for both groups. NK cell cytotoxicity in the asbestos treated groups was depressed at 2 and 7 days post exposure (73% and 43% of control, respectively) and recovered to control values by day 28. The relative and absolute numbers of NK cells recovered were below air control values for the duration of the study. Specifically, the percentage of control NK cell values in asbestos-treated groups were 64%, 36%, 73%, and 76% for days 2, 7, 28, and 56, respectively. The effects of asbestos appear to be relatively specific for NK cells since the relative and absolute number of T cells were generally unaffected at these times. These data suggest that asbestos alters NK cell activity via a cellular depletion mechanism rather than a functional alteration.

Guinea pigs have traditionally been used to identify contact sensitizers. However, these methods are expensive and require subjective analysis of erythema, which makes evaluation of dyes difficult. Mouse models such as the mouse ear swelling test (MEST) and lymph node cell proliferation (LNCP) assay eliminate these problems. However, the usefulness of these methods for detection of moderate and weak sensitizers has been controversial. To evaluate contact sensitizing potential of several dyes, we examined several modifications of the MEST, and a LNCP assay. A strong sensitizer, 2,4-dinitrofluorobenzene (DNFB), was tested along with several reportedly weak sensitizers (formalin, glutaraldehyde, dispersive orange 3) and dyes of unknown sensitizing potential (disperse blue 3, disperse red 11, solvent red 1). Almost all of the MEST procedures detected the strong sensitizer. However, caution had to be taken to optimize concentration and sensitizing routine so as to detect a response. Weak sensitizers were not detected using the MEST. None of the three compounds of unknown sensitizing potential were positive using the MEST. Preliminary results from the LNCP assay indicate success in detecting both strong sensitizers and the weaker sensitizer, formalin. The LNCP assay proved to be more sensitive as a preliminary screen for weaker sensitizers. This abstract does not necessarily reflect EPA policy.
INVESTIGATION OF THE MECHANISMS OF CONTACT HYPERSENSITIVITY INDUCTION UTILIZING THE MURINE LOCAL LYMPH NODE ASSAY. House, R.V., Thomas, P.T., and Gerberick, G.F. Life Sciences Research, TriState Research Institute, Chicago IL and Human and Environmental Safety Division, The Procter & Gamble Co., Miami Valley Labs, Cincinnati, OH.

We are currently involved in the validation of the murine local lymph node assay (LLNA) as an alternative model for assessment of contact sensitization. Studies are underway to date the relative utility of this assay for mechanistic evaluation of the induction process. In the present study, animals were treated with the known sensitizer oxazoline (OZA) at concentrations of 0.001, 0.05, and 0.1% daily for four days with quantitation of cellularity and cytokine production by the draining lymph node cells assessed on Day 5. Exposure to OZA resulted in a dose-related increase in cellularity as early as Day 2 of induction; this increase peaked by Day 5. In vitro analysis identified cytokines, and their kinetics of production, by the lymph node cells revealed a dose-related increase in production of IL-2 by cells from mice treated with OZA. A dose-related increase in production of IL-3 by lymph node cells isolated from OZA-treated mice was apparent by Day 2 of treatment and peaked on Day 3. In comparison, treatment with OZA had no effect on production of TNF by lymph node cells until Day 4 of treatment, when exposure to all three doses of OZA resulted in a significant production of TNF. These results suggest a modulation of cytokines in the local lymph nodes during the induction of contact hypersensitivity. Studies are currently in progress to further define the cytokine production profile in both the lymph nodes and epidermal cells of animals treated with contact sensitizers.

COMPARATIVE MEASUREMENTS OF ALLERGIC CONTACT HYPERSENSITIVITY RESPONSES TO XENOBIOTICS. M.L. Stern, T.A. Brown and A.E. Munson. Pharmacology and Toxicology, Medical College of Virginia/VCU, Richmond, VA.

A total of 17 chemical compounds were tested for the ability to induce allergic contact hypersensitivity in Balb/CJF mice. Of those chemicals 8 were also tested in guinea pigs. In the mouse all the chemicals were assayed with a radioisotopic assay employing 125I-labeled oxysol. The second assay in mice was a modification of the mouse ear swelling test (MEST). The radioisotopic assay was used as one assay for testing in the guinea pig. The second assay was a visual evaluation of the challenged area of the guinea pig in which erythema and edema were rated for a hypersensitivity score. Of the 17 compounds tested in the mouse, 7 induced a positive response detected by one or both assays. Of the 8 compounds tested in the guinea pig three induced a positive response. All compounds which induced a response in the guinea pig also induced a response in the mouse. One compound which induced a response in the mouse could not be detected in the guinea pig by either assay method. All the chemicals which induced a response in the mouse could be detected by the MEST. One chemical was detected by the MEST and not the radioisotopic assay. The role of Freund's complete adjuvant in the testing for contact hypersensitivity has not yet been clearly established. Eleven of the 17 compounds were tested with and without adjuvant pretreatment. The pretreatment of mice with Freund's complete adjuvant aids in the detection of contact hypersensitivity with the radioisotopic assay. The usefulness of adjuvant pretreatment with the MEST is less clear.

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MURINE MODELS FOR CONTACT SENSITIZATION. SL Rodenberger, P. W. Ledger, M.E. Prevo. ALZA Corp., Palo Alto, CA. Sponsor: HI Malbach. A method combining murine ear swelling and lymph node cell proliferation has been evaluated as a quantitative measurement of delayed contact sensitization. The method involves 1 or 4 occluded, 24h abdominal induction applications; the use of Pseudomonas toxin as an adjuvant; topical challenge to the ear; ear measurements at 0, 24, 48 and 72h post-challenge; and injection of 125I-UDR at 48h. By inclusion of vehicle-induced/article-challenged control groups, positive responses can be objectively recognized by standard statistical analysis. Intended for use in screening for the sensitization potential of therapeutic agents, the model was evaluated with known sensitizers tetracaine and naloxone. Axillary lymph node cell proliferation was more sensitive than ear swelling. Performing both 1 and 4 inductions was essential for tetracaine; strong sensitizers gave enhanced lymph node responses after 1 induction, whereas naloxone sensitization was only apparent after 4 inductions. Ear swelling responses increased after 4 inductions of tetracaine as compared to 1 induction, however ear swelling response was not observed after 1 or 4 inductions of naloxone. After 1 or 4 inductions of a strong sensitizer, oxazoline, lymph node and ear swelling responses were observed. This method allows quantitative evaluation of contact sensitization as compared to the more qualitative scoring methods in traditional sensitization studies for less labor, cost, time, and animals. It is adaptable to modifications such as coadministration of immunomodulators.


In prior studies we showed that the abrogation of toxicity of Cy in mice by PA was associated with the concomitant potentiation of immune response including macrophage number and function. In this study the effect of Cy on peritoneal macrophages and its recovery by PA was assessed at ultrastructural level. At high dose (350 mg/kg), Cy significantly reduced the macrophage number, adherence ability, nitroblue tetrazolium reduction ability and phagocytosis against sheep EBC. Macrophages treated with Cy showed vacuolation in cells, reduction in the number of lysosomal bodies and mitochondria, and highly damaged or hazy membranous work with disintegrated endoplasmic reticulum. The mitochondria were swollen and showed reduced number of cristae. Macrophages from the animals treated first with PA (1 µg/animal, twice weekly for 2 wk) and then with Cy showed normal cells with the reappearance of lysosomal vesicles, mitochondria packed with cristae, and other cell organelles having normal appearance; the number and functions were also increased. The recovery was relatively lower when PA was given after the Cy. PA treated alone increased the cell size and mitochondrial number. Our study shows that PA can alter the morphology and physiology of macrophages in such a way that either they escape from the toxic effect of Cy or repair themselves more efficiently.

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MODULATION OF MACROPHAGE CELL SURFACE PROTEINS BY O,O,S-TRIMETHYL PHOSPHOROTHIOATE (OOS-TMP). K.E. Hodgar and D.D. Ellefson, School of Medicine, University of Southern California, Los Angeles, CA.

Exposure to OOS-TMP was shown to macrophage (Ma) differentiation. Characterization of the expression of Ma cell surface markers, Mac-1, Mac-2, Ia and F4/80 was conducted via cytometricity. These studies showed that F4/80 expression was decreased within 4 hr and the reduction was maximal at 72 hr. In addition, within 2 hr after treatment, the protein recognized by F4/80 was cleaved to a smaller molecular weight. Alternatively, the percentage of peritoneal cells (PC) which co-express Mac-1 and Ia was decreased 12 hr after treatment and again the alteration was maximal at 72 hr. The PC expressing Mac-1 was decreased at days 1 and 3 following treatment. Alternatively, the percentage of Mac-1-positive PC that also expressed Mac-2 was elevated 1 day after treatment. Therefore, OOS-TMP modulates Ma cell surface proteins in a rapid, but transient manner. Additional studies were conducted and showed that the trypsin inhibitory capacity (TIC) of peritoneal lavage fluid was reduced by in vivo administration of OOS-TMP. Also, in vitro exposure of purified α2-macroglobulin to OOS-TMP inhibited the TIC of this molecule. Supported by ES 44337.

HEATED LINOLEIC ACID ANILIDE: TOXICITY AND RELEVANCE TO TOXIC OIL SYNDROME. M F Khan, B S Kaphalia, A Palafax, T R Jerrells and G A S Anass. Department of Pathology, University of Texas Medical Branch, Galveston, TX.

Toxic oil syndrome (TOS) is epidemiologically related to ingestion of cooking oil adulterated with 2% aniline. The etiology of TOS is still unknown. Fatty acid anilides (FAA) have been implicated as the toxic agents responsible for TOS, although it is not clear whether FAA as such or their decomposition products are the causative agents. In the present study, the toxicity of linoleic acid anilide (LAA) and the heated (200°C for 30 min) linoleic acid anilide (HLAA) in rats and their relevance to TOS. Male Sprague-Dawley rats were given 250 mg/kg of LAA or HLAA in mineral oil (750 μg/kg) via gavage, on alternate days for 2 weeks (a total of 7 doses). Control rats received an equal volume of vehicle only. The animals were sacrificed at day 1, 7, 14 and 21 after the last dose. While lung, spleen and kidney weight showed significant increases at certain time points, liver weight decreased at day 1 in the HLAA treated group. Red cell counts and hemoglobin content decreased at day 1 in both LAA and HLAA groups, while platelet counts showed an increase. Serum LDH, GOT and GPT activities were significantly decreased up to day 7 in both LAA and HLAA groups, however these changes were more prominent in the HLAA treated group. These enzyme levels recovered to control levels at day 28. Both LAA and HLAA treated groups showed a decrease in serum IgM levels, whereas IgA levels significantly increased in both groups at all the time points observed and were more pronounced in the HLAA treated group. Similarly, IgG levels also showed increases in both the groups. Alterations in the lymphocyte subpopulations were also observed. While T-cell population decreased, B-cell population remained unchanged. Among T-cell subsets, T-helper cells did not show any change while T-suppressor cells decreased significantly at day 1 and 7 but regained control levels at day 28. The changes observed in this study as a result of LAA and HLAA exposure and more so by HLAA indicate a role for fatty acid anilides in the etiology of TOS.

A 10 DAY INTRAVENOUS TOXICITY STUDY OF RECOMBINANT HUMAN MACROPHAGE COLONY STIMULATING FACTOR (M-CSF) IN C57BL/6 MICE. C W Johnson, J P Nachtman, S E Mills, H L Moons, R D McCabe and R E Cimprich, New York, NY.

M-CSF is a dimeric protein that promotes proliferation and differentiation of macrophages, and thereby may prove therapeutic in the treatment of cancer or infection. To assess safety of M-CSF, C57BL/6 mice received daily i.v. boluses of excipient (4 mice/sex/group) or M-CSF (3 mice/sex/group) at dose levels of 3, 10, 30, 60, or 75 mg/kg/day for 10 days. The maximum tolerated dose was 30 mg/kg/day and one mouse each died at 60 and 75 mg/kg/day. All animals were necropsied on day 11. The in-life no effect level was 3 mg/kg/day and in general, both sexes showed similar effects at similar doses. Body weights tended to be elevated in response to M-CSF treatment. M-CSF related hematologic changes included macrocytic, normochromic anemia and thrombocytopenia. M-CSF treatment was associated with increases in tissue macrophages including hypertrophy/hyperplasia of reticulumendothelial cells, and macrophage infiltrates in the cecum, colon, epididymides, bone marrow, heart, kidneys, liver, lymph nodes, mesentery, nerve, pancreas, rectum, muscle, skin and urinary bladder. Additional effects were increased hepatic and splenic hematoepoiesis and cecal edema. Hepatic and splenic changes correlated with increased weights in these organs. Cecal edema and fluid filled intestines correlated with in-life abdominal swelling. Since the MTD observed in this study is significantly higher than the doses used in the clinic, M-CSF appears to have a broad safety margin and to be relatively safe.

THE ROLE OF SYSTEMATICALLY ADMINISTERED ALPHA INTERFERON ON NON-SPECIFIC PULMONARY HOST DEFENSE. G L Rosewell, R Ranahan, W Craig, P Barbera, E Corsini, P Thomas, C E Comment and M I Luster. NIH, RTP, NC and IIT Research Institute, Chicago, Il.

Alpha interferon (IFNα), a naturally occurring cytokine with multiple biological activities, has proven therapeutic potential in a variety of infections and malignancies. Although in vitro studies have demonstrated an antiviral effect by IFNα against most respiratory viruses, clinical trials have not been uniformly successful. Studies have suggested the potential toxic effects of systemically administered IFNα on cells thought to be involved in host defense against influenza; the lung NK cell and the alveolar macrophage (AM2). Following a single treatment (10,000 U/mouse), IFNα enhanced NK cell activity and yet no enhanced production of AM2 cytokines. These findings correlated with a delay in the morbidity of mice exposed to influenza. To determine whether the AM2 was refractory to IFNα or did not respond due to inadequate concentrations reaching the respiratory epithelium, these cells were exposed in vitro to IFNα. While AM2 responded to the positive control IFNγ, no cytostatic activity was demonstrable by these cells in the presence of IFNα. In contrast, peritoneal macrophages (PM2) treated in vitro with IFNα, as well as PM2 isolated from animals treated in vivo, demonstrated substantial cytostatic activity. In conclusion, systemically administered IFNα can stimulate non-specific host defenses (NK cells) and this route can provide adequate drug concentrations into the interstitial compartment of the lung. IFNα administered systemically increased resistance to influenza suggesting that the NK cell may have a role in the control of this infection. Regardless of whether systemically administered IFNα are protective to the pulmonary epithelium into air sacs, the AM2 appears to be refractory to its stimulatory effects at concentrations that substantially up-regulate PM2 as well as splenic and pulmonary NK cells. These studies demonstrate the potential activity and limitations of systemically administered IFNα at controlling immune responses in the lung.
Male F344 rats were exposed 5 days/wk to a simulated urban profile of NO\textsubscript{2} (an 0.5ppm background level for 16 hours, a 6 hr exposure spike, during which the concentration rose to 1.5 ppm, and then held at 1.5 ppm for 2 hr, and returned to background, and a 2 hr down time for service). Weekend exposures omitted the spike. Control rats were exposed to filtered air. Spleens cells were removed after 1 and 3 wks and 3, 12, 18 and 16 mos of exposure and assessed for NK cell activity and blastogenic response to T cell mitogens, phytohemagglutinin and concanavalin A, and to the B cell mitogen Salmonella typhimurium glycoprotein. NO\textsubscript{2} significantly depressed the NK cell response in rats exposed for 3 wk at the 25:1 (p < 0.01) and 50:1 (p < 0.05), but not 100:1 effector to target cell (E/T) ratios. NK cell activity was not altered following 1 wk or 3, or 12 mos of exposure. Significant suppression (p<0.05) was observed following 18 mos of exposure but only at the 50:1 E/T ratio. NO\textsubscript{2} did not significantly affect responses to mitogens at any time. Mitogen responses were clearly depressed in the 12 and 18 mos exposure groups due to aging. The results indicate a transient suppression of NK cell activity due to environmentally relevant NO\textsubscript{2} exposures but no effect on B or T cell blastogenic responses. (This abstract does not necessarily reflect EPA policy.)

We have developed in vitro immunotoxicological screening assays based on culture of defined immune cell populations and utilizing cDNA probes to cytokines and their receptors to monitor altered mRNA expression in response to immunomodulatory agents. Administration of two model compounds, Bioxim and Cyclosporin A, to murine peritoneal macrophage and splenocyte cultures resulted in distinct, reproducible patterns of altered cytokine expression (Meredith et al. Hum. Toxicol. 8 411 (1989)). We now present data to compare molecular biological analyses following administration of test compounds in vivo and in vitro. Bioxim induced transient expression of IL-1α, IL-1β and TNFα mRNA in macrophages both in vitro and in vivo; turn-over of actin, a housekeeping gene, was unaffected. Cyclosporin A inhibited IL-2 mRNA expression both in vivo and in mixed lymphocyte culture in vitro. Azathioprine in vivo inhibited the expression of both IL-2 and actin mRNA in thymocytes indicating its non-specific/cytotoxic mode of action; in vitro azathioprine inhibited both mononuke and lymphokine mRNA in a dose dependent manner. Tributyltin oxide partially inhibited the expression of IL-2 receptor mRNA both in vivo and in vitro. These data validate earlier results obtained using in vitro screening systems. (Partially supported by the CEH RAP programme and by UK Ministry of Agriculture Fisheries & Food.)

Bone marrow (BM) is the source of immune effector cells, and thus its normal function is vital to immunocompetence. Alteration in BM is a side-effect of exposure to certain xenobiotics. However, investigation of BM function is often neglected in immunotoxicology studies, primarily due to the time-, labor-, and material-intensive nature of the assays. In addition, scoring of BM colony-forming assays are often subjective. For this reason we are developing and validating assays to assess BM function as measured by cellular proliferation, based upon serum-free liquid suspension microcultures. This system is simple, reproducible, and quantitative. BM cells cultured with rIL-3, rGM-CSF, or rGM-CSF all displayed a significant dose-related increase in cellularity, whereas cells cultured with rIL-2 or rTNF were not affected. Significant increase in cellularity was noted at 3 days of culture, with optimum growth at 5 days. Cultures grown in serum-free medium displayed greater sensitivity to the cytokine-induced growth without a concurrent increase in background cellularity. Preliminary studies which included azidothymidine (AZT) in the cytokine found a dose-related decrease in cellular proliferation, with significant suppression of cell growth at AZT levels higher than 1 μg/ml. These studies suggest the utility of liquid BM cultures in assessment of chemical-induced myelotoxicity.

We have developed in vitro immunotoxicological screening assays based on culture of defined immune cell populations and utilizing cDNA probes to cytokines and their receptors to monitor altered mRNA expression in response to immunomodulatory agents. Administration of two model compounds, Bioxim and Cyclosporin A, to murine peritoneal macrophage and splenocyte cultures resulted in distinct, reproducible patterns of altered cytokine expression (Meredith et al. Hum. Toxicol. 8 411 (1989)). We now present data to compare molecular biological analyses following administration of test compounds in vivo and in vitro. Bioxim induced transient expression of IL-1α, IL-1β and TNFα mRNA in macrophages both in vitro and in vivo; turn-over of actin, a housekeeping gene, was unaffected. Cyclosporin A inhibited IL-2 mRNA expression both in vivo and in mixed lymphocyte culture in vitro. Azathioprine in vivo inhibited the expression of both IL-2 and actin mRNA in thymocytes indicating its non-specific/cytotoxic mode of action; in vitro azathioprine inhibited both mononuke and lymphokine mRNA in a dose dependent manner. Tributyltin oxide partially inhibited the expression of IL-2 receptor mRNA both in vivo and in vitro. These data validate earlier results obtained using in vitro screening systems. (Partially supported by the CEH RAP programme and by UK Ministry of Agriculture Fisheries & Food.)

The use of the antifungal drug, amphotericin (Am), is associated with a high incidence of severe fever and chills. TNF-α has been implicated in these reactions, however, studies have been limited by the need for an appropriate biological response model. This report addresses the design and implementation of an in vitro model of Am stimulated release of TNF-α from human blood monocytes. Human blood monocytes, purified with ficoll-hypaque, were plated at a rate of 10⁵ cells per well of 24-well plates. Subsequently, nonadherent cells were removed and the adherent cells (primarily monocytes) were treated with a suspension of Am in culture medium containing DMSO (0.04% v/v). All media and reagents were endotoxin negative by the limulus assay. Culture supernatants were collected at 4 hours and TNF-α was measured with a biossay dependent upon the cytokotoxicity of TNF-α for a mouse fibroblast line. Two different Am concentrations are represented two separate experiments with MNC from different donors. In experiment 1, the mean and range of concentrations of TNF-α were 25 (20-28), 22 (20-28), 50 (40-60), and 141 (125-178) pg/ml in supernatants of MNC cultures treated w/ 0, 0.1, 1.0, and 10.0 μg Am per ml culture medium. In experiment 2, the means and ranges were 13 (6-22), 25 (20-32), 35 (32-40), and 89 (71-100) pg/ml in supernatants of MNC cultures treated with 0, 1.25, 2.50, and 5.00 μg Am per ml culture medium. Medium with DMSO, alone, did not stimulateTNF-α release. At clinically achievable concentrations (1-5μg/ml), Am produced a concentration dependent release of TNF α from human MNC in culture. System features including 1) concentration/response profiles of MNC from different donors, and 2) the effects of different Am treatments on TNF α release, may be directly applicable to the design of therapeutic regimens with reduced clinical toxicities.
INVESTIGATION OF THE MECHANISM FOR LEYDIG CELL TUMORIGENESIS BY LINURON IN RATS. J C Cook, L S Mullin, E F Stula, M A Applegate, and L B Biegel. The Du Pont Co., Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE.

Linuron, an herbicide, produced a concentration-dependent increase in Leydig cell adenomas in Cr:CDBR (CD) rats fed 0 (5%), 50 (16%), 125 (29.7%), and 625 (56.1%) ppm for 2 years. A nongenotoxic mechanism was investigated since linuron was negative in tests for genotoxicity. Linuron is structurally related to the antianabolic flutamide which has been shown to produce Leydig cell tumors. To investigate whether linuron possesses antianabolic activity, growing and adult CD rats were treated for two weeks with either linuron or flutamide; accessory sex organ weights and serum hormone levels were measured. Serum hormone levels were also evaluated from PI and PI CD male rats in a multigeneration reproduction study with linuron. Linuron appears to possess antianabolic activity based on the following: 1) decreased accessory sex organ weights; 2) increased serum estradiol and luteinizing hormone (LH) levels in linuron-treated adult rats; and 3) competed with [3H]-testosterone for binding to the androgen receptor. These data are consistent with the effects seen with flutamide and support the hypothesis that linuron is producing Leydig cell tumors via an antianabolic mechanism where sustained hypersecretion of LH appears to be responsible for the development of hyperplasia/adenomas.


Possible inhibitory effects of the naturally occurring antioxidant γ-oryzanol(γ-02), phyto acid (PA), and N-tritratoclitine-16,18-dione (TTAD) were investigated in a multi-organ bioassay system (DDB model). Animals were given two i.p. injections of 1000 mg/kg b.w. 2,2'-dihydroxy-di-n-propylnitrosamine (DHPN) followed by two i.g. administrations of 1500 mg/kg b.w. N-ethyl-N-hydroxethylnitrosamine (ENHN), and then three s.c. injections of 75 mg/kg 3,2'-dimethyl-4-aminobiphenyl (ZMAB) for the initial 3 weeks. Starting one week after the last injection, animals were treated with dist containing 1% γ-02, 2X PA, 0.2X TTAD or basal diet alone for 32 weeks. Animals were sacrificed and complete autopsy was performed at the end of week 36. Histological examination revealed that γ-02 and PA enhanced lung and urinary bladder carcinogenesis, respectively. On the other hand, TTAD inhibited hepatocarcinogenesis and pancreatic carcinogenesis. These results indicate that naturally occurring antioxidants showed different modifying activities on different organ site carcinogenesis. In conclusion, this multi-organ bioassay system is very useful for detecting not only enhancing but also inhibitory effects of the carcinogenesis of unknown chemicals.


Validity of the multi-organ bioassay system was investigated in F344 male rats using sodium phenobarbital (PB) and N,N-dibutyl-nitrosamine (DBN) as model compounds. After sequential treatment with diethylnitrosamine (DEN), N-methylnitrosourea (MNH) and 2,2'-dihydroxy-di-n-propylnitrosamine (DHPN), rats were fed 500 ppm PB in the diet or 50 ppm DBN in the drinking water. The rats were sacrificed 18 and 24 weeks after the commencement. Upon histopathological investigation, PB was found to increase significantly the incidences of hyperplasia and adenomas of the thyroid and the numbers and areas of GST-P1 foci in the liver. DBN increased the lung tumor incidences at week 18, and brought about significant increases in both numbers and areas of lung tumors per rat at week 24. Furthermore, DBN enhanced the occurrences of hyperplasias and papillomas of the esophagus as well as hyperplasia for the forestomach at both time points. In addition, significant numbers of esophageal carcinomas and lingual papillomas developed in the group given DBN after pretreatment with the three carcinogens at week 24. Assessment of lesion yield appears indicating the advantage of the multi-organ bioassay system for detection of cancer modifiers within a limited time period.
MULTI-ORGAN BIOASSAY SYSTEM: RESULTS FOR KNOWN CARCINOGENS AND PROMOTERS.


Effects of 11 known carcinogens or promoters on pre-neoplastic and neoplastic lesion development were investigated in a multi-organ bioassay system (MND model). Male F344 rats were sequentially treated with N-diethylnitrosamine (100mg/kg b.w., i.p., single), N-methyl-nitrosourea (200mg/kg b.w., i.p., 4 times, wk 1-2) and 2, 2'-dihydroxy-di-n-propyl-nitrosamine (0.1% in drinking water, wk 3-4) for multi-organ initiation and then received dietary supplementation with one of the chemicals for 16 weeks. All rats were killed at the end of week 20, and subjected to complete autopsy. Histopathological examination clearly showed that carcinogenic or promoting sites of 11 chemicals reported were well correlated with the results of the present experiment. In addition, clofibrate was shown to enhance urinary bladder while inhibiting thyroid carcinogenesis. Caprolactam showed no effects as expected from earlier findings. The results thus indicated that the system could be useful as a medium-term multi-organ bioassay system for detecting carcinogenic and tumor-promoting potential of test agents with unknown target sites.

SUSCEPTIBILITY TO SODIUM L-ASCORBATE (Na-AsA)- PROMOTION IN 2-STAGE URINARY BLADDER CARCINOGENESIS (UBC) OF ODS-CD/og (ODS) RATS WHICH DO NOT SYNTHESIZE L-ASCORBIC ACID (AsA). S Mori1, T Murai1, Y Takeuchi1, Y Kurata1, S Makino1, Y Hayashi1 and S Fukushima1, 1 Aburahi Lab. Shionogi Res. Lab. & Co., Ltd., Shiga, and 1st Dep. Pathol. Nagoya City Univ. Med. Sch. Nagoya, Japan.

As the relationship between AsA including Na-AsA and cancer is regularly discussed, we investigated whether male ODS rats have susceptibility to Na-AsA-promoting or AsA-co-promoting effect in N-butyl-N-[(4-hydroxybutyl)nitrosamine (BBN)-initiated UBC and the level of susceptibility compared with that in male F344 rats with AsA-synthesizing ability. Experiment 1: ODS and F344 rats given 0.05% BBN for 2 wks and following by 5% Na-AsA for 32 wks. F344 rats were sensitive to the Na-AsA-promotion in UBC, but ODS rats were not. Na-AsA elevated the urinary pH and the urinary concentrations of Na+ and total AsA in both strains. Experiment 2: the ODS and F344 rats were given 5% Na-AsA for 8 wks. Na-AsA increased the DNA synthesis in urinary bladder epithelium of F344 rats, but not that of ODS rats. Experiment 3: ODS and F344 rats were given 5% AsA, 5% NaHCO3, and 5% AsA+3% NaHCO3 for 32 wks after 2 wks-UBC-initiation. AsA did not promote UBC in both strains. NaHCO3 and NaHCO3+AsA promoted the UBC in F344 rats, but not in ODS rats. The results indicate that the administration of Na-AsA or AsA does not influence the UBC in ODS rats.

TOXICITY AND LACK OF CARCINOGENIC ACTIVITY OF SODIUM AZIDE IN F344/N RATS: M A ABDO, J K HASEMAN and M P JOKINEN, NIEHS, RESEARCH TRIANGLE PARK, NC.

Sodium azide is used in the manufacture of explosives and automobile safety air bags. Toxicity and carcinogenicity studies were conducted by administering 0, 5 or 10 mg/kg sodium azide in distilled water by gavage once daily, 5 days per week for up to 133 weeks to groups of 60 male and 60 female rats. Dose related depression in mean body weight was observed throughout the study. Survival of high-dose rats of both sexes was significantly reduced as compared to controls. There were no chemical-related increase in tumor incidence. Significant decreases were observed in the incidence of mononuclear cell leukemia and adrenal gland pheochromocytoma in male rats, and mammary gland fibroadenoma and pituitary gland neoplasms in female rats. These decreases could be partly explained by the reduced survival of the high dose group. Chemical-related brain lesions (necrosis of the cerebrum and/or thalamus) occurred at a significantly increased incidence in the high dose rats of each sex.

STUDIES ON THE MECHANISMS OF ACRYLONITRILE INDUCED GASTROINTESTINAL DAMAGE: TIME COURSE OF MOLECULAR INTERACTION AT THE GASTROINTESTINAL TISSUES OF RATS. A E Ahmed, S Z Abdel-Rahman and A Nour Al-Deen, Department of Pathology, University of Texas Medical Branch, Galveston, TX.

Acrylonitrile (VCN) is extensively used in polymer industries. Increase in gastrointestinal (GI) cancer following VCN exposure is reported. There is a paucity of information regarding the mechanisms of the carcinogenic effect of this compound on the GI tissues. Time course for the interaction of [2,3-C14] VCN with GI tissues of male Sprague-Dawley rats following a single oral dose of 45.3 mg/kg was studied. Total tissue radioactive uptake was maximal at 15 min following VCN administration (403 pmole VCN eq/mg tissue). This level sharply declined at one hour after treatment (214 pmole VCN eq/mg tissue) and another maximum was observed at 6 hr (77 pmole VCN eq/mg tissue). A similar pattern was observed for radioactive soluble metabolites in GI tissues. Radioactivity binding to DNA was maximal at 15 min (7.5 pmol VCN eq/mg DNA) and a minimum at 72 hr after VCN administration (1.9 pmole VCN eq/mg DNA). Radioactivity binding to protein was maximal at 30 min after dosing. Radioactivity in the GI tissues observed at early times indicate direct absorption and interaction of the intact VCN molecule, while the delayed accumulation observed at 6 hr, suggest the formation of blood born metabolite(s). The covalent interaction indicate limited ability of the GI tissue to protect itself against acrylonitrile and/or its reactive intermediates, making GI more susceptible to acute as well as genetic damage. (Supported by NIEHS Grant #ES01871).
STUDIES ON THE MECHANISMS OF ACRYLONITRILE-INDUCED REPRODUCTIVE TOXICITY: MOLECULAR INTERACTION IN TESTICULAR TISSUES. S. Z. Abdel-Rahman, A. Mour Al-Deen and A. E. Ahmed. Department of Pathology, University of Texas Medical Branch, Galveston, TX.

Acrylonitrile (VCN), a commonly used monomer in polymer industries, has been shown to induce malignancy in extra hepatic tissues including GI, lungs and prostate. Irreversible interaction of (VCN) or its reactive metabolites with tissue macromolecules may be responsible for its carcinogenicity. Little information is available on the effect of VCN with reproductive system. The objective of this study is to investigate the time course for interaction of VCN with DNA and protein in testis of rats. Male Sprague-Dawley rats were given a single oral dose of [2, 3-35Cl] VCN (46.5 mg/kg sp. act. 2.8 mCi/mg). Total tissue radioactive uptake was maximal (39 pmole VCN eq/mg tissue) at 0.5 hour after VCN administration and minimal (1.9 pmole VCN eq/mg tissue) at 72 hours. Maximum accumulation of radioactivity in the soluble fraction of the testicular tissue was observed at 0.5 hour (24.3 pmole VCN eq/mg tissue) and minimal at 72 hours following dosing. The molecular interaction of VCN metabolites with testicular proteins was constantly minimal at all times (1.5 ± 0.3 pmole VCN eq/mg tissue). Binding of radioactivity to testicular DNA was maximal at 0.5 hr after VCN treatment (29 pmole VCN eq/mg DNA). Radioactivity was detected on DNA molecules at 72 hours after dosing (8 pmole VCN eq/mg DNA). The results suggest that VCN is able to act as a multi-potent carcinogen by alkylating DNA in extra hepatic target tissues, and may affect the male reproductive tissues leading to serious reproductive abnormalities. (Supported by NIEHS Grant #S01871).

STUDIES ON THE MECHANISMS OF NITROOSOURA INDUCED HEPATOMIC TOXICITY. CCNU-INDUCED INHIBITION OF DNA SYNTHESIS AND ITS PREVENTION BY TRANS-STILBENE OXIDE (TSO). J-P Loh, G I Hussein, and A. E. Ahmed. Department of Pathology, University of Texas Medical Branch, Galveston, TX.

CCNU (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea) is an alkylating agent with known hepatotoxicity. Previous studies in our laboratory have indicated that induction of hepatic metabolic enzymes has been protective against CCNU induced hepatotoxicity. The objective of this study is to determine the effect of CCNU on hepatic DNA synthesis in rats and investigate if the enzyme inducer TSO can alter this effect. Male Sprague-Dawley rats were treated with either TSO (350 mg/kg, daily for 5 days, i.p.) or corn oil prior to administration of a single oral dose (50 mg/kg) of CCNU. Animals were then treated with 3H-thymidine (0.5 µCi/g, sp. act. 27.5 Ci/mmole) 2 hr before sacrifice at the various time intervals following CCNU administration (6, 9, 24 hr). At the time of sacrifice livers were removed and DNA synthesis was determined. CCNU induced significant inhibition of DNA synthesis at 6 hr (60% of control) and the rate of DNA synthesis was still inhibited (60% of control) at 48 hr following CCNU treatment. TSO induced an increased rate of DNA synthesis (194% of control). Animals treated with TSO prior to CCNU administration showed no CCNU-induced inhibition of DNA synthesis. In these animals a marked increase in DNA synthesis (255% of control) was observed at 6 hr following CCNU administration. Our study indicates that TSO acts as a protective agent against CCNU-induced inhibition of DNA synthesis either by induction of the detoxification mechanisms for CCNU or by induction of unscheduled DNA synthesis in the liver. (Supported by NIEHS # S01871)


Bemtradine (SC-33643), a diuretic antihypertensive agent, was studied for its carcinogenicity in a two year bioassay in Charles River CD rats via dietary ad libitum 150, 150, and 450 mg/kg for up to 97 weeks. Bodyweights were decreased by week 105 by (compared to controls) 5-15% in the female and 10-12% in the male dosage groups. Prolactin values were significantly increased in 150 and 450 mg/kg females. The compound caused significant increased incidence of liver, thyroid (both sexes), and mammary (females only) neoplasms. Bemtradine and its metabolites in man and rats (SC-36741, desethylbemradine) were tested and found to be nongenotoxic in Ames, mouse lymphoma TK+/− (bemtradine only), rat primary hepatocyte UDS, CHO/GFPRT, CHO cytogenetics, and in vivo mouse micronucleus assays.

Finally, in an altered hepatic foci promotion assay in female Charles River CD rats, bemradine was found to be a promotor, though not as potent as phenobarbital.

We concluded that bemradine is a nongenotoxic carcinogen which acts by a hormonally modulated promotional activity.

Alpha-1b globulin is necessary for d-limonene promotion of male rat kidney tumors. D.R. Dietrich and J.A. Swenberg. Departments of Environmental Sciences and Pathology, University of North Carolina, Chapel Hill, NC.

In a two-year bioassay, d-limonene (dL) was shown to induce kidney tumors only in the male rat but not in female rats or male and female mice (NTP, Technical Report 347, 1990). dL is also one of the many compounds known to induce alpha-1b globulin (A2U) nephrotoxicity in male rats (Swenberg et al., Toxicol. Apol. Pharmaco., 97, 1989) with the exception of the A2U deficient NFR rat (Dietrich and Swenberg, Tcotoxicologist, 10, 1990). The objective of this study was to show that dL promotes renal tumors only in the presence of A2U. In an initiation-promotion assay, male F344 and NBR rats were treated with 500 ppm N-Ethyl-N-Nitrosourea (ENH) in the drinking water for 2 weeks followed by 150mg dL/day for 30 weeks, administered in corn oil by oral gavage 5 days per week. An initiation-control, promotion-control, and a vehicle control group was included for both strains. Cell proliferation was assessed via BrU-labeled mitomycin m-pumps after 5 and 30 weeks of dL promotion, respectively. No tumors were found in any of the NBR groups, whereas a ten-fold increase in the incidence of renal adenomas and atypical hyperplasias was found in the EHEN-dL treated F344 rats in comparison to EHEN rats treated with EHEN-in corn oil. A five-fold increase of P2-rabbit labeling index was found in all dL treated F344 rats, with no difference between treatment groups was found in the NBR rats. This is thus concluded that dL promotes renal tumors only in the presence of the male rat specific urinary protein A2U. Since A2U is a species and sex specific protein that is causal for both the cytotoxic and carcinogenic response, extrapolation of dL carcinogenicity data from rat to species other than humans, is not warranted.
Methapyrilene is a non-genotoxic liver carcinogen specific to the rat. An alteration in mitochondrial number and morphology has been observed in rat hepatocytes exposed to methapyrilene, both in vivo and in vitro. This biological phenomenon may be due to the effect of methapyrilene on mitochondrial protein and DNA syntheses. To test this hypothesis, studies have been initiated to examine the effects of methapyrilene on isolated rat liver mitochondria. Mitochondria were isolated from untreated rats, then exposed to 0-100 uM methapyrilene. Ethidium bromide and chloramphenicol were used as controls for inhibition of DNA and protein syntheses, respectively. DNA and protein syntheses were measured using 3H-thymidine and 3H-leucine incorporation. No effects on DNA or protein syntheses were observed in mitochondria isolated from untreated rat hepatocytes. Studies were conducted in order to evaluate the ability of isolated mitochondria to metabolize 14C-methapyrilene. The metabolites were analyzed by thin layer chromatography with radioactive detection. Preliminary results demonstrated that functional mitochondria do not metabolize methapyrilene to any significant degree. To allow for cellular metabolism of methapyrilene, studies are in progress to evaluate DNA and protein syntheses in hepatocyte mitochondria isolated from rats following exposure to methapyrilene.

Identification of specific chromosomal aberrations associated with transformation of mesothelial cells is important in elucidating the mechanism of action of agents such as asbestos. We have established strains of normal rat mesothelial cells in culture that spontaneously transform with a high frequency. Cytogenetic analysis of these strains revealed that at late passage (p20-p34), trisomy of chromosome 1 was present in >80% of the cells in all 4 strains examined, whereas at early passages (p8-p10), only 15-44% of the cells had trisomy 1. This indicates that the frequency of trisomy of chromosome 1 had increased in the population as a function of passage, suggesting that cells with trisomy 1 had a selective growth advantage under in vitro culture conditions and that this alteration was associated with transformation. A commercially available rat mesothelial cell line (4/4 RM4, ATCC), was also found to have a duplication of a portion of the long arm of chromosome 1. Rat chromosome 1 contains linkage groups syntenic with human chromosome 11, which has also been observed to be trisomic in human mesothelioma. To determine if chromosome 1 alterations have relevance to the transformed phenotype in vivo, a neoplastic cell line was established from a spontaneous rat mesothelioma. At passage 15, trisomy of chromosome 1 was observed in this line in 26% of the cells. However, when these cells were injected into nude mice, 99% of the cells from the resulting tumor contained an additional copy of chromosome 1. Therefore trisomy 1 also conferred a selective growth advantage in vivo and/or was associated with the malignant subpopulation in the tumor derived cell line. These studies indicate that chromosome 1 contains gene(s) involved in transformation of rat mesothelial cells.

The pathogenesis of mineral fiber-induced mesothelioma in both man and experimental rodent models is poorly understood. Fiber-induced mesotheliomas in rats and humans are biologically and morphologically similar, however, it is not known if the cellular and molecular pathways for transformation in the two species are also similar. Recent findings have suggested that concurrent expression of platelet-derived growth factor (PDGF) and its receptor in human mesothelioma cells may be an essential element in the transformation of these cells. We have examined the expression of PDGF α and β receptors and their ligands (PDGF A and B chains) in cell lines derived from asbestos-induced rat mesotheliomas. These cells were found to express transcripts for the PDGF β receptor and to specifically bind PDGF BB homodimer. However, the concomitant expression of PDGF B was not detected. No expression of PDGF α receptors could be detected at either the RNA or protein level in the transformed cells, and transcripts for PDGF A were not expressed. In addition, PDGF (AA, AB, and BB) was not mitogenic for normal rat mesothelial cells cultured in low serum. Taken together, these results indicate that in the rat, autocrine stimulation of growth by PDGF is not operative and suggests that the mechanism of transformation for this cell type may be different for rat and man.

The carcinogenic sensitivity of the medaka (Oryzias latipes) is being probed through the systematic exposure of this small fish to an array of chemical carcinogens. The carcinogens being tested represent several chemical classes including aromatic amines. The aromatic amines to which medaka have been exposed include: aniline, 4-chloroaniline, o-anisidine, 2,4-diaminotoluene, and 2-naphthylamine. Medaka were exposed to aqueous solutions of these chemicals for four weeks followed by 22 weeks of depuration. At the end of depuration, 60 animals from each treatment were analyzed for evidence of carcinogenicity. Strong carcinogenic responses were observed with aniline and 4-chloroaniline, causing epitheliomas of the gas bladder. Weaker carcinogenic responses were observed with o-anisidine and 2,4-diaminotoluene, causing liver neoplasms with o-anisidine and eye and gas bladder neoplasms with 2,4-diaminotoluene. Results from the 2-naphthylamine exposure will also be presented. These results indicate that the medaka is sensitive to aromatic amines and may be a useful model to assess the carcinogenic hazard posed by compounds in this chemical class.
Binding of carcinogens to DNA and repair of induced lesions are not random processes. Methods to isolate adducted DNA should facilitate study of the distribution of DNA damage and repair within the genome of mammalian cells. Thus, a rabbit polyclonal antibody raised against DNA adducted with (±)-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE-I) was tested for its ability to alter the electrophoretic mobility of adducted DNA through agarose. The IgG fraction of the antiserum retarded the migration of DNA fragments containing carcinogen adducts, but did not affect the mobility of non-adducted DNA. Fab fragments and IgG coupled to ferritin did not promote retardation to the same extent as IgG alone. Levels of DNA adduction as low as 0.6 adducts per 1000 bp were effective in causing retardation of 3.2 kb fragments. This method might be useful in studies of preferential binding of BPDE-I to replicating genes and DNA repair kinetics. Supported by USPHS/NIH grants P01 CA42765 and R01 ES03602.

AFB1 is a carcinogenic mycotoxin that requires activation to the corresponding 8,9-epoxide for activity. In addition to being present in foodstuffs, AFB1 can contaminate respirable grain dusts and thus the respiratory system is a potential target for carcinogenesis. In the present study, we have investigated the role of polyacrylamide aromatic hydrocarbon-inducible forms of cytochrome P-450 in the pulmonary and hepatic microsomal activation (DNA binding) and detoxification ([3H]AFM and [3H]AFQ formation) of [3H]AFB1. In rabbit lung microsomes, BNF treatment did not affect the Vmax for DNA binding of [3H]AFQ formation, but the Vmax for [3H]AFM formation was increased by 60%. The Km values for these reactions were not affected by BNF treatment. In liver microsomes, the Vmax for DNA binding and [3H]AFM formation were increased by BNF treatment (to 2.3 and 3.3 times control, respectively) while Km values were unaffected. The Vmax for [3H]AFQ formation was not affected by BNF treatment, but the Km was increased to 4.5 times control. These results suggest a role for the IA subclass of P-450 isozymes in the biotransformation of AFB1 to AFM1 in lung and liver and in the bioactivation of AFB1 in the liver. (Supported by the Medical Research Council of Canada)


A slow acetylator phenotype has been associated with increased incidence of bladder cancer in humans. To determine if a phenotypic slow acetylator results in increased DNA damage, the human/mouse bladder carcinogen, 4-aminobiphenyl, was given in drinking H2O for 20 g to congenic mouse strains C57BL/6J and BALB/c-NTac (rapid- and slow-acetyltransferase genotype, respectively). The levels of covalently-bound N-((deoxyguanosin-8-y)-4-ABP DNA adducts, believed to initiate tumours, were quantitated in the liver and bladder by F-post-labeling analysis. DNA adduct levels increased with dose in male and female liver and male bladder. At comparable doses, adduct levels were two-fold higher in female liver than in male liver, but liver adduct levels were independent of acetylator genotype. In contrast to the liver, adducts were about two-fold lower in female bladder DNA compared to male. Also in contrast, DNA-bladder adducts were significantly higher in rapid-acetylator females at both 75 and 150 ppm dose levels; median adduct levels were 20% higher in slow-acetylator male bladders compared to their rapid counterparts. These results are consistent with the increased tumorigenicity of 4-ABP to liver of female mice and bladder of male mice, but suggest factors other than acetylator phenotype limit the extent of 4-ABP-adduct formation in mice.

ACTIVATION OF AFLATOXIN B1 (AFB1) BY ARACHIDONIC ACID (AA)- AND NADPH-DEPENDENT MECHANISMS IN GUINEA PIG TISSUES. L Liu, R K Stewart and T E Massey. Dept. Pharmacology and Toxicology, and Medicine, Queen's University, Kingston, ON, Canada. Sponsor: J Brodeur.

Pulmonary and renal cytosolic lipoxigenases (LOX) and microsomal prostaglandin synthase (PHS), as well as cytochrome P-450 monoxygenases (P-450), were able to activate [3H]aflatoxin B1 ([3H]AFB1) to a DNA-binding metabolite, although pulmonary PHS-catalysed activity was very low. Renal microsomal AA-dependent DNA binding was inhibited 85% by 0.1 mM indomethacin, and 61% by 0.1 mM nordihydroguaiaretic acid (NDGA). Purified soybean LOX- and pulmonary LOX-mediated DNA binding were totally inhibited by NDGA, but not by indomethacin. In lung preparations, the Vmax of P-450-catalysed [3H]AFB1-DNA binding was higher than that of LOX (85.8 ± 54.1 and 2.68 ± 0.7 pmol/mg/min, respectively), but their Km values were similar (37.2 ± 22.6 and 21.2 ± 12.6 µM, respectively). In kidney, Km values of LOX and P-450 for AFB1-DNA binding were lower than that of PHS (15.4 ± 9.3, 23.6 ± 8.9 and 55.7 ± 27.1 µM, respectively), whereas Vmax values of PHS and P-450 were higher than that of LOX (1.14 ± 0.55, 1.50 ± 0.55 and 0.22 ± 0.11 pmol/mg/min, respectively). These results suggest that LOX in lung and kidney have relatively high affinity towards AFB1, and that LOX and PHS may play a significant role in pulmonary and renal bioactivation of AFB1, especially at low substrate concentrations. (Supported by the Medical Research Council of Canada)

The protective effects of naturally occurring dietary modulators against experimentally induced chemical carcinogenesis are well established. In this study, the effect of dietary modulators benzylisothiocyanate (BIT), ellagic acid (EA), indole-3-carbinol (IC) and catechin (CC) on 7,12-dimethylbenz[a]anthracene (DMBA) induced gastrointestinal (GI) tract nuclear anomalies (NA) was investigated. Groups of mice were gavaged with BIT (60 mg/kg), EA (50 mg/kg) IC and CC (100 mg/kg) or DMSO (10 ml/kg), 18, 24 and 2 hrs before DMBA administration. 24 hrs after DMBA administration all mice (DMSO treated and appropriate controls) were sacrificed and the GI tract NA quantitated. Data clearly shows that DMBA increased the incidence of NA formation in the GI tract in a dose dependent manner. Pretreatment of mice with BIT, EA, IC and CC, significantly (p < 0.001) inhibited DMBA induced duodenal NA. Dietary modulators or DMSO alone did not induce the NA formation. BIT pretreatment exerted maximum inhibition (48%) followed by EA (42%), IC (38%) and CC (30%), when compared to DMSO. Our data is in agreement with previous reports that dietary inhibitors modulate carcinogen DNA adduct formation and experimentally induced chemical carcinogenesis. (Abstract does not necessarily reflect EPA policy)

1307 MIREX IS A POTENT NONPHORBOL ESTER-TYPE TUMOR PROMOTER IN MOUSE SKIN. G J Moser and R C Smart, Dept. of Toxicology, North Carolina State University, Raleigh, NC.

The organochlorine pesticides, mirex, kepone and chlordane which varied greatly in their ability to stimulate epidermal protein kinase C (PKC) activity in vitro were evaluated as complete skin tumor promoters in female CD-1 mice. Chlordane (200 μM) stimulated epidermal PKC activity to its maximum velocity as the potent skin tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA), while kepone (200 μM) stimulated PKC activity to a velocity approximately one half that of TPA and mirex (200 μM) which is structurally similar to kepone did not stimulate PKC activity. Mice were initiated with 200 nmol 7,12-dimethylbenz[a]anthracene and topicaly treated thrice weekly with either 2 nmol TPA, 2 nmol chlordane, 250 nmol kepone, or 25, 50, 100, or 200 nmol mirex. Chlordane and kepone did not promote tumors; however, mirex promoted tumors at all dose levels in a dose-dependent manner. At 20 weeks 25 nmol mirex produced an average of 0.2 tumors per mouse with a 10% incidence of tumor bearing mice while 200 nmol mirex produced an average of 16.2 tumors per mouse with a 96% incidence of tumor bearing mice. Unlike the phorbol ester, TPA, tumor promoting doses of mirex did not induce epidermal ornithine decarboxylase activity or increase epidermal DNA synthesis as determined by the incorporation of 3H-thymidine. Multiple applications of 2 nmol TPA produced epidermal hyperplasia as indicated by an increase in the number of non-cornified cell layers (6-7) while 200 nmol mirex produced a marginal response by increasing the number of cell layers from 1-2 in control mice to 3 cell layers in mirex-treated mice. These data indicate that mirex is a potent nonphorbol ester-type tumor promoter in mouse skin.

1306 A DERMAL CARCINOGENICITY STUDY OF MAGNES®-100 NONOMER. R A Barie, R M Pedercini* and N A Friedman. American Cyanamid Company, Wayne, NJ and *Exxon Biomedical Sciences, Inc., East Millstone, NJ

MAGNES® (Methyl acrylamidoglycolate methyl ether), like other acrylate monomers, caused edematous lesions of the rat forestomach after acute gavage and extensive forestomach hyperplasia after repeated gavage. Gastric hemorrhage and congestion but no hyperplasia were seen in dogs after repeated gavage. Considering these effects as the result of local irritation, this study was conducted to evaluate the potential carcinogenic effects of MAGNES® when applied to the skin at irritating and non-irritating concentrations.

Groups of 50 male C3H/HeNGr 1BR mice were given 25 ul of 0.3, 9 or 30% MAGNES® in acetone, 3 times per week for 24 months. Treatment had no effect on survival of body weight. General irritation effects observed during treatment included desquamation and atonia. Dermal irritation was observed by microscopic examination as slight acanthosis in the 30% treatment group. No dermal neoplastic lesions were observed for any treatment group. No treatment related microscopic pathology was observed in other major tissues. Therefore, MAGNES® was not carcinogenic when given to mice by the primary route of industrial exposure. These results further support the conclusion that rat forestomach hyperplasia caused by MAGNES® has little relevance to humans.

1308 INHIBITION OF AFLATOXIN AND HETEROCYCLIC AMINE GENOTOXICITY IN VIVO AND IN VITRO BY CHLOROPHYLLIN. V Breinhold, R Dashwood, J Hendrick and C Bailey, Dept. Food Science & Technol., Oregon State Univ., Corvallis, OR.

Chlorophyllin (CHL), a derivative of the common dietary phytochemical chlorophyll was examined for its potential as an anticarcinogenic agent in rainbow trout. The food-borne genotoxins aflatoxin B1, 3-amino-1-methyl-5H-pyrido[4,3-b]-indole (Trp-P-2) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) were all mutagenic in the Ames Salmonella assay in the presence of trout liver S9, and CHL at 0.3-1 micromole/plate showed potent dose-responsive inhibition of mutagenicity. CHL also strongly inhibited aflatoxin B1-8,9-epoxide mutagenicity without S9, indicating a trapping or scavenging mechanism. In vivo, aflatoxin B1 showed dose-linear liver DNA adduction which was progressively inhibited by increasing dietary CHL in the 0-2000 ppm range. Binding was 70% inhibited at 2000 ppm CHL fed for 7 days with aflatoxin B1. These results suggest that this common dietary factor should be a potent inhibitor of carcinogenesis by these genotoxins in the trout model. Tumor studies with over 7000 trout are in progress to test this hypothesis. (Supported by USPHS grants ES00210, ES03850, GA34732).

Petroleum middle distillates fuels (PMD) are broadly defined as mixtures of hydrocarbons boiling between approximately 350-700°F. PMD contain low levels of polycyclic aromatic hydrocarbons, but nevertheless consistently elicit evidence of weak tumorigenic activity in mouse skin (Biles et al., Toxicology 53:301, 1988). This activity is characterized by low tumor yields (<10-25%) and median latencies exceeding 2 years. PMD are not mutagenic nor are they tumor initiators, but they are weak tumor promoters in CD-1 mice (McKee et al., FAAT 12:748, 1989). PMD also elicit extensive skin irritation in mice with repeated and prolonged exposure. Collectively, these data suggest that PMD may induce tumors by a secondary mechanism, such as chronic skin irritation or injury. To study this, two-year dermal carcinogenicity studies were conducted with three PMD. The PMD were administered in two 37.5 ul doses/week to groups of 50 male C3H mice. Skin irritation was evaluated both grossly and by histopathology. Despite similar levels of chronic skin irritation, only two of the three PMD were tumorigenic to mouse skin. These data along with previous studies (Freeman et al., Toxicologist 10:194, 1990) suggest that irritation alone (at least as defined in this work) is not sufficient to explain the tumorigenic potential of PMD.


To further assess the role of skin irritation in the dermal carcinogenic process, two petroleum middle distillates (PMD) were tested by altering the dosing regimen depending on the level of skin irritation. The PMD were administered undiluted in twice weekly doses of 37.5 ul to groups of 50 male C3H mice. For one PMD, one group of mice was dosed continuously for 2 years. A second group was dosed intermittently; dosing was suspended upon the onset of marked irritation and reinstated when the irritation had resolved. Among the continuously dosed animals 22/50 mice developed skin tumors whereas no tumors developed in mice treated intermittently. The second PMD was tested in a single group of 40 mice. After 1 year of testing, dosing was suspended due to severe skin irritation. No tumors were present at that time. The study continued without resumption of dosing for another year, and the skin irritation never completely resolved. Among these mice, 16/40 developed squamous cell tumors of the skin.

Repeated and prolonged exposure appears to be a prerequisite for tumor formation. It also appears that the tumorigenicity of the PMD is secondary to toxicity to the skin, the nature of which remains undefined, but as noted in the preceding paper, it does not appear to be skin irritation alone.
1313 DETERMINATION OF RESTING BLOOD PRESSURE IN UNRESTRAINED CYMONOGLUS MONKEYS USING IMPLANTED TELEMETRIC TRANSMITTERS. WA Mann G Welzel and LB Kinter. SmithKline Beecham Pharmaceuticals, Dept. Toxicology, King of Prussia, PA 19406. Sponsor: T. Leonard

Monitoring of cardiovascular functions in the unrestrained monkey is necessary for characterization of the toxicological effect of drugs on the cardiovascular system without the overlay of stress artifacts associated with prolonged restraint or excessive handling. In this study a simplified method of direct monitoring of blood pressure and heart rate in conscious unrestrained cynomolgus monkeys (Macaca fascicularis) using implantable blood pressure transmitters having a useful life of ~6 months (Data Sciences, Inc., St Paul, MN) was evaluated. This methodology allows recording from multiple animals over an extended period of time at investigator-determined intervals. In anesthetized monkeys the telemetry-transduced parameters agreed well (±5%) with values simultaneously obtained using a calibrated transducer system. In 3 conscious monkeys blood pressures and heart rates were determined every 10 minutes for 4 consecutive days. Average (±SD) daily values for blood pressure and heart rate were: systolic 101±9 mmHg, diastolic 60±7 mmHg, mean 81±6 mmHg and heart rate 127±22 bpm. These values are lower than those generally reported for anesthetized or conscious, restrained cynomolgus monkeys. Each monkey exhibited a diurnal rhythm that was reproducible from day to day, with blood pressure and heart rate higher during waking hours. We conclude that implantable blood pressure telemetry affords a simple and reliable method for determining cardiovascular functions in conscious unrestrained cynomolgus monkeys over prolonged time intervals. The approach provides continuous, quantitative cardiovascular data during initial range-finding toxicology studies in exotic species.


B-Agonists (BAs) and methylxanthines (MXs) are often used concurrently in the treatment of asthma. Although clinically beneficial, this combined use is reported to produce adverse cardiac effects. In this study, we examined the cardiotoxic effects of several drug combinations of BAs and MXs in male Sprague-Dawley rats (>500g). Groups of rats (N = 7) were given a single sc injection of either isoproterenol (ISO, 15 µg/kg), fenoterol (40 µg/kg) or terbutaline (TER, 0.4 mg/kg) alone or in combination with amphetamine (AM) (20 mg/kg, given 20 min prior to each of the BAs) with appropriate controls. Separate groups of rats received prednisone (4 mg/kg) for 3 days before ISO and/or AMN treatment. Blood samples were obtained from the orbital venous plexus of representative numbers of rats from each group for serum electrolyte determinations. One rat each from ISO-AMN-and TER-AMN-treated groups died within 30 min of treatment. Hearts were removed and placed in buffered formalin 48 hours after treatment for subsequent histopathologic examination. The severity of myocardial necrosis induced by each of the BAs was increased when they were given in combination with AMN. Prednisone pretreatment did not enhance the ISO-induced myocardial injury. Serum iron was significantly reduced in all treatment groups. Calcium and potassium levels were unaffected. Results indicate that the combined administration of BAs and MXs increases cardiotoxicity in rats.


To study early changes in chronic mitral insufficiency, to assess the ability of MR to produce toxic myocardial and pulmonary changes, and of taurine to reverse these early changes, we surgically induced MR in 13 clinically normal dogs. Four dogs were treated with taurine to reduce MR effects; 4 were to be treated by standard medication and 5 received no treatment. MR caused electrocardiographic abnormalities and a reduction in myocardial adenosine triphosphate (ATP), suggesting MR-induced heart damage. Taurine partially ameliorated the ATP reduction. AM recovered by segmental bronchial washings (SBW) 2-months after MR phagocytized less sheep erythrocytes, suggesting an early lung response. SBW soluble protein and hydroxyproline were marginally increased at 2-4 months, corresponding to the development of slight histologic edema. Although there was no cytologic or histologic pulmonary inflammation, total lung collagen was increased in dogs with MR. MR models early myocardial damage and lung responses. Taurine partially ameliorates its effects. [Research supported by Amer. Heart Assn., Kans. Affil., Grant-in-Aid No. G-1.]


As part of the preclinical safety evaluation process, an investigational macrolide antibiotic, LY281389, was examined for autonomic activity in isolated smooth and cardiac muscle preparations, and for cardiovascular effects by intravenous infusion in anesthetized beagles dogs. Concentration-dependent antagonism of acetylcholine and angiotensin I (guinea pig ileum), norepinephrine (rat vas deferens), and isoproterenol (guinea pig atria) were observed at LY281389 concentrations >10^{-9} M. At concentrations of LY281389 >10^{-6} M, the response of the guinea pig ileum to field stimulation was also inhibited approximately 65-100%, indicative of potential anticholinergic or a-adrenergic activity. In anesthetized dogs, the predominant effect of LY281389 was an increase in heart rate at doses >200 µg/kg. LY281389 also produced slight increases in mean pressure, and shortening of the P-R interval. In summary, LY281389 possesses non-selective receptor antagonist activity in vivo, and produces cardiovascular stimulation in anesthetized dogs. These results indicate that, in addition to potent antitubercular activity, the macrolide, LY281389, may exert unexpected actions on cardiovascular function.
A relevant animal model to aid establishing safety guidelines for workplace inhalation exposures to cardiovascular agents was evaluated by measuring responses to aerosols of saline, a known β-agonist (isoproterenol), and a representative "inert" dust (clay). A method has been developed which uses a telemetry system to non-invasively monitor heart rate (HR) and electrocardiogram (ECG) waveform effects during whole-body inhalation exposures of a group of 4 unrestrained, conscious monkeys. Saline aerosol, at concentrations up to 6.5 mg/L, had no HR or ECG effects. Heart rates decreased slightly during exposure to clay aerosol at concentrations of 5 µg/L and higher. A dose-response relationship was demonstrated for isoproterenol aerosol. Mean heart rate increased 0%, 15%, 18%, 27%, or 54% during exposures to 0.6, 1.0, 1.4, 2.5, or 4.9 mg isoproterenol/L. These results, plus the anatomical similarity of the monkey respiratory tract to that of man, give confidence in using this model to extrapolate to people.

HISTOCHEMICAL AND CHEMICAL STUDY OF ALLYLAMINE + β-AMINOPROPIONITRILE VASCULAR TOXICITY. P. J. Boor, M. Fagasson*, P. Cicio*, M. Trent and S. Awasthi, Univ. of Texas Medical Branch, Galveston, TX, and Univ. of Bologna*, Bologna, Italy.

Allylamine (AA) is a cardiovascular toxin that causes acute subendocardial necrosis and chronic vascular changes. β-aminopropionitrile (βAPN) is the toxic component of Lathyrus odoratus, the toxic sweet pea causing lathyrisim. We recently described a synergistic vascular toxicity of AA + βAPN. This study was a histochmical survey of aorta with selected biochemical analysis of that model. Male rats given AA(100mg/kg) + βAPN(15g/kg)/day by gavage for ten days were killed on days 1, 3, 5, and 10. Aortas were removed, fast-frozen and sectioned; a battery of histochemical techniques included cytosolic enzymes [lactate dehydrogenase (LDH), glucose-6-phosphate dehydrogenase (G6P-DH), etc.], membrane markers [Mg-ATPase, Ca-ATPase, 5′AMPase], mitochondrial NADH-DH, alizarin red S for Ca**+, and elastic tissue and proteoglycan stains. Early changes were limited to a marked, diffuse medial increase in G6P-DH and NADH-DH beginning at day 1. Striking loss of cytosolic and membranous enzymes was seen in necrotic area of mid-media abruptly at day 10, but non-necrotic areas of aorta and surrounding vessels showed persistently increased NADH-DH and G6P-DH staining. Elastic tissues and proteoglycan were unchanged. Chemical analysis at 10 days confirmed a marked overall increase in microsomal and cytosolic G6P-DH, and showed marked increase in superoxide dismutase, postmitochondrial glutathione peroxidase, and mitochondrial malondialdehyde formation. We take these changes to indicate early oxidative stress of the vascular smooth muscle, followed by abrupt necrosis of the media between day 5 and day 10. (Supported by NIH Grant HL-26189).

PHENOTYPIC MODULATION OF AORTIC SMOOTH MUSCLE CELLS BY ALLYLAMINE: INFLUENCE OF SUBSTRATUM AND GROWTH CONDITIONS IN VITRO. K. Ramos and G. Liu, Depts. of Veterinary Physiology/Pharmacology and Medical Physiology, Texas A&M University, College Station, TX and Dept. of Molecular Biology, American Red Cross, Rockville, MD.

Aortic smooth muscle cells (SMC) modulate from a contractile to a proliferative phenotype upon subchronic exposure to allylamine (AAM). Our goal was to assess the influence of substratum and growth conditions in vitro on the expression of the AAM-induced proliferative phenotype. Incubation of growth-arrested SMC on plastic with 10% serum for 24 hr increased thymidine incorporation by 124.1 ± 21.7% in controls and 223.6 ± 48.8% in AAM cells (n=5, p<0.05). Enhanced growth rate was observed on glass, but not plastic when AAM cells were seeded in the presence of 1 or 10% serum for up to 10 days (n=3). Increased growth rate of AAM cells was not due to enhanced plating efficiency since comparable attachment were observed for up to 180 min. Reseeding of control cells on AAM-coated substrates afforded these cells a growth advantage comparable to that observed in uncoated AAM cultures. Thus, selective growth advantage of AAM cells on glass may be related to differences in extracellular matrix deposition. (Supported by NIH grant ES 04849).
COMPARATIVE CARDIOTOXICITY OF ALLYLANE (AA) AND DOXORUBICIN (DOX) IN MYOCARDIAL SLICES. AR Parrish, NG Shipp, RT Dorr, AL Gandolfi and K Brendel. Department of Pharmacology, Univ. of Arizona, Tucson, AZ.

An in vitro myocardial slice system was used to study the comparative toxicities of DOX and AA, both potent cardiotoxins. Both agents were toxic in a concentration (10^4,10^5 10^6 M) and time- (6,12,24 h) dependent fashion. Viability parameters used to assess toxic insult included protein synthesis, LDH content and release, ATP content and GSH content. The relative toxicities of each compound were similar at each concentration and timepoint. Agents were also studied to block the toxicity of each compound. Semicarbazide, a monoamine oxidase inhibitor, was successful (>75%) at alleviating the damage induced by allylamine. ICRF-187, an iron chelator, alleviated doxorubicin toxicity (>65%) for certain viability parameters (LDH loss, ATP content) but not others (protein synthesis). This work shows the myocardial slice system to be effective for both comparative and mechanistic (toxicity blocking) work in cardiotoxicity. (NIH GM 38290)

OXIDATIVE STRESS & LIPID PEROXIDATION IN ACUTE ALLYLANE-INDUCED CARDIOVASCULAR TOXICITY. S Awasthi and P J Bloor. Univ. of Texas Medical Branch, Galveston, TX.

Allylamine is an aliphatic amine utilized in synthesis of pharmaceuticals: its derivatives are used as veterinary analgesics and therapeutic agents for treatment of fungal infections. Allylamine causes aortic and myocardial lesions and is metabolized to acrolein in vivo and in vitro. Acrolein may cause allylamine's toxic effects, since it acts as a strong peroxidizing agent. To investigate possible involvement of free radicals in allylamine toxicity, we conducted a time course study following administration of allylamine (150 mg/kg) to rats by gavage. Animals were sacrificed at 1, 3 & 5 hrs after allylamine treatment, and subcellular fractions of aorta, epicardium and endocardium were assayed for enzymes of defense system, lipid peroxidation, thiol status and OH radical generation. A striking increase in malonaldehyde formation was found in aortic microsoma, with marked depletion of protein bound, total and free -SH content. A significant increase in O2 generation was found in aorta, epicardium and endocardium, but other changes were not so marked in epicardium and endocardium. Levels of glutathione peroxidase and catalase were not significantly altered. In an in vitro experiment, liposomes incubated with acrolein (0.5 - 2 mM) showed a proportional increase in lipid peroxidation in liposomal membrane. Results suggest that in vivo treatment with allylamine causes damage to aortic microsoma, and the mechanism can possibly be explained on the basis of acrolein-induced lipid peroxidation, membrane dysfunction, and oxidative stress. (Supported by NIH Grant #HL-26189).

CELLULAR AND MOLECULAR BASIS OF ALLYLANE-INDUCED AORTIC SMOOTH MUSCLE CELL (SMC) PROLIFERATION. RC Bowes, CHart and K Ramos. Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.

Repeated cycles of aortic injury upon exposure to allylamine (AAM), a selective vascular toxin, modulates aortic (SMC) from a contractile to a proliferative phenotype. The present studies were conducted to evaluate the cellular/molecular basis of this response. Sprague-Dawley rats (175-200 g) were gavaged daily with AAM (70 mg/kg) or tap water for 20 days. Post-confluent subcultures of SMC from control and AAM-treated animals were processed for analysis of incorporating into phosphatidic acid (PA) and c-Ha-ras mRNA expression. Decreased incorporation into PA was observed in secondary cultures of SMC from AAM-treated animals (n=4). This alteration correlated with a 20-fold reduction in c-Ha-ras transcript levels (n=3). The mitogenic responsiveness of AAM cells relative to controls was markedly enhanced. However, growth arrest inhibited 3H-thymidine incorporation to a greater extent in AAM cells (n=5). Because the ras oncogene may modulate SMC proliferation, these results suggest that alterations in PA metabolism by ras contribute to the deregulation of SMC proliferation induced by AAM. (Supported by NIH grant ES 04849).

BLOOD PRESSURE EFFECTS IN SPRAGUE-DAWLEY RATS WITH SUBCHRONIC ORAL ADMINISTRATION OF FPL 63547XX, AN ANGIOTENSIN-CONVERTING ENZYME INHIBITOR. A J Jacobs, P Habeek and B Clark. Department of Drug Safety Evaluation, Fisons Pharmaceuticals, Rochester, NY.

FPL 63547XX is the ester prodrg of a thidiazoline angiotensin-converting enzyme (ACE) inhibitor. As a class, ACE inhibitors have been demonstrated to be effective for the treatment of hypertension and congestive heart failure primarily through inhibition of the renin-angiotensin-aldosterone system. This investigation has examined the effects of subchronic daily oral administration of FPL 63547XX on systolic blood pressure (SBP) in normotensive Sprague-Dawley (S-D) rats at dose levels used in a ninety-day toxicity study (0, 150, 300 and 1000 mg/kg/day). SBP was determined weekly during the treatment (126 days) and recovery (77 days) phases of the study using an indirect tail-cuff method. SBP was decreased similarly in all three treatment groups within three days of initiation of dosing. An approximately 30 percent reduction in SBP was achieved by day 9. The reduction in SBP was maintained throughout the second day following discontinuation of dosing. Control levels were reached within three weeks. Creatinine clearance was reduced at the end of the treatment phase for males administered FPL 63547XX but returned to control levels at the end of the recovery phase. Subchronic administration of FPL 63547XX to normotensive S-D rats had a profound and sustained reduction in SBP and creatinine clearance were demonstrated to be reversible upon discontinuation of dosing.

Episomatic hemorrhax is a relatively rare fatal condition which affects predominantly male mice. Characteristic features are hemorrhagic diathesis with frequent exsanguination into the chest cavity, hemorrhagic cardiac myopathy, and prothrombin complex deficiencies. The etiology has not been previously reported. New interest has prompted us to report findings from an outbreak of episomatic hemorrhax which smoldered in the National Institutes of Health mouse colonies from 1971 through 1974. During that time, mortality due to hemorrhax ranged from 0-26% per month in male breeders. Microscopic heart lesions included edema, multifocal hemorrhage, necrosis, acute inflammation, and fibrosis. The disease occurred only in hysteresis-derived mice. The outbreak was abruptly halted by increasing sodium bisulfite in the feed to 20 ppm. The complete syndrome including hemorrhagic cardiac myopathy was readily reproduced in germ-free male mice given a vitamin K-free diet, and in conventional male and female mice given sodium warfarin in the drinking water. We concluded that the cause of episomatic hemorrhax and hemorrhagic cardiac myopathy was vitamin K deficiency.


Paltokin (PTX) may create a cation-permeable pore by interacting with the sarcolemmal Na'-K' ATPase, the site of action of cardiotonic steroids. We compared the cardiotonic steroids cyarin, ouabain, digoxin, and the aglycone, strophanthidin, in antagonizing the depolarizing action of PTX. The resting membrane potentials of excised frog sartorius muscles were measured in vitro by standard electrophysiological techniques. Cardiotonic steroids were tested in the presence of 10'M PTX in Tyrode and 0.1% bovine serum albumin. One hour after the addition of PTX to the medium, PTX alone produced depolarization from -81 mV ± 6; pretoxin, to -27 mV ± 13. In the presence of 10'M cardiotonic steroids, PTX produced depolarizations to -72 mV ± 6 with cyarin, -67 mV ± 7 with ouabain, -55 mV ± 5 with digoxin, and to -11 mV ± 8 with strophanthidin. These results suggest that the presence and number of sugar moieties on the steroid nucleus can influence their antagonistic effect on the depolarizing action of PTX in frog sartorius muscle.

EFFECT OF PALTOKIN ON REGIONAL HEMODYNAMICS IN THE RAT. G W Parker, and T J Taylor. U. S. Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD. Sponsor: D A Crenshaw

Paltokin (PTX) causes long-lasting vascular smooth muscle contraction in vitro. Little is known, however, about the duration of this effect in vivo. We investigated the duration of the hemodynamic effects of a sub-lethal dose of PTX (0.01 µg/Kg; i.v. bolus) in urethane-anesthetized F344 rats (n=6) instrumented for the measurement of arterial blood pressure and regional blood flow (pulsed doppler) of the distal aorta (hindquarter) and renal artery. PTX caused an immediate increase in mean arterial blood pressure (MAP), reaching a maximum of 113 ± 11% (p<0.01) above baseline (expressed as % change). This was accompanied by an immediate decrease in renal flow (RF, -67 ± 5%) and hindquarter flow (HQR, -36 ± 5%), and an increase in renal vascular resistance (RVR, 652 ± 144%) and hindquarter vascular resistance (HQR, 197 ± 43%) (p<0.05). RVR was initially, but only transiently, greater than HQR (p<0.05). MAP (40 ± 4%; p<0.01) remained elevated 1 hour after toxin, as were RVR and HQR (85 ± 3% and 236 ± 84%, respectively; p<0.05). HQR (-46 ± 11%; p<0.05), but not RF (-18 ± 9%); HQR, also remained decreased 1 hour after toxin. Thus, PTX induced a long-lasting hypertensive response that was due largely to increased regional vascular resistances.

THE EFFECTS OF U-80,816B ON THE ARTERIAL PRESSURES (AP) AND ELECTROCARDIOGRAMS (ECG) OF CONSCIOUS DOGS. EKJ Leong, DA Rapp, CP Sabaati, and KT Kirton. Safety Pharmacology Research. The Upjohn Company, Kalamazoo, MI.

U-80,816B, [1-(4-(5-methyl-1H-imidazol-1-yl)-2-butylnyl]-pyridilidone hydrobromide], is a partial cholinergic agonist which may be useful for treatment of cognitive disorders associated with senile dementia and Alzheimer's Disease. For drug safety evaluation, beagle dogs were infused intravenously (3 or 10 µg/kg/min), or given a bolus oral dose (600 or 1200 µg/kg). At 10 µg/kg/min., 3 of 3 dogs developed cholinergic symptoms in 10 min., prominent heart rate (HR) and PR interval (PR) changes in 54 ± 11 min., but without significant AP changes. However, at 72 ± 14 min. having received an average cumulative dose of 720 µg/kg, A-V blocks occurred. Stopping the infusion or giving an IV injection of 0.1 mg/kg of atropine sulfate alleviated the blocks. At 3 µg/kg/min., 3 of 3 dogs developed cholinergic symptoms in 81 ± 35 min., without causing significant changes in APs and ECGs, and A-V blocks. Two dogs ingested a single dose of 1200 µg/kg developed cholinergic symptoms within 25 min. A-V blocks occurred in 1 of 2 dogs in approximately 70 min. At 600 µg/kg, only cholinergic symptoms were observed. The results indicated that the IV to PO dose ratio for A-V blocks was 1 to 1.66 and the trends of HR and PR changes are useful indicators for clinical monitoring in human trials.
PARAMETERS OF EMETINE CARDIOTOXICITY IN THE ISOLATED, PERFUSED RAT HEART. S J Pan and A B Combs. Division of Pharmacology and Toxicology, College of Pharmacy, University of Texas, Austin, TX.

The alkaloid emetine is still very useful as the emetic in ipecac syrup and it is effective to treat for certain anemic infections. At normal doses, the acute toxicity hazard of emetine is minimal. However, cardiac toxicity can occur with chronic use, or with abuse as may occur with bulemics. The purpose of these studies is to use an in vitro perfused heart technique to study the mechanisms of emetine cardiotoxicity. Baseline dose- and time-response data have been collected for comparison with later mechanistic studies. Male Sprague Dawley rats (400-600 g) were anesthetized with pentobarbital. After heparin, the hearts were removed and perfused on a Langendorff apparatus with 80 cm H2O afterload. In the drug-treated hearts, emetine was added at concentrations of 0.75, 0.37, 0.23, and 0.14 μM. Parameters measured included time until the ventricle stopped beating, coronary flow, and LDH activity in the coronary effluent. In other studies, propranolol was used as a pretreatment before 0.37 μM emetine. The time for ventricular arrest was inversely proportional to the dose. Consistently, the area would keep beating during ventricular arrest. Measurement of the time to arrest was not possible with the lower doses because of arrhythmias. Emetine was discontinued upon arrest and the hearts would usually start beating regularly within 5 to 15 minutes. The 0.37 μM concentration had a greater effect on reducing coronary flow than did the 0.75 μM concentration. On the other hand, the higher concentration had much the greater effect on LDH release. Pretreatment with a concentration of propranolol (1.5 μM) that minimally decreased the heart rate was ineffective in preventing ventricular arrest or in preventing increases in LDH release. Dr. Combs is the Bergen Brunswig Centennial Fellow in Pharmacy.


Previous work has established that α-ketoglutaric acid (α-KG) protects against cyanide-induced toxic effects in the central nervous system. In this experiment a-KG was used to protect against NaCN-induced blood chemistry changes. α-Ketoglutaric acid in low (500 mg/kg) and high (5.0 gm/kg) doses was administered with or without NaCN (2.30 mg/kg). Blood samples (N = 5) were drawn at 0, 5, 15, 30, and 60 min for analyses. Blood samples were analyzed for changes in pH, Pco2, Po2, excess base, K+, and lactate values. Blood pH was decreased significantly (P < 0.05) 5 min after treatment with cyanide alone. Transient decrease in blood pH was observed in α-KG + NaCN treatment. NaCN treatment alone caused a transient decrease in Pco2. No significant changes in Pco2 levels were observed in other treatments. After NaCN treatment base excess was greatly decreased after 5 min and still decreased at 60 min. The decrease in base excess was moderate and limitless and returned to normal at 15 min after treatment with α-KG + NaCN treatment. Base excess remained within the normal limit in other treatments. A significant increase in K+ level was observed only at the five minute point time treatment with NaCN alone. Blood lactate levels increased in the 5 & 15 min samples in the NaCN only group. It appears that α-KG inhibits NaCN-induced drop in blood pH, lactate production, and K+ level.

BENZO[a]PYRENE-INDUCED ALTERATIONS IN H-THYMIDINE INCORPORATION IN CULTURED AORTIC SMOOTH MUSCLE CELLS. S D Nandan, S K Crum and K Ramos. Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.

Polycyclic aromatic hydrocarbons may contribute to the pathogenesis of human atherosclerosis. Since alterations in smooth muscle cell (SMC) proliferation are thought to mediate the atherogenic process, the present studies were conducted to evaluate the effects of BaP on DNA synthesis in cultured rat aortic SMC. 'H-thymidine incorporation was measured in cycling cultures incubated with BaP (0.3 - 30 μM) for 3, 12 or 24 hr. Low concentrations of BaP (0.3 and 3 μM) caused a time-dependent increase in 'H-thymidine incorporation. In contrast, 30 μM BaP decreased the incorporation of thymidine at all times tested (n = 3). This reduction appeared to be both time- and dose-dependent. In vivo toxicity studies showed that cultured aortic SMC isolated from Sprague DAWley rats treated with BaP (10 mg/kg) for 8 weeks incorporated more thymidine than cells from control animals (n = 5). Collectively, our results show that BaP modulates aortic SMC proliferation and raise the possibility that such alterations contribute to BaP-induced atherogenesis. (Supported by NIH grant ES 04849).

THE CARDIOTOXIC EFFECTS OF ANABOLIC-ANDROGENIC STEROIDS ON PRIMARY NEONATAL CELL CULTURES. R B Melchert, T J Herron, S K Davis, and A A Welder. University of Oklahoma, College of Pharmacy, Oklahoma City, OK.

In the last few years, anabolic-androgenic steroids have been classified as schedule IV drugs of abuse in some states. Currently, they are known to be abused by professional and college athletes as well as adolescents to improve exercise performance. Although many of the adverse effects of these steroids are well-defined, the cellular mechanisms of cardiotoxicity are not known. In this study, the concentration- and time-dependent effects of testosterone cypionate (TC), stanozolol (S), and fluoxymesterone (F) were described. Evaluation of drug effects were made in 4-day old primary myoccardial cell cultures exposed to 1 x 10^-4 M, 1 x 10^-3 M and 1 x 10^-9 M concentrations of TC, S, and F each for 1, 4, and 24 hr. Cellular injury was evaluated by induction of morphological alterations, neutral red retention, tetrazolium (MTT) formazan production, and lactate dehydrogenase (LDH) release in primary myocardial cell cultures obtained from 3-5 day old Sprague-Dawley rats. Although morphological integrity was maintained for 1 hr, at 24 hr some morphological alterations including vacuoles, granules, or pseudopodia were seen in each concentration of all three steroids. In the evaluation of neutral red retention only 1 x 10^-4 M TC showed a significant decrease in ability to retain the dye compared to untreated controls at 24 hr. Also at 24 hr, the MTT assays revealed similar results in that only the 1 x 10^-4 M TC treatment group showed a significant decrease in the ability to convert MTT to MTT formazan when compared to untreated controls. Unlike the myoccardial cell cultures treated with the three concentrations of both S and F, significant LDH release was seen with those cultures treated with 1 x 10^-4 M TC as compared to the untreated controls at 4 and 24 hr. Furthermore, of the three anabolic-androgenic steroids tested (TC, S, and F), TC was the most cardiotoxic steroid in our primary myocardial cell model. From these results, a concentration- and time-dependent cardiotoxic profile was established for each anabolic-androgenic steroid. (This work was supported by NIDA FIRST award DA05699).
Cocaine was once thought to be a relatively safe and nonaddicting elixir. However, now it is known that cocaine use causes dependence, withdrawal, and life-threatening cardiac events. A complicating factor in evaluating cocaine cardiototoxicity is that other drugs may be used concomitantly with cocaine. Methamphetamine (METH) is commonly found in the blood and urine upon autopsy in individuals with cocaine-associated cardiac deaths. In this study, primary myocardial cell cultures obtained from 3-5-day-old Sprague-Dawley rats were used to evaluate the concentration and time-dependent additive adverse effects of METH and cocaine on the heart. After the cells had been maintained in culture for 4 days, they were exposed to $1 \times 10^{-5}$ and $1 \times 10^{-3}$ M cocaine alone; $1 \times 10^{-5}$ and $1 \times 10^{-3}$ M METH alone; and the combinations of $1 \times 10^{-3}$ M cocaine + $1 \times 10^{-5}$ M METH and $1 \times 10^{-5}$ M cocaine + $1 \times 10^{-5}$ M METH. Lactate dehydrogenase release (LDH), morphology, and beating activity were evaluated after exposure to the drugs for 1, 4, and 24 hr. With all treatment groups for the first 4 hr, LDH release was not significantly different from untreated controls. Significant LDH release ($p < 0.001$) was exhibited at 24 hr with $1 \times 10^{-3}$ M cocaine alone, $1 \times 10^{-3}$ M METH alone, and $1 \times 10^{-3}$ M cocaine + $1 \times 10^{-5}$ M METH. For 24 hr of treatment, cellular injury (necrosis, vacuolization, and granulation) induced by $1 \times 10^{-3}$ M and $1 \times 10^{-3}$ M cocaine alone was extensive and minimal, respectively. When $1 \times 10^{-3}$ M METH was added to $1 \times 10^{-5}$ M cocaine, the injury was severe. No measurable beating activity was observed at 1, 4, and 24 hr exposure to $1 \times 10^{-3}$ M cocaine alone and $1 \times 10^{-3}$ M cocaine + $1 \times 10^{-5}$ M METH. At 1 hr, beating activity after treatment with $1 \times 10^{-5}$ M cocaine alone was not significantly different from untreated controls. Addition of METH (1 x $10^{-5}$ M) potentiated cocaine-induced ($1 \times 10^{-5}$ M) depression of contractile activity at 4 and 24 hr. Therefore, METH may interact with cocaine at the cellular level to directly potentiate myocaridal injury. (Supported by NIDA FIRST Award #DA05699).

Previous studies in this laboratory have shown that benz[a]pyrene (BaP)-induced aortic lesions in Japanese quail correlate temporally with alterations in the profile of endogenous aortic protein phosphorylation. The present studies were conducted to evaluate the phosphorylation of exogenous histone by calcium/phospholipid-dependent (PKC) protein kinase in the particulate and cytosolic fractions of aortic homogenates upon exposure to BaP. Male Japanese quail (160-210 g) were exposed to BaP (10 mg/kg) or MCT oil for 8 weeks. Exogenous histone phosphorylation by PKC in the particulate fraction of aortic homogenates from control animals was 34 ± 4.2 pmol/mg/min, while that of homogenates from BaP-treated animals was reduced to 12 ± 2.8 pmol/mg/min ($n = 3$). In contrast, a marked increase in PKC activity was observed in the cytosolic fraction of aortic homogenates from BaP-treated animals. These changes were consistent with the pattern of endogenous aortic protein phosphorylation induced by BaP. Collectively, our results suggest that alterations in signal transduction may be involved in the induction of vascular lesions by BaP. (Supported by NIH grant ES 04849).

MODULATION OF PROTEIN PHOSPHORYLATION IN RAT AORTIC SMOOTH MUSCLE CELLS BY 2,3,7,8-TEXACHLORODIBENZO-P-DIOXIN(TCDD) IN VIVO. T J Weber, X Ou, C Hart, T R Narasimhan, S H Safe and K Ramos, Departments of Veterinary Physiology/Pharmacology and Medical Physiology, Texas A&M University, College Station, TX.

TCDD may enhance the progression of experimentally-induced atherosclerotic lesions. Because the induction of aortic lesions by other polycyclic aromatic hydrocarbons is associated with alterations in the phosphorylation of aortic proteins, the present studies were conducted to evaluate the pattern of in vitro protein phosphorylation mediated by calcium-phospholipid-dependent (PKC) and cAMP-dependent (PKA) protein kinases upon subchronic exposure to TCDD. Female rats (200-230 g) were injected weekly with 10 µg/kg TCDD or corn oil for 4 weeks. Aortic smooth muscle cells (SMC) were isolated from control and TCDD-treated animals and established in secondary culture. A significant increase in PKC-mediated phosphorylation of endogenous histone was observed in the cytosolic fraction of SMC from TCDD-treated animals. In contrast, TCDD treatment caused a 40% reduction in PKA-mediated phosphorylation in both the cytosolic and particulate fractions. These changes correlated with changes in the phosphorylation of endogenous proteins. Our results show that TCDD modulates the phosphorylation of aortic proteins.

DEVELOPMENTAL TOXICITY STUDY OF DIETHYLENE GLYCOL (DEG) IN CD-1 MICE. H K Bates, CL Price, MC Marr, CB Myers, JJ Heindel, and BA Schwartz. Research Triangle Institute and the *National Toxicology Program/NIHES, RTP, NC.

Craniofacial malformations were observed in the offspring of CD-1 mouse breeding pairs exposed to DEG in drinking water for approximately 4 months (Williams et al., Fundam. Appl. Toxicol., 14, 622-626, 1990). In this study, DEG was administered by gavage to timed-pregnant Swiss (CD-1) mice (25-31/group) on gestational days (GD) 6-15 at dose levels of 0, 1250, 5000, or 10,000 mg/kg/day. Maternal clinical signs, water and food consumption, and body weights were recorded at regular intervals throughout gestation. There was no significant effect on maternal weight gain. Relative food consumption was decreased in the 10,000 mg/kg/day dose group from GD 6-12. Relative (g/kg/day) water intake was significantly increased during and post-treatment in the 5000 and 10,000 mg/kg/day dose groups. Renal lesions were noted in 3/28 animals at 10,000 mg/kg/day, one of which was sacrificed moribund. Mean maternal kidney weight was increased in the 5000 and 10,000 mg/kg/day groups. At laparotomy on GD 17, implant status, fetal weight, sex, and morphological development were evaluated. Exposure had no effect on pre- or post-implantation loss. The only significant developmental effect was a decrease in fetal body weight in the 10,000 mg/kg/day group. Examination of the fetuses for external, visceral and skeletal malformations did not reveal any significant effects. DEG caused minimal developmental toxicity at the maternally toxic dose of 10,000 mg/kg/day. Developmental toxicity did not occur in the 5000 mg/kg/day group although maternal toxicity was evident. [NTP/NIHES Contract No. NO1-ES-82555]
EG-induced developmental toxicity was previously reported in rats and mice (Price et al., Toxicol. Appl. Pharmacol. 81, 113-127, 1985). In this study, artificially inseminated rabbits were dosed with EG on gestational days (gd) 6-19 at 0, 100, 500, 1000 or 2000 mg/kg/day (23-24 does/group) at 5 ml/kg. Maternal clinical signs, water consumption and body weights were recorded throughout gestation. At necropsy (gd 30), maternal liver, kidney and uterine weights were taken. Kidneys were examined histologically. Corpora lutea and implants were recorded; all live fetuses were examined. At 2000 mg/kg/day, 42% of does died, three delivered early and one aborted; renal lesions in the cortical tubules included intraluminal oxalate crystals, epithelial necrosis and tubular dilatation and degeneration. There were no other signs of maternal toxicity at this dose, and none at lower doses. There was no evidence of developmental toxicity at any dose tested, including no effects on pre- or postimplantation loss, fetal body weight, or on fetal malformations or variations. The NOAEL was therefore 1000 mg/kg/day for maternal toxicity and >2000 mg/kg/day for developmental toxicity in this study. Supported by NTP/NIAMS Contract NOI-ES-95255.

Effect of dibutyl phthalate on the F344 rat with and without in utero exposure. J Killinger, R Melnick, A Basaran, J Hite, M Ryan, A Sawhney, and P Kurtz. Battelle, Columbus, OH; NIEHS, Research Triangle Park, NC.

Dibutyl phthalate (DBP) was administered in diet at 0, 2500, 5000, 10,000, 20,000, and 40,000 ppm to rats for 13 weeks starting at 6 weeks of age with no prior DBP exposure or after exposure to 0 or 10,000 ppm of DBP in utero to 8 weeks of age. Vaginal smears prior to necropsy and epididymal sperm motility and concentration and testicular spermatic concentration at necropsy were evaluated. Histopathology was done on all collected tissues. Blood was evaluated for hematology and clinical chemistry in all animals and for testosterone and zinc in males. Testicular zinc and testosterone and liver peroxisomal palmitoyl CoA oxidase activity were also measured. In general animals given 10,000-40,000 ppm DBP without in utero exposure were affected more severely than animals with in utero exposure. Dose-related effects included reductions in body weight and food consumption; mild regenerative anemia; hyperlipidemia; changes in liver function including increases in palmitoyl CoA oxidase activity which correlated with liver weight increases and cytochemical alterations at all dose levels; reduced serum testosterone and testicular zinc; increased serum zinc; and adverse effects on epididymal sperm and testicular germinal epithelium at 210,000 ppm. (Sponsored by NTP Contract No. NOI-ES-75184).
A microprocessor-based system for calculation and delivery of cutaneous doses as a part of developmental toxicity studies has been developed. The system utilizes a dual syringe diluter/dispenser (Hamilton MicroLab® Model 941) attached to an IBM PS/2 Model 50 personal computer (PC). Dosages, based on individual animal body weights, are calculated on a host computer using the body weights obtained during gestational dosing as scheduled in the study protocol. The individual doses are downloaded to the PC from the file created on the host computer. The PC program uses the calculated dose, specified in microliters, to control the diluter/dispenser for precise application of accurate volumes. In addition, the PC selects the syringe appropriate for dosing control or treated animals. Thus, the dual syringe capability prevents cross contamination of control and test substances. The system can be operated as a “closed system” to reduce the potential exposure of the technician to the test substance. Safety is enhanced by use of a foot pedal for dispensing the test substance which facilitates animal handling and further reduces the potential for chemical exposure. In conclusion, this system provides greatly enhanced accuracy, consistency and safety of application of cutaneous doses over manual methods, and furthermore, can effectively be used for gavage or intraperitoneal dosing.


The developmental toxicity of pentostatin was determined in CD rats and New Zealand White rabbits. Rats were given single iv doses of 0, 0.01, 0.10, or 0.75 mg/kg pentostatin on gestation days (GD) 6-15, and rabbits were given 0, 0.005, 0.01, or 0.02 mg/kg on GD 6-18. Rats had decreased body weight gain and food consumption during treatment at 0.10 and 0.75 mg/kg, with increased postimplantation loss at 0.75 mg/kg. Decreases in fetal body weight and numbers of ossified cervical centra were observed at 0.75 mg/kg. The incidence of skeletal malformations was significantly increased at 0.75 mg/kg. In rabbits, greater body weight loss than vehicle controls and decreased food consumption occurred at 0.02 mg/kg during the treatment period. The incidence of death, abortion, and early delivery was also increased at 0.02 mg/kg. No effects of pentostatin were observed on fetal body weight or survival, or visceral or skeletal malformations or variations. Thus, pentostatin was teratogenic in rats but not in rabbits, and caused fetal toxicity in both species at maternally toxic doses.


The developmental toxicity, including the teratogenic potential of an antimicrobial organosilicon quaternary ammonium chloride (Dow Corning® 5700 Hydrolysate) was evaluated in rats. Groups of 25 pregnant CD® rats were administered either 100, 300 or 1,000 mg/kg/day of test material orally by gavage as a single daily dose on days 6 through 15 of gestation at a volume of 10 ml/kg. The control group received the vehicle only. Cesarean examinations were performed on all females on gestation day 20, followed by teratologic evaluation. Antemortem observations of the treated dams were not considered a result of treatment. A slight but statistically significant increase in liver weights was observed at the 1,000 mg/kg/day dosage level. Patterns in body weight changes and food consumption were not indicative of maternal toxicity. At cesarean section, the male to female ratio at the 1,000 mg/kg/day dosage group was significantly different from the control group. However, this was the only difference noted among the treated animals and was not considered biologically significant. Based on these findings, the no observable effect level of DC 5700 Hydrolysate, when administered to gravid rats, was considered to be 300 mg/kg/day for maternal and 1,000 mg/kg/day for developmental toxicity.


To determine the potential developmental toxicity of hydroquinone (HQ), pregnant rats were given 0, 30, 100, or 300 mg/kg HQ by gavage on the 6th through the 15th days of gestation. Maternal effects included slight, but significant (p<0.05), reductions in body weight gain and feed consumption for the dams given 300 mg/kg HQ during the treatment period. Reproductive indices, i.e., pregnancy rate, number of corpora lutea, implantation sites, viable fetuses, early and late resorptions, fetal sex ratio, pre- and post-implantation losses, and gravid uterine weights were not affected by treatment with HQ. A slightly reduced (p<0.05) mean fetal body weight seen at the 300 mg/kg dose level correlated with a slight reduction in maternal body weight gain. The occurrences of external, internal soft tissue and skeletal malformations or variations were not statistically different between the control and the HQ-treated groups. HQ was not selectively toxic for the developing conceptus and should not be considered a developmental toxicant in rats. The NOEL for both maternal and developmental toxicity was 100 mg/kg, while 300 mg/kg was a NOAEL. Supported by the Chemical Manuf. Assoc. HQ Panel.

To determine the potential developmental toxicity of HQ, pregnant New Zealand White rabbits (18 mated per dose group) were administered in aqueous solution 0, 25, 75, and 150 mg HQ/kg/day by gavage on gestation days (gd) 6 to 18. Cесarean sections were performed on gd 30. No adverse effects of treatment were evident from physical observations, liver or kidney weights, premature delivery incidence, or cesarean sectioning data. The NOEL for maternal toxicity was 25 mg/kg/day; doses greater than this adversely affected food consumption and/or body weights of dams during the treatment period. In the 150 mg/kg/day dose group, total incidences of external, visceral, and skeletal findings for fetuses did not differ statistically from controls. Slight increases were found, however, in the incidences of ocular and minor skeletal malformations (microphthalmia, vertebral/rh defects, angulated hyoid arch) on both a per fetus and a per litter basis. Under the conditions of this study, HQ at 150 mg/kg/day, produced negligible developmental alterations in the presence of maternal toxicity. The NOEL for developmental toxicity was 75 mg/kg/day.

(Work supported by the Chemical Manufacturers Assoc. HQ Panel)


Studies in other laboratories have demonstrated that high doses of recombinant human interferon-α (rhIFN-α) are associated with increased abortion rate in monkeys. Accordingly, the potential developmental toxicity and abortifacient activity of another recombinant human interferon, rhIFN-γ, were investigated. Female cynomolgus monkeys received daily subcutaneous injections of 0, 3, 30, or 150 μg rhIFN-γ/kg weight from day 20 to 80 of gestation, inclusive. No evidence of maternal toxicity, embryotoxicity, fetotoxicity, or teratogenicity was observed in the low-dose or mid-dose groups. No treatment-related maternal toxicity was seen in females that received 150 μg/kg, but an increased abortion rate (7/10) was observed. This abortifacient effect was apparently not related to the presence of antibodies to rhIFN-γ, which were detected in animals from all three treatment groups. The limited number of fetuses in the high-dose group did not permit a complete assessment of the teratogenic potential of the test compound, but no evidence of teratogenicity was observed in the fetuses available for examination. Since the abortifacient effect was observed only in high-dose females at doses far exceeding the maximum human clinical dose, this finding does not preclude safe use in humans; however, the qualitatively similar findings with rhIFN-γ and rhIFN-α suggest that they may have common mechanisms of action following high-dose administration.

MATERNAL AND NEONATAL SAFETY OF SYNTHETIC HUMAN RELAXIN ADMINISTERED TO NEAR-TERM PREGNANT RHESUS MONKEYS. PK Working, MS Golub* and JD Green. Dept. of Safety Evaluation, Genentech, Inc., South San Francisco, CA and "California Primate Research Center, Davis, CA.

Synthetic human relaxin (hFLx-2) was administered to near-term pregnant rhesus monkeys (N = 5-6 per group) at doses of 0, 0.1, or 2.0 mg hFLx-2/kg to assess possible adverse effects on dam and neonate. Daily infusions began as soon as cervical softening was detected in the dams and continued until 12 hours prior to delivery. Maternal cardiovascular parameters, blood pressure and fetal heart rate were measured during the dosing period, and maternal health and neonatal growth and development, including a multi-item neurobehavioral test battery, were assessed for two weeks postnatally. There was no evidence of a treatment-related hypotensive response in the dams, nor were any adverse effects detected in any other maternal, fetal or infant endpoint reflecting growth, health, activity or maturation, including fetal heart rate, clinical pathology parameters, uterine involution, and infant growth and behavioral indices in the two weeks following birth. Subsequently, the reproducitve fitness of the adult females was monitored for one year after treatment. There were no group differences in resumption of menses, conception rate, length of gestation, maternal weight gain or pregnancy outcome, or the health of the mothers and newly delivered infants postnatally. In summary, no treatment-related adverse effects were seen in general health and subsequent reproductive fitness in adult female rhesus monkeys exposed intravenously or in growth and development of infants exposed in utero to hFLx-2.

DEVELOPMENTAL TOXICITY OF METHACRYLAMIDE (MAC) IN MICE. JD. George, CJ Price, MC Marr, CB Myers, JJ Heindele, and BA Schenck. Research Triangle Institute, and National Toxicology Program/NIH, Research Triangle Park, NC.

MAC, a monomer used in the manufacture of acrylic resins, was tested for developmental toxicity in Swiss mice (n=27-30/group). MAC (0, 60, 120 or 180 mg/kg/day, po) was administered on gestational days (gd) 6-17. On gd 17, fetuses were weighed and examined for malformations (external, visceral, and skeletal). Postimplantation loss/litter was slightly but significantly increased (17% vs 5.5% for control) at 180 mg/kg/day. Fetal weight was decreased (85-93% of controls) at ≥120 mg/kg/day. There was no effect of treatment on malformation incidence. Dams (180 mg/kg/day) exhibited decreased weight gain during treatment, decreased body weight at sacrifice on gd 17, and increased relative liver weight (≥120 mg/kg/day). Maternal food and water consumption were not significantly affected, and no clinical signs of neurotoxicity were observed. In summary, 60 mg/kg/day MAC was a no observed adverse effect level (NOEL) for both maternal and developmental toxicity. Mild maternal toxicity and clear evidence of developmental toxicity were observed at doses ≥120 mg/kg/day MAC. Supported by NTP/NIH Contract No. N01-ES-95255.
Methanol has been documented as a teratogen in studies of chronic inhalation exposure. 3000 ppm, during the period of organogenesis (days 6-15, NEDO, 1986) or 20000 ppm throughout gestation (Nelson et al. 1985). Produced skeletal and visceral abnormalities in rats. Infurna & Weiss (1986) exposed pregnant rats during days 15 to 17 days or 17 to 19 of gestation by offering a 2% solution as drinking fluid overnight. Behavioral abnormalities were detected in the pups. However, data about acute exposures to methanol are lacking. Our study was directed specifically to the period of organogenesis. Day 10 was chosen as the day of exposure (day of the vaginal plug = day 0 and animals were sacrificed on day 20). Doses of 1.3, 2.6, 5.2 ml/kg body weight were given to 3 groups of animals with an additional group serving as controls. The data showed a significantly higher proportion of affected litters following 1.3, 2.6 ml/kg than controls. No evidence of maternal toxicity was observed. Hemorrhage was the most consistent anomaly in the pups. In accord with segment II study guidelines, 5.2 ml/kg was chosen as the maternal toxic dose to complete the dose-response function.

CS2 is a solvent in the rubber industry and an intermediate used in the viscose process in the manufacture of mercerized rayons, rayon and cellulose. While inhalation is the major route of human exposure, an approximate 60 ppm CS2 NOEL was extrapolated from an oral developmental toxicity study in rabbits and became a standard-setting force in a recently adopted 4 ppm PEL. Therefore, a more appropriate developmental inhalation toxicity study of CS2 vapor was conducted in New Zealand white rabbits. Twenty four doses/group were exposed to either 0, 60, 100, 300, 600 or 1200 ppm CS2 vapor for 6 hrs/days on gestation days (G-day) 6-18. Does received a laparotomy and an examination of their uterine contents on G-day 29. Live fetuses were sexed, weighed and examined externally. All fetuses received visceral, cephalic and skeletal examinations. Mortality and decreased maternal body weights were observed at 1200 ppm. Increases in early resorptions and decreases in viable fetuses and their weights were observed at 600 and 1200 ppm. Increased late resorptions and fetal malformations were seen in the 1200 ppm group only. Therefore, the developmental NOEL in this study and the more appropriate NOEL for setting related CS2 inhalation standards is 300 ppm CS2. (Supported by the Inter-industry Council on CS2)

Methanol (MeOH) and MeOH/gasoline blends are proposed alternative motor vehicle fuels. High doses (≥10,000 ppm) of inhaled MeOH have been shown to be fetotoxic in the rat (Nelson et al., Fund. Appl. Toxicol. 5:727-736, 1985). We exposed pregnant CD-1 mice to 5,000 or 15,000 ppm (study I) and 2,000 or 5,000 (study II) ppm MeOH via inhalation for 7 hr/day on days 6-15 of gestation. Animals had water but not food during exposure. Sham-exposed, unexposed, and food-deprived controls were included. MeOH exposure did not produce maternal toxicity, but all exposure groups gained less weight than fed or food-deprived unexposed controls. Most of the litters of dams exposed to 15,000 ppm were completely resorbed and 38% of fetuses surviving to day 17 had exencephaly. In both studies, exposure to 5,000 ppm resulted in exencephaly in about one-third of the litters and 5-10% of all fetuses. At 2,000 ppm, 1 of 220 fetuses had exencephaly. No exencephaly occurred in controls. Maternal plasma [MeOH] was 2,000 and 8,000 μg/ml at the end of the first period of exposure to 5,000 and 15,000 ppm MeOH, respectively. Mice appear to be more sensitive than rats to MeOH developmental toxicity, possibly due to differences in blood [MeOH].

Concentrations of arsenic (As), copper (Cu), manganese (Mn), selenium (Se), and zinc (Zn) in the livers and kidneys (wet tissue) from 178 aborted fetuses, and 118 age-matched nonaborted fetuses were analyzed by atomic absorption spectroscopy. Aborted fetuses were also examined grossly and microscopically, and for infectious agents. Aborted fetuses had significantly less liver concentrations of Cu (P < 0.01), Mn (P < 0.001), Se (P < 0.05) and Zn (P < 0.01) than nonaborted fetuses. Kidney concentrations of As (P < 0.05), and Se (P < 0.05) of aborted fetuses were significantly less than nonaborted fetuses, but kidney Cu and Zn levels were significantly greater (P < 0.05) for aborted fetuses than in nonaborted fetuses. No significant correlations were evident for tissue trace element concentrations and fetal breed or etiologic agents of abortion. Myocardial degenerative lesions correlated with low liver Mn (P < 0.05), Zn (P < 0.05) and low kidney Se (P < 0.005), as well as for tissue mineralization and high kidney Mn (P < 0.01) Zn (P < 0.005) and As (P < 0.05) concentrations. A positive correlation between degenerative skin lesions and kidney Zn (P < 0.0001) was observed. It was concluded that trace elements may play a role in both infectious and noninfectious bovine abortion. (Support: CDA; Sask Wheat Pool; NSERC OGP0036697)
There has been considerable interest in the role of indirect (i.e., maternally-mediated) effects on the embryo/fetus following exposure to toxicants, as it is likely that prenatal death or abnormal development can result from either direct or indirect effects or a combination of these. Preliminary to experiments examining the relationship of chemical teratogenesis and maternal stress, we exposed pregnant mice to restraint stress by the protocol of Beyer and Chernoff (Teratogen. Carcinogen. Mutagen. 6:419, 1986). Mated female CD-1 strain mice were restrained (R) in a supine position for 12 h on gestation day 9; controls were unrestrained (U) or unrestrained plus 12 h food/water deprived (UD).

All litters were examined on day 18 for gross and skeletal defects. The same defects noted by Beyer and Chernoff (1986), i.e., fused ribs, supernumerary ribs, and exencephaly, were seen in fetuses from R and UD dams at levels of 7, 28, and 6.5% vs. 0.5, 5, and 0%, respectively. Only extra ribs (2%) were seen in U fetuses. However, we also observed median facial clefts in 2 and bent tail in 1 of 169 fetuses from 13 R litters but none in 202 fetuses from 17 U or UD litters. More litters are being examined to determine if these new stress-associated defects can be produced consistently.

The distribution of the 4S polycyclic aromatic hydrocarbon-binding protein (PBP) was shown to localize to specific cell types with xenobiotic metabolizing capabilities. Immunohistochemical studies with polyclonal antisera were carried out in tissues of C57BL/6 and DBA/2 male mice. In the liver, the PBP showed no distributional preference and was detected in hepatocytes located in all regions of the lobule. In the lung, the localization of the PBP was cell-specific. It was found in epithelial lining cells of airways and in Type II alveolar cells. The levels in lung were lower than in liver, as had been found previously by Western blotting and ligand binding studies of tissues. Hypotheses are suggested for the physiological role of the PBP which account for its wide distribution in species, mouse strains, and tissue types but limited cell-type distribution. These include modulation of xenobiotic metabolizing activities not associated with TCDD or the Ah receptor, involvement in PAH immunotoxicity, or activity as a PAH carrier protein.

Recent interest in testing chemicals for developmental neurotoxicity has led to several suggested testing paradigms. To explore whether, the Chernoff-Kaylock assay (C-K) could be used as a first screen for developmental neurotoxicity, the results of a C-K assay workshop (Terat. Carcinogen. & Mutagen. 7, 1987) were compared to data on known developmental neurotoxicants listed in the Catalog of Teratogenic Agents (6th Ed., T. Shephard, John Hopkins Press, 1989). Forty-one chemicals listed in the C-K assay data base were also listed by Shepard. 90% of the chemicals listed as developmental neurotoxicants were positive in the C-K assay. Since the C-K assay is not designed to specifically detect nervous system effects, 95% of the "positive" endpoints were associated with reduced viability in utero, at birth, or postnatally. 40% demonstrated reduced birth weights or decreased postnatal weight gain. 9.8% (4/41) of the developmental neurotoxicants were negative in the C-K assay. The negative results for these chemicals (dexamethasone, griseofulvin, DMSO, EDTA) could be attributed to differing routes of exposure, inadequate dose levels, or species-specific susceptibility. The data suggest that the C-K assay may be useful as a screening test for chemicals which have potential for altering development of the nervous system.
CONJUGATES OF BENZO(A)PYRENE PRODUCED BY THE LIVER ARE MEDIATORS OF TOXICITY. G Y KweI and SE Irwin. Center for Toxicology and Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ. Sponsor: F.C. Kaufman.

The present study is aimed at evaluating the production of water-soluble conjugates of benzo(a)pyrene (BaP) by the liver and their transport and processing by extrahaepatic target tissues. Male Sprague-Dawley rats were injected i.p. with 3H-B(a)P, 1 μmol/kg, and the profile of BaP conjugates in bile, urine and various tissues determined. Over 90% of the radioactivity in tissues and body fluids was accounted for as conjugates of BaP. To evaluate the capacity of BaP conjugates to bind to target tissue macromolecules, radiolabelled conjugates, produced in vitro from isolated hepatocytes or collected in vivo from bile of rats injected with 3H-B(a)P, were separated on TLC plates and used as reagents. Lung slices from New Zealand rabbits and Sprague-Dawley rats were incubated with varying concentrations of sulfate, glucuronide and glutathione conjugates, and binding to protein and DNA determined. Significant binding was observed with all three classes of conjugates; however, highest binding was noted with the glutathione conjugates. Toxicity and mutagenicity of the conjugates were measured in the Ames Salmonella assay and two mammalian mutagenicity systems (Chinese hamster V79 and mouse lymphoma L5178Y cell lines). The toxicity of sulfate and glucuronide conjugates was greatly enhanced by addition of sulfatase or β-glucuronidase to culture media. The data indicate that hydrolysis of the conjugates is necessary for BaP toxicity and suggest a novel mechanism for BaP-induced carcinogenesis. Supported in part by NCI Grant CA-20807 and NIEHS Grant ES-05022.

LIPOXYGENASE CATALYZED METABOLISM OF BENZO(A)- PYRENE AND BENZO(A)PYRENE-7,8-DIOL IN HUMAN SKIN. Rajesh Agarwal and Hassan Muhittar. Department of Dermatology, Case Western Reserve Univ. and Department Veteran Affairs Medical Center, Cleveland, OH.

The ubiquitous environmental pollutant benzo(a)-pyrene (BP) is a known skin carcinogen. It is activated by cytochrome P-450 and epoxide hydrolase to proximate carcinogenic form BP-7,8-diol, which is further metabolized to the ultimate carcinogenic form BP-7,8-diol-9,10-epoxide. In tissues, such as the skin, where P-450-dependent activity is low, alternate pathways may play an important role in the activation of chemical carcinogens and development of cancer. In this study lipoxygenase (LO) catalyzed metabolism of 7,8-BP and 7,8-diol in human skin was examined. Incubation of 7,8-BP with arachidonic acid and epidermal cytosol resulted in the metabolism of BP. HPLC analysis showed that 1,5-, 3,6-, and 6,12-quinones are the major metabolites. Under similar conditions the incubation of 7,8-BP-7,8-diol resulted in the formation of anti- and syn-7,8,9,10-tetrol and other metabolites. These metabolic reactions were inhibited (up to 80%) by nordihydroguaiaretic acid whereas indomethacin had no such effect. Incubation under anaerobic conditions significantly decreased the metabolism. The metabolites thus formed were shown to act efficiently toward epidermal protein. 7-Linoleic acid and 15-hydroperoxyec- osatetraenoic acid but not oleic acid also supported this metabolism. Our data show that human epidermis can metabolize BP and BP-7,8-diol through arachidonic acid dependent LO pathway.

C57BL/6 mice were given ip injections of either corn oil, benzo(e)pyrene (BeP; 50 mg/kg), or 2,3,7,8-tetrachlorodibenzo-p-dioxin in corn oil (TCDD; 4 μg/kg), killed after 24 h, and hepatic microsomes were prepared for experiments. P450-mediated activities of these microsomes were characterized using several biochemical tests. The Ames test was used as an assay to measure changes in P450-mediated activation of several individual promutagens. Microsomes from each of the 3 treatments were used as the activating system, and the index of mutagenicity was the chemical concentration at 50% of the maximum number of excess revertants (EC50). With aflatoxin B1 (AFB1) as a promutagen, the EC50 associated with BeP treatment was 40% higher than the EC50 associated with corn oil pretreatment. In contrast, TCDD pretreatment was associated with a 20% decrease in EC50 relative to the corn oil controls. Rates of erythromycin and aminopyrine demethylation were lower in BeP-pretreated mice than in corn oil controls (23 and 20%, respectively). Finally, rates of generation of 10-hydroxy warfarin from (R)-warfarin were depressed 34% in BeP-pretreated mice relative to corn oil controls.

1362 BENZENE(PYRENE PRETREATMENT OF C57BL/6 MICE RESULTS IN INCREASED BIOACTIVATION OF AFLATOXIN B1 W.F. Ma and J.O. Babish. Department of Pharmacology, NYS College of Veterinary Medicine, Cornell University, Ithaca, NY. Sponsor: D. Lisk

C57BL/6 female mice were given ip injections of either corn oil, BeP (50 mg/kg), or TCDD (4 μg/kg) and killed after 24 h. Salmonella typhimurium strain TA100 was used to measure bioactivation of promutagens by hepatic microsomes from each of the 3 treatments. The concentration of promutagen producing a response equal to 50% of the maximum number of excess revertants seen over a 100-fold concentration range (EC50) was used to compare the treatments. For the promutagen aflatoxin B1 (AFB1), BeP-pretreatment resulted in an 80% increase in EC50 over the corn oil controls. As expected, in contrast to corn oil pretreatment TCDD pretreatment was associated with a 20% relative decrease in bioactivation of AFB1. For the promutagens BeP, 7,12-dimethylbenz(a)anthracene, 3-methylcholanthrene, and 1,2-dimethylbenz(a)anthracene, the EC50 values associated with BeP pretreatment were decreased relative to the controls (61, 66, 89 and 65%, respectively). TA100 revertants induced by these chemicals with TCDD pretreated microsomes were 660, 350, 390 and 130%, respectively, higher than the controls. Since the effects of BeP and TCDD in this study were discordant, it appears that changes in bioactivation of promutagens brought about by BeP pretreatment are not mediated through the Ah receptor.

1363 METABOLISM OF DIBENZ(A,J)ACRIDINE AND 7H-DIBENZO-(C,G)CARBAZOLE BY CUNNINGHAMELLA ELEGANS. J.B. Reid and D. Warshawsky. University of Cincinnati Medical Center, Department of Environmental Health, Cincinnati, OH.

Polycyclic and N-heterocyclic aromatic (NHA) compounds are widespread environmental pollutants and can be found in former coal gasification sites. It is important to investigate the role of natural organisms with respect to toxic/carcinogenic byproducts and as biodegradative tools for remediation. The filamentous fungus, Cunninghamella elegans, is known to degrade polycyclic aromatic compounds but little is known about the ability of this organism to degrade NHA. Strain 9245 converted 36 to 39% of dibenz(a,j)acridine (DBA) and 35% of 7H-dibenzo(c,g)carbazole (DBC) to water-soluble, ethyl acetate-insoluble conjugates or metabolites in 120 hours. The majority of the remaining material was associated with the organism. Deconjugation of DBA water-soluble products with β-glucuronidase and aryl sulfatase yielded metabolites which were ethyl acetate soluble (10 and 15% of the original water soluble materials, respectively). DBA, DBC and benzo(a)pyrene are almost completely adsorbed by the organism within the first 20 hours of incubation. Water soluble metabolites were extracted into the aqueous phase at a relatively constant rate reaching about 1/3 of the original concentration in 400 hrs. These experiments indicate that fungi can degrade DBA and DBC. Supported by NIHES grant P42ES04908.


Hydroxymethylarenes (HMA's), major metabolites of strongly carcinogenic methylarenes in rat liver, have potent carcinogenicity. HMA's have been demonstrated by us to be activated by rat liver cytosolic sulfotransferases (ST's) with formation of reactive sulfate esters and bind covalently to the exocyclic amino groups of adenine and guanine residues of calf thymus DNA through their methylene carbons with loss of a sulfate anion. Three ST's that activate HMA's existed in rat liver cytosol. A major protein with the highest HMA-sulfating activity was purified to homogeneity and named STa. STa was identified as a new isozyme of hydroxysteroid ST's and demonstrated by cDNA cloning and sequence analysis to consist of 283 amino acid (AA) residues (Mr 33124). The deduced AA sequence of STa shared a homology of 74% with that of the rat liver cytosolic senescence marker protein SMP-Z whose enzymatic function has never been demonstrated since it was first reported by Chatterjee, et al. in 1981. An extremely strong homology (90% on an average) was found in their four local AA sequences corresponding to 60% of the total sequences.
OXIDATION OF HFC-134a BY CYTOCHROME P450IIIE1.

1,1,1,2-Tetrafluoroethane (HFC-134a), a non-ozone-depleting CFC-alternative undergoing toxicological evaluation, is oxidatively defluorinated by rat hepatic microsomes. Phenobarbital or Aroclor 1254 decreased the specific activity of defluorination. However, induction of P450IIIE1 in rats by pyridine (100 mg/kg, ip, 4 d) increased the rate of HFC-134a metabolism by microsomes 8-fold (Vmax 47 vs. 6 nmol F/mg protein/15 min) and increased specific activity (nmol F/nmol P450) 4.2-fold. Immunoquantitation showed about a 4.5-fold increase in microsomal P450IIIE1 in pyridine-treated rats. p-Nitrophenol (p-NP) and aniline competitively inhibited HFC-134a defluorination by microsomes (Ki: 36 µM p-NP; 115 µM aniline). Pyridine also potently induced HFC-134a defluorination by rabbit liver microsomes. Purified rabbit liver P450IIIE1 defluorinated HFC-134a (18.2 nmol F/nmol P450/60 min) rapidly compared to IA1 (5.3 nmol F/nmol P450/60 min); rates with IB1 and IA2 were negligible. We conclude that P450-dependent oxidation of HFC-134a is catalyzed primarily by P450IIIE1. Thus, it is likely that chronic exposure to inducers of P450IIIE1 (e.g. ethanol, trichloroethylene, pyridine) prior to exposure to HFC-134a, or related tetrahaloethanes, in vivo will increase the metabolic yield of F- and organics such as trifluoroacetate. The toxicological consequences of accelerated HFC metabolism are presently under study.
Hydrochlorofluorocarbons (HCFCs) are being developed as substitutes for ozone-depleting chlorofluorocarbons; because widespread human exposure to HCFCs may be expected, it is important to evaluate thoroughly their toxicities. The metabolism of 2,2-dichloro-1,1,1-trifluoroethane (HCFC-123), given by ipalation (5% for 2 h), was studied in rats by 19F NMR spectroscopy. We found that HCFC-123 is bioactivated to an electrophilic intermediate (trifluoroacetyl chloride) that reacts covalently with liver proteins in vivo to form a single, stable adduct. This adduct was identified as trifluoroacetylxamine. Trifluoroacetic acid was the only urinary metabolite detected. Identical results were obtained with rats exposed to 2-bromo-2-chloro-1,1,1-trifluoroethane (halochloro), an analog of HCFC-123. Also, the pattern of microsomal and cytosolic proteins immunoactive with hapten-specific, anti-trifluoroacetyl protein antibodies was identical in livers of HCFC-123- and halochloro-exposed rats. Halothane causes an idiosyncratic, and sometimes fatal, hepatitis that is associated with an immune response against several trifluoroacetate-like liver proteins (neocaritins) in humans. The present findings raise the possibility that humans exposed to HCFC-123 or structurally related HCFCs or halogenated anesthetics may be at risk of developing an immunologically mediated hepatitis that is unlikely to be detected in animal toxicity studies of HCFCs.

Chloroacetic acids are produced in drinking water as a result of disinfection processes. Chloroacetic acids are also metabolites of widely used and toxic halogenated hydrocarbons. Thus, chronic human exposure to these chemicals is likely to occur. The objective of the present study was to examine the toxic effects of monochloroacetic acid (MCA), dichloroacetic acid (DCA) and trichloroacetic acid (TCA) in a 90-day subchronic study in rats via oral exposure by drinking water. In each treatment group, dosage was 1/4 of LD50 per day. Control rats received distilled water only. After 90 days, major organs were removed, fixed, paraffin embedded and stained. Light microscopic examination of the major organs revealed variable degrees of alterations in the lung and liver of all three treated groups. In the liver, morphological changes were predominantly localized to the portal triads, which were mildly to grossly enlarged with random bile duct proliferation, extension of portal veins, fibrosis, edema and occasional foci of inflammation. In the lungs, foci of pulmonary vascular inflammation were generally found on the periphery of small pulmonary veins and were more prevalent in all three treatment groups than in control rats. Morphological changes in the testes and brain were seen only in the DCA treated group. Seminiferous tubules in the testes were severely atrophic, contained enlarged Sertoli cells, very few spermatocytes and no mature sperm. Focal vacuolation was present in the forebrain and brainstem. These studies indicate that toxicity increased with this dose regimen in the following order: DCA > TCA > MCA.

Trichloroacetic (TCA) and dichloroacetic (DCA) have been shown to be hepatocarcinogenic in mice. DCA caused a focal necrosis not seen in TCA-treated mice, whereas TCA caused excessive lipid peroxidation that was less marked in mice. These effects were not observed in rats. This study examines the metabolism of TCA and DCA to identify mechanisms of action. Male Fischer 344 rats and B6C3F1 mice were administered oral doses of 5, 20, or 100 mg/kg 14C-TCA and DCA (pH to 7). An excretion balance was constructed by assaying the urine, feces, exhaled air, and carcass for radioactivity; blood concentration over time profiles were also collected. In rats and mice administered DCA, less than 1% of the parent compound was recovered in the urine. The conversion of DCA to CO2 was much greater in rats. However, a larger percentage of the dose was incorporated into proteins in mice. DCA blood concentrations in rats given the high DCA dose were much higher than in mice, suggesting a high first-pass effect in mice. The metabolism of TCA was found to be similar in rats and mice. More than half of any single dose of TCA was excreted unchanged in the urine. Likewise, blood concentration over time curves were found to be similar in rats and mice. DCA was detected in the urine of TCA treated animals, providing proof of a reductive dechlorination of the compound. This reductive dechlorination of TCA would generate a free radical that initiates lipid peroxidation and may be involved in the evolution of tumors. DCA, on the other hand, is extensively metabolized to dechlorinated products, i.e. glycolate. This metabolism might lead to harmful effects in the hepatocyte via oxidation-reduction reactions that generate hydrogen peroxide. (This work is funded by N.I.E.H.S. #R01 ES04648-01A1).
Chloral hydrate [$\text{C}_2\text{H}_5\text{CH(OH)}_2$] has been used as a hypnotic for over a century, and is currently used in pediatric procedures such as dentalistry, CAT or MRI scans, and EEG. In spite of, or perhaps because of its long record of use, little modern toxicological evaluation of the drug has been undertaken, particularly as regards chronic hazards. It is a metabolite of trichloroethylene (TCE), which is known to be genotoxic, and is carcinogenic in rodents. Chloral hydrate itself, like other reactive aldehydes, is mutagenic and causes cyrogenic abnormalities, spindle inhibition, SCE's and DNA strand breaks in \textit{vivo} and \textit{in vitro}. It has also been shown in a limited study to induce liver tumors in young mice, after a single dose lower than that usually given therapeutically to children. A statistical analysis of the tumor incidence with time, and calculation of plausible bounds on the predicted cancer risk have been undertaken using methods based on the Armitage-Doll multi-stage model of cancer causation. The form of the model used reflects the expectation that exposures to carcinogens early in life may have greater effect than exposures of adults. Although the uncertainty of these predictions is high due to the limitations of the data, they clearly indicate that the drug should not be used without careful evaluation of the risks and benefits. The dose typically given to children is 60 mg/kg. This intake is equivalent to drinking 1 liter of water contaminated with 5 ppb TCE (the MCL) daily for 500 years, if all the TCE is converted to chloral hydrate. These calculations suggest that widely used but inadequately evaluated drugs may represent a risk factor for human cancer which is substantial relative to other more clearly recognized risks such as environmental contamination.

Trichloroethylene (TCE) was selected as an impurity warranting a health-based residue limit because it is considered a probable carcinogen. The PMA procedure for setting health-based residue limits for organic volatile solvents in pharmaceuticals was applied to TCE. Data pertaining to chemical and physical properties, use and occurrence, biodisposition, toxicology and effects in people were collected and evaluated. A weight-of-evidence test suggested that use of a safety factor approach, rather than use of statistical models, was most appropriate for TCE. Acceptable daily intake (ADI) values calculated for oral, parenteral and inhalation routes and encompassing different toxicological endpoints ranged from 2 to 45 mg/day. Based upon a consideration of all of the data for TCE, and the ADI values derived from the data, a health-based residue limit of 2 mg/day is considered to be appropriate as a lifetime daily average for chronically administered pharmaceuticals, i.e., pharmaceuticals intended for continuous use for a year or more in a lifetime.

Metabolism of $^{14}$C(UL)TRI in rats results in a significant portion of label being incorporated as $^{14}$C-glycine into proteins, such as hemoglobin and albumin, presumably by transamination of TRI-derived $^{14}$C-glyoxylate. Glyoxylate can form $\alpha$, $\beta$-unsaturated carboxylic acid Schiff bases with amino groups. We have examined N-terminal amino groups of albumin isolated from rats dosed with TRI, as well as rat albumin incubated with rat hepatocytes and $^{14}$C(UL)TRI for the presence of this type of adduct. Using a Cu$^{2+}$ catalyzed transamination technique, 40% of the \textit{in vivo} and 0% of the \textit{in vitro} albumin label could be accounted for as this N-terminal Schiff base. This indicates an intracellular site for Schiff base formation with glyoxylate in response to TRI metabolism in the rat. The \textit{in vitro} results are consistent with a different type of extracellular protein adduct observed with TRI metabolism. We are following this up by examining albumin lysolecithin-$\epsilon$-amino groups from rats dose with TRI for similar adducts. Our aim is to develop adducted hemoglobin and albumin into a useful dosimeter biomarker of TRI exposure and intracellular hepatocyte glyoxylate levels.

(Supported by EPA CR-815216)

Recently we have reported that DCVC sulfoxide is a much more potent nephrotoxin than DCVC and causes depletion of reduced nonprotein thiol concentrations in both liver and kidney. \textit{In vitro} incubation of DCVC sulfoxide with GSH resulted in the formation of S-[1-chloro-2-(S-glutathione)-vinyl]-L-cysteine sulfoxide (DCVC-GSH), a product formed by the Michael addition of GSH to DCVC sulfoxide followed by the loss of HCl. A metabolite of DCVC sulfoxide which has HPLC retention time and positive and negative ion FAB/MS spectra similar to that of DCVC-GSH was excreted in the bile of rats given DCVC sulfoxide (100 mg/kg, i.p.). The majority of DCVC-GSH was excreted within 1 hr after DCVC sulfoxide administration with no conjugate detected beyond 2 hr. These results provide evidence that DCVC sulfoxide acts as a Michael acceptor \textit{in vivo} and suggest that DCVC sulfoxide nephrotoxicity may be due to similar interactions of DCVC sulfoxide with renal macromolecules. (Supported by NIEHS grant T32 ES07015)
Male B6C3F1 mice were exposed for 8 hrs to PERC vapor concentrations of 25, 100, 500, and 1000 ppm. Blood concentrations of PERC and plasma concentrations of its major metabolite, trichloroacetic acid (TCA) were measured during and post-exposure. These concentration time course data were used in the development of a physiologically based pharmacokinetic (PBPK) model for PERC and TCA. The enzymatic rate constant, Vmexc (mg/kg/hr), was estimated by adjusting the value of Vmexc until the predicted PERC blood concentration time course curves fit with observations. A preliminary estimate of the Vmexc value is between 0.5 to 1.5 mg/kg/hr for these PERC inhalation exposures. Currently, the stoichiometric yield and kinetic rate constants for TCA are being determined. These data will be used to develop a compartmental model for TCA for use in refining the estimate Vmexc.

The C₈ CTFE oligomer component of a novel hydraulic fluid (3.1 oil) is more toxic than the C₆ CTFE oligomer component. The toxicity of 3.1 oil is believed to be related to the conversion of neutral CTFE oligomers to their corresponding halogenated fatty acids. Male Fischer 344 rats were given 7 daily oral gavage doses (1.25 g/kg) of two batch-formulated 3.1 oils (3.1 oil-C₈ and 3.1 oil C₆:C₈) and pure CTFE C₆ (trimer) and C₈ (tetramer) oligomers, respectively. All rats exposed to test compounds for 7 days demonstrated significant 2-fold increases in liver weight over controls. A significant 2-fold greater amount of tetramer than trimer was found in the livers of rats dosed for 7 days with tetramer, trimer and 3.1 oil-C₈, respectively. After respective 24 h and 7 day dosings, the amount of tetramer acid formed in the liver was 2X and 10X the amount of trimer acid formed, respectively. In addition to the formation of tetramer acid, rats treated with tetramer also formed comparable amounts of trimer acid. This data indicates that toxicity induced by the tetramer component of 3.1 oil may be due to the resulting persistent high concentrations of halogenated fatty acids.

An important consideration in health risk assessments of halocarbon solvents is the validity of species to species comparisons of the uptake, disposition, and elimination of the chemicals following their ingestion. Therefore, the relative pharmacokinetics in species of widely varying size was evaluated following the administration of perchloroethylene (PER). Unanesthetized male Sprague-Dawley rats and beagle dogs were administered PER at doses of 1 or 10 mg/kg. The halocarbon was administered in polyethylene glycol (PEG) in a single bolus either orally (po), or by intraarterial administration (ia) through an indwelling carotid arterial cannula. Blood samples were collected from an indwelling cannula in the jugular vein at intervals up to 48 hr following dosing and PER concentrations analyzed by headspace gas chromatography. Maximum blood concentrations of PER in both species were achieved in 5 and 20 min for ia and po administrations, respectively. For each dose and route of administration, the terminal elimination half-lives of PER in rats were significantly shorter than in dogs and the area-under-the-blood-concentration-time-curve (AUC) was lower in rats relative to dogs. There are therefore significant differences in the pharmacokinetics of PER in rats and dogs, which should be taken into consideration in making interspecies comparisons of kinetic or toxicity data for PER exposure (Supported by APOSR 870748).

A subchronic (28-day) inhalation evaluation of orthochlorobenzylchloride (OCBC) was performed to establish a no observable effect level (NOEL) to support the worker safety program. Five male and five female Wistar rats were exposed to vapor of OCBC at dose levels of 0.01, 0.03 and 0.10 mg/L for 6 hours/day, 5 days/week. Effects were limited to the high dose, primarily in the male animals. A reduction in weight gain (78%) food consumption (69.3%) and water consumption (78.6%) was observed at 0.10 mg/L. Hemoglobin and red blood cell count were increased in males (P<0.01). Packed cell volume was increased in males (P<0.01) and females (P<0.05). The myeloid/erythroid ratio was increased in both male and female rats (P<0.01) at the high dose of OCBC. Histopathological changes were found in the respiratory system. Metaplastic alterations of the epithelium were observed in the nasal mucosa and trachea of all animals exposed to the high dose level. All animals from the high dose group demonstrated epithelial hyperplasia and some keratinization of the larynx. Lung involvement included degeneration of the bronchial epithelium in all female rats and 3/5 males at 0.10 mg/L OCBC. Also, one male rat had focal bronchiolar epithelial hyperplasia and a second had focal squamous metaplasia. Lymphoid hyperplasia of the threbrobronchial lymph nodes was noted in 3/5 males and 1/5 female rats at the high dose. These findings indicate that OCBC is an irritant vapor with a NOEL of 0.03 mg/L. Changes in red blood cell parameters suggest that OCBC has an adverse effect upon the hemapoietic system. Also, the occurrence of threbrobronchial lymphoid hyperplasia may indicate that OCBC is a lung sensitizer.
Chloroform (CHCl₃), a drinking water contaminant, increases the frequency of thyroid tumors in rats. The mechanism of CHCl₃-induced thyroid tumor development is not known. This study examined the effects of CHCl₃ on serum concentrations of thyroid (T₄) and triiodothyronine (T₃) in male F-344 rats at times ranging from 3 days to 1 year. After 3 days of daily dosing with CHCl₃ at 500 mg/kg in corn oil (5 ml/kg, p.o.), serum T₄ levels fell to 40% of control values. When rats received CHCl₃ (180 mg/kg/day) by gavage in aqueous emulsion (4% Emulphor, 5 ml/kg), no changes were seen in serum T₄ or T₃ concentrations at 3 or 7 days. In the long-term study, rats were provided drinking water containing CHCl₃ at 0 (distilled water), 900 or 1800 mg/L resulting in an average daily dose of 0, 60 or 121 mg/kg/day, respectively. After one year of treatment with CHCl₃ at 1800 mg/L in drinking water, serum T₄ values were decreased by 17% relative to controls. The slight decrease (4.4%) in rats treated with 900 mg/L was not statistically significant. No changes in serum T₃ levels were noted at any time. Decreased T₄ may lead to increased thyroid stimulating hormone (TSH) secretion by the pituitary due to decreased feedback inhibition by T₄. This could result in goiter and possibly thyroid tumor promotion. Alternatively, decreased T₄ might reflect decreased T₃ secretion via direct pituitary effect of CHCl₃. This condition would not be expected to promote thyroid tumors. Analysis of serum TSH will contribute important information regarding the mechanism of CHCl₃-induced suppression of serum T₄ levels in F-344 rats. This abstract does not necessarily reflect EPA policy.

The mechanism initiating hepatic fibrogenesis following exposure to carbon tetrachloride (CCl₄) is unknown. We postulated that exposure of hepatocytes to CCl₄ in vivo, phenotypically alters those cells that are not lethally damaged by this hepatotoxic agent. This study was conducted to determine if this alteration would be manifested in vitro as an increased capacity of these hepatocytes to synthesize collagen.

Hepatocytes were obtained by in situ perfusion of livers in rats treated for two, three, or four weeks with CCl₄. Hepatocytes were then maintained for eight days under non-proliferating conditions. Collagen biosynthesis was determined after incubating the cells for 24 hour with [5-¹⁴C]-proline by selective enzymatic digestion of collagen. Collagen biosynthesis increased significantly in all cultures of hepatocytes from CCl₄-treated rats. Synthesis, expressed relative to total protein synthesis, increased from 1.27 ± 0.34 (control) to 3.70 ± 1.57 (2 wks), 4.06 ± 0.10 (3 wks), 4.80 ± 0.22 (4 wks). Hepatocellular RNA probed with a cDNA specific for type I procollagen indicated that CCl₄-exposed hepatocytes contained higher levels of type I procollagen mRNA. These data support the hypothesis that CCl₄ exposure alters hepatocytes to synthesize greater levels of collagen. Supported by NIH grant DK337947.

Carbon tetrachloride (CCl₄) is a highly toxic industrial solvent with pronounced effects on liver and brain. CCl₄ is enzymatically cleaved to produce free radicals that attack membrane components including proteins. Earlier reports indicated that CCl₄ affects 
C⁴⁺ regulated events in the brain. Hence, the present study was initiated to determine whether 
CCl₄ affects inositol 1,4,5-triphosphate (IP₃) receptor binding and protein kinase C (PKC) activity in rat brain, since IP₃ and PKC are known to be involved in signal transduction. 

YH-IP₃ binding was determined using rat brain microsomes whereas 
PKC activity was determined in the soluble fraction. 
CC₄ in vitro decreased 3H-IP₃ binding to 
microsomes in a concentration dependent manner with an ICₕ₀ of about 80 uM. CCl₄ at 1 uM concentration inhibited PKC activity by 50%. Thus perturbations in the binding of IP₃ to its receptor 
sites and modulation of PKC activity by CCl₄ in vitro suggests that CCl₄ may exert neurotoxicity by altering signal transduction pathways. (Supported by NIH/MBRS grant # RR03047)

SUBCHRONIC TOXICITY OF 1,3-DICHLORO-2- 
PROANOL IN THE RAT. G C Jersey, W J Breslin, 
and G J Zielke. The Toxicology Research Laboratory, 
The Dow Chemical Company, Midland, MI.

The purpose of this study was to evaluate the potential subchronic oral toxicity of 1,3-dichloro-2-propanol in rats. Groups of male and female Sprague-Dawley rats were gavaged with 0, 0.1, 1, 10 or 100 mg/kg/day of 1,3-dichloro-2-propanol, 5 days/week for 13 weeks. Decreases in body weight gain, feed consumption and hematologic parameters, increases in liver and kidney weights, alterations in serum chemistry and urinary parameters, gross pathologic changes in the stomach and histopathologic changes in the stomach, kidney, liver and nasal tissue were observed at 1000 mg/kg/day in males and females. The changes in serum chemistry were considered secondary to renal and hepatic alterations observed in high dose animals. At 10 mg/kg, increased liver weights in males and females and histopathologic changes in the stomach, kidneys and liver in males were observed. The treatment-related effects observed at 10 mg/kg were less frequent and/or less severe than the effects observed at 100 mg/kg. No effects were observed at 0.1 or 1 mg/kg in males or females.

DISPOSITION AND LIVER SUBCELLULAR DISTRIBUTION OF [14C]-TRICHLOROETHYLENE (HCB) IN MALE AND FEMALE RATS. M Charbonneau, Département de Médecine du Travail et d'Hygiène du Milieu, Université de Montréal, Québec, Canada.

HCB causes liver porphyria in female but not in male rats. Disposition and liver subcellular distribution of HCB in female rats was compared to that in male rats. Animals were given HCB (100 mg/kg, po, 10 ml/kg in corn oil) for 5 consecutive days; [14C]-HCB (12.5 μCi/kg) was given on last day. Urine was then collected for 24 hr and animals were killed. Radioactivity was measured in kidney and liver homogenates, urine, plasma, and abdominal fat. No differences between sexes were observed for fat, plasma, and urine levels. Urinary pentachlorophenol excretion was, however, higher in female rats whereas urinary HCB excretion was higher in male rats. Radiolabel levels in liver and kidneys were higher in male than in female rats. Male rats 

10-Day Repeated Dose Inhalation Toxicity Study 
with Benzyltriphenylphosphonium Chloride (BTTPC). 
K Valentine, M.C. Carakostas, and J.P. Hansen. 
Du Pont, Haskell Laboratory for Toxicology and 
Industrial Medicine, Newark, DE.

BTTPC is a curing agent used in the production of 
fluoroelastomers. A 10-day inhalation study was 
conducted to determine the target organ effects of 
BTTPC. Groups of 10 male CrI:CD® rats were exposed to 
BTTPC at 5.1, 15 or 60 mg/m³ for 6 hr/day, 5 
days/wk for 2 weeks. Blood, urine and tissue samples 
were obtained for analysis of clinical chemical 
and anatomic pathology parameters after the 10th exposure and after a 14-day recovery period. During the exposures, 
concentration-related decreases in body weights occurred in all exposure groups. Clinical pathology data revealed that 
leukopenia, increased red blood cell mass parameters, and increased serum protein and hepato-
cellular enzyme activities occurred at 15 and 60 
mg/m³; these changes were transient and had resolved after 14 days of recovery. Microscopic lesions were found in the lungs and nasal cavities of rats from all exposure groups. The lesions consisted of hypertrophy and hyperplasia of bronchial and bronchiolar epithelium, and necrosis and inflammation of nasal olfactory and respiratory epithelium. Resolution of tissue injury occurred in the lungs but was incomplete in the nose; necrosis and inflammation of nasal respiratory and olfactory epithelium and respiratory epithelial metaplasia were evident following a 14-day recovery. Based on these data, a no-observable ef

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fect level for BTTPC was not demonstrated.

The house fly is a suitable model to determine comparative pharmacokinetics for polychlorinated biphenyl (PCB) congeners, but data are not yet entirely suitable for the development of a complete pharmacokinetic model. The rapidly metabolized PCB 18 (2,2',5-trichlorobiphenyl) was topically administered to the dorsal thorax of 3-day old female house flies in 1.0 ml acetone. Flies were maintained in groups of 3 in 25 ml scintillation vials lined with filter paper to trap the excrement and reduce reabsorption of PCB by contact. At specified times, flies were anesthetized, removed from the vial, rinsed with acetone, bled and dissected for extraction with hexane-acetone followed by digestion. Disappearance of the applied dose was rather rapid with 98, 18, 15, 7 and 4% of the dose being recovered in the acetone rinse at 0, 2, 4, 13 and 24 hours, respectively. Concentrations in the hemolymph also declined between 2 and 24 hours, while cuticle, head, ovaries, and thoracic muscle levels peaked at 4 hours. Radioactivity in the alimentary canal peaked at 13 hours, containing 11% of the dose. Excrement contained 10, 27, 33 and 39% of the dose at 2, 4, 13 and 24 hours, respectively. There is evidence for polar metabolites in the excrement and tissues. These data will help to construct a pharmacokinetic model to explain previous results.


The toxicity of 11 polychlorinated biphenyl (PCB) congeners was measured as a function of the longevity of 3-day old female Musca domestica. The following PCB congeners were administered topically at 7.5, 10 or 15 ug in 1.0 ml acetone on days 1 and 3: 18 (2,2',5); 28 (2,4,4'); 49 (2,2',4,4'); 84 (2,2',3,3,6); 95 (2,2',3,5,6); 101 (2,2',4,5,5'); 105 (2,3,3',4,4'); 110 (2,3',3,4,6); 118 (2,3',4,4,5); 126 (3,3',4,4,5) and 153 (2,2',4,4,5,5). Daily mortality data, collected until the last of 30 flies died, were analyzed by life-table methods. PCB's 18, 95 and 153 did not significantly decrease mean longevity at 10 ug, but the results for PCB 95 were ambiguous. Although PCB 18 did not result in significant toxicity at 10 ug, it reduced the life span more than any other congener (87%) when administered at 15 ug. PCB 84 caused the smallest significant reduction in life span (15%). The order of toxicity (decrease in mean survival time) was: PCB 18 (15 ug) > 49 > 118 > 126 > 110 > 105, 28 > 153 (15 ug), 101 > 84. Congeners chlorinated in the 2,5- positions on one or both rings are the least toxic and those substituted with 2,4-, 3,4- and 2,3,6- are the most toxic to house flies. Since it has not been possible to demonstrate an Ah receptor in flies, the relative equipotency of coplanar congeners probably represents a more general mechanism of toxicity.


Certain sulfur-containing metabolites of PCBs are retained in the Clara cells and in the airway lumen of rodent lung due to their interaction with a secretory 13 kDa protein. We have now isolated a cDNA encoding the rat lung PCB-binding protein. The identity of the encoded protein is supported by expression of the cDNA in COS-1 cells where homologues from transfected cells show specific binding of 4,4'-bis[(2H)methylsulfonyl]-2,2',5,5'-tetrachlorobiphenyl, a high affinity ligand for the PCB-binding protein. Also a monospecific antiserum to the PCB-binding protein recognizes a 13 kDa protein in the homogenates of transfected cells but not in the corresponding fraction of mock transfected cells. Interestingly, DNA sequence analysis and prediction of amino acid sequence reveals that the PCB-binding protein shares 53% positional amino acid identity with uteroglobin, a progesterone-binding protein found in rabbit uterus and lung. The kinship of these proteins suggests novel approaches to elucidation of the physiological role of the PCB-binding protein and of its possible role in PCB-induced lung toxicity.

DIFFERENTIAL IMMUNOTOXICITY OF CHLORINATED DIPHENYL ETHER CONGENERS IN DBA/2 AND C57BL/6 MICE. L Howie, R Dickerson, and S. Safe. Dept. of Vet. Phys. & Pharm., Texas A&M Univ., College Station, TX.

Chlorinated diphenyl ethers (CDPEs) are potentially toxic impurities in commercial grade pentachlorophenol. The dose-response effects of the following congeners on the splenic plaque-forming cell response to sheep red blood cells (SRBCs) were determined in DBA/2 mice: 2,3',4,4',5-hexachloro-, 2,3',4,4',5-pentachloro-, 2,2',4,5,5'-pentachloro-, and 2,2',4,4',5,5'-hexachloro-DEPE. Previously reported data indicated that 2,3',4,4',5-hexachloro-DEPE was the most potent congener in C57BL/6 mice, and this compound was also the most immunotoxic in DBA/2 mice. However, the differences in the potency of the individual CDPEs in the two mouse strains ranged from 100-fold for the toxic 2,3',4,4',5-substituted congener to equivalent potencies for the 2,2',4,5,5'-substituted compound. In contrast, hepatic microsomal AHH and EROD activities were also induced in the C57BL/6 mice but not in the Ah nonresponsive DBA/2 mice. These results suggest that additional mechanisms that do not involve the Ah receptor may be mediating immunotoxicity in DBA/2 mice. (Supported by N.I.H., ESO4917).
ASSOCIATION WITH HEXABROMOBIPHENYL (HBB) ALTERS THE RATE OF ENTRY OF LOW-DENSITY LIPOPROTEIN (LDL) INTO FIBROBLASTS. S I Jang and J A Bernstein. Department of Environmental and Industrial Health -Toxicology, University of Michigan, Ann Arbor, MI

LDL appears to play a role in the transport of lipophilic halogenated hydrocarbons, such as HBB, into peripheral cells. To study how LDL facilitates the entry of HBB into cells, the entry of [125I]LDL (reconstituted with 3H-cholesterol linolate) and LDL associated with [14C]HBB were compared in wild type (K1) and mutant (ldIA-7) Chinese Hamster Ovary cells. The mutant cells lack functional LDL receptors. LDL can enter into K1 cells by both receptor-mediated and non-receptor-mediated pathways. LDL can only enter into ldIA-7 cells by non-receptor-mediated pathways. Results showed that LDL entered wild type cells rapidly at a rate of 56 ng/hr during the first 30 min. Between 30 and 90 min, the rate approached zero. After 90 min, the initial rate was resumed and this rate was maintained up to 2.5 hr. For mutant cells, LDL entered the cells at a steady rate of 16 ng/hr which continued for 2.5 hr. The entry of LDL associated with HBB showed a biphasic kinetic curve with the same rate of uptake for both cell types. During the first 45 min, the rate of entry was 245 ng/hr, After that, the rate decreased to 200 ng/hr and remained constant up to 2.5 hr. The results indicated that the LDL not only can carry HBB and enter into cells, but also that the rate of entry was 4X and 16X greater than that without HBB association for wild type and mutant cells, respectively. It is postulated that association with HBB alters the hydrophobicity of LDL resulting in enhanced LDL entry into CHO cells. Since LDL associated with HBB enters at the same rate in both cell types, this enhanced entry probably occurs via non-receptor-mediated pathways.

UPTAKE OF TETRABROMOMETHANE VAPOR IN THE RAT. A F Eidson, A R Detl, R F Henderson. Inhalation Toxicology Research Institute, Albuquerque, NM

Tetrabromoethane (TBE) is a dense liquid used in industry to separate minerals by density. It is toxic to liver and kidney when administered by inhalation or ingestion. We are conducting inhalation studies in F344/N rats to determine the uptake and retention of TBE and to develop toxicokinetic models of TBE metabolism. Rats were exposed at 0.3 ppm or 3.0 ppm TBE for up to 6 hr, and sacrificed in groups of three at 0.5, 1, 2, 4, or 6 hr during exposure, or at 1, 2, or 8 hr after the end of exposure. Blood, fat, kidney, and liver tissues were analyzed for TBE content by vacuum distillation and gas chromatographic/mass spectrometric assay of a toluene extract relative to a triple-labeled TBE internal standard. Only low levels of TBE were found in kidney or liver. TBE accumulates and concentrates after exposure ceased. No TBE was found in blood of rats exposed at either vapor concentration. An additional experiment using TBE spiked into whole blood in vitro showed no metabolism within 10 min after mixing. These results suggest rapid tissue metabolism of inhaled TBE vapor or the vapor is poorly absorbed. [Research was supported by NIEHS via Interagency Agreement ES-20692 with U.S. DOE/OHER Contract No. DE-AC04-76EV01013]
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