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Preface

This issue of the Toxicologist is devoted to the abstracts of the presentations for the platform, poster/discussion, and poster sessions of the 28th Annual Meeting of the Society of Toxicology, held at the Atlanta Hilton and Towers, Atlanta, Georgia, February 27–March 3, 1989.

The issue also contains a Keyword Index (by subject or chemical) to the titles of all the presentations, beginning on page 293.

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ESTIMATING TIMES TO INITIATION ONSET AND MALIGNANCY USING A TWO-STAGE MODEL OF CARCINOGENESIS. W-Y Tan and T B Starr. Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

Technological advances in detection of genetic markers provide the opportunity to identify and quantitate initiated cells in certain target organs for carcinogenesis. When such information is obtained from long-term survival/sacrifice experiments with cause of death unspecified, six distinct outcomes may be resolved. Natural deaths can occur without initiated cells present, with initiated but no malignant cells present, or with malignant cells present. Similarly, scheduled sacrifices can occur without initiated cells present, with initiated but no malignant cells present, or with malignant cells present. We report on (1) a generalization of the Moolgavkar-Venzon-Knudson two-stage model that utilizes this information in accounting for competing risks of death, and (2) development of a semi-parametric procedure for estimating model parameters and resulting distributions of times to the onset of initiation and malignancy. Our approach accommodates pharmacodynamic factors such as exposure-induced alterations in cell division rates. Also, for the first time, it permits observations on the presence/absence of initiated cells at necropsy to be utilized in estimating the parameters of a mechanistic model of carcinogenesis. Finally, the approach should prove useful in assessing the relative merits of alternative experimental designs.


Awake F344 rats (N=8) were exposed in a plethysmograph to 1.0 ppm 14O2 for 2 hr and removal of O2 by the rat was determined by a downstream O3 monitor. The rats inhaled 1276 nmole of O3 during the exposure and removed 54.2 ± 4.2% (S.E.) of inhaled O3. A mean of 99.4 nmole of 14O was recovered in the total respiratory tract (TRT) of the rats which was distributed as follows: 49.3%, nasopharynx; 6.5%, trachea/larynx; and 44.0%, lungs. Rat data were compared to O3 removal data in resting humans (Gerry et al., J Appl. Physiol. 65:393, 1988) using the following factors: the total inhaled 14O (moles/hr), the TRT uptake as a fraction of the amount of O3 inhaled, and the TRT (distal to the glottis on larynx/trachea plus lung) uptake as a fraction of the amount of the inhaled 14O. The fractional amount of O3 entering the blood was assumed to be negligible similar in rats and humans, and the residual 14O in tissues was assumed proportional to the O3 dose during exposure. The calculated (RT dose of O3 was 56.5 and 25.5 pmole/cm2 of surface area/hr in rats and humans, respectively. Thus, there was 2.1 fold higher average O3 dose in the LRT of rats versus humans. (This abstract does not necessarily reflect EPA policy.)

CARCINOGENIC RISK ASSESSMENT OF HEXACHLOR. E I Caversen and J H Brantner. Dynarac Corporation, Rockville, MD, and California Department of Health Services, Toxic Substances Control Division, Sacramento, CA.

The carcinogenicity of heptachlor was assessed to provide health-based criteria to risk managers at hazardous waste sites. Based on five human epidemiological studies (Wang and MacMahon, 1974a; D'Urso et al., 1981; Shindell and Ulrich, 1986; MacMahon et al., 1988), there is insufficient evidence that heptachlor is a human carcinogen. In the NCI (1977) bioassay, the incidence of hepato-cellular carcinoma was increased in male and female B6C3F1 mice fed heptachlor for 80 weeks and held for an additional 10 weeks; however, no increase in the incidence of hepatic and other tumors was noted in Osborne-Mendel rats fed heptachlor for 80 weeks and held for an additional 30 weeks. These data suggest that heptachlor acts as a promoter rather than as an initiator or complete carcinogen. Hepatic tumor incidence in rats was shown to be a threshold mechanism and promotion is considered to be a threshold mechanism. Using the B6C3F1 mouse hepato-cellular carcinoma data (NCI, 1977) as a threshold mechanism, the 5% lower bound on the dose associated with an incremental lifetime risk of 10−6 for human cancer induced by heptachlor was estimated to be 8.7 x 10−3 mg/kg/day, of which corresponds to a maximum exposure level (MEL) of 0.609 x 10−3 mg/kg/day for a 70-kg human. For a nonthreshold mechanism, the NOAEL in female rats was 25.7 ppm of heptachlor in the diet (NCI, 1977) when corresponding to a dosage of 1.29 mg/kg/day. To extrapolate to humans, a correction factor of 1.4 for body weight differences is used and a safety factor of 1,000 is applied, resulting in a MEL of 126 x 10−3 mg/kg/day for a 70-kg human. The two approaches to calculating risk result in a 200-fold difference in the MEL.

QUANTITATIVE RISK ASSESSMENT OF NON-CANCER HEALTH EFFECTS FOR ACRUTE EXPOSURE TO AIR POLLUTANTS. C V Alexeeff and D C Lewis. California Department of Health Services, and Engineering Science, Berkeley, CA.

Current procedures for establishing exposure levels to airborne toxiconactants apply intuitive uncertainty factors (UF) to observed non-carcinogenic responses. Limitations of this procedure led to the development of a more comprehensive approach. The new approach used human and animal data published in the literature. A log-probit dose-response (DR) relationship was assumed, and confidence limits were calculated to account for experimental sample size. When available, chemical- and species-specific factors were used to account for differences in exposure duration, absorbed dose, species sensitivity, and response severity. 1-hour ambient concentration limits (ACLs) were estimated for NH3, AsH3, C6H5Cl, CCl4, Cl2, H2, SO2, CH2Cl2, C2Cl4, HCHO, HCl, HCN, HF, N2, and phosgene. In comparison to the UF method, chemicals with a steep DR (e.g. phosgene) had higher ACLs when the DR information was used. ACLs calculated using shallow DR data (e.g., chlorine), or a default DR value (e.g., phosgene), were lower than those using UFs. Thus the traditional UF method suggests safe exposure levels that are higher or lower in comparison to the method utilizing all available data for the chemical. (Supported by USEPA #88-02-4398).

Bendictin, the major therapeutic agent for nausea and vomiting during pregnancy, was implicated in 1980 as a causative agent in fetal malformations. Lawsuits and eventual removal of Bendictin from the marketplace followed. Our meta-analysis of all the published studies of Bendictin use and birth defects revealed a pooled relative risk for birth defects of 0.99. Temporal trends in birth defects showed no changes with the withdrawal of the drug, while hospitalization rates for nausea and vomiting of pregnancy double. These national data suggest that the therapeutic regimen was both safe (with respect to birth defects) and effective.

DEVELOPMENT OF EXPOSURE CRITERIA FOR SILVER. N. J. HALE, R. J. SEIDMAN. California Department of Health Services, Toxic Substances Control Division, Sacramento, CA and J. DURDA. ICF-Clement Inc., Fairfax, VA.

The earliest indication of excess silver exposure in humans is argyria, a bluish-black discoloration of the skin resulting from silver deposition in the dermis. This condition evidently develops prior to any adverse health effects resulting from excess silver intake and it has been used as an end point for development of regulatory criteria. Silver criteria previously developed have been based on the assumption 50% to 100% of ingested silver is retained and that argyria occurs following ingestion of a critical amount of silver. Based upon reports that a large fraction of absorbed silver is excreted, dose rate not the total intake of silver would appear to be a better indicator for the expression of argyria. When intake rate exceeds excretion rate, silver accumulation leads to silver deposits. Applied Action Levels (AALs) for silver were developed using dose rates from case studies of argyria related to the historical use of silver as a pharmaceutical agent.

ENVIRONMENTAL EXPOSURE CRITERIA FOR SELENIUM. S. M. DIZIO, M. J. HALE. California Department of Health Services, Toxic Substances Control Division, Sacramento, CA and M. MCKEEVE. ICF-Clement Inc., Fairfax, VA.

Selenium (Se) has been found at hazardous waste sites in California. Accordingly, exposure criteria for Se in air and drinking water were developed in accordance with principles listed in the California Site Mitigation Decision Tree. The criteria were based on an epidemiological study of a population in an area of China with endemically high Se levels in soils and food crops. In developing target Se concentrations in air, soil and ground water at hazardous waste sites, additional factors that must be addressed include: (1) natural occurrence of selenium in soils in central California; (2) Se's status as an essential nutrient; (3) uptake of Se from soil or ground water into food crops or crops utilized for grazing animals; (4) bioaccumulation of selenium in the food web; (5) special susceptibility of certain species such as birds and fish to Se; (6) exposure, especially to children, via soil contact; (7) chemical species of Se present and factors that may affect chemical speciation of Se.

NEUROCHEMICAL ACTIONS OF PLANT AMINO ACIDS LINKED TO HUMAN MOTOR-NEURON DISEASES. S. M. Ross and P. S. Spencer, Institute of Neurotoxicology, Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

Beta-N-methylamino-L-alanine (BMMA) and beta-N-oxyl-L-alanine (BOAA) are chemically and neuropharmacologically non-neurotoxic amino acids present in the neurotoxic seeds of Cynara cardunculus L. and Lathyrus sativus L., respectively. Heavy exposure to seeds containing BMMA has been etiologically linked to the Western Pacific amyotrophic lateral sclerosis/Parkinsonism-dementia complex, while BOAA, an excitatory amino acid, has been causally related to the pathogenesis of human neurolathyrism, a form of upper-motor-neuron degeneration. Both diseases are associated with selective degeneration of motor neurons and cause non-convulsive locomotor disorders in exposed animals. Investigations have revealed that BOAA might mediate acute neurotoxicity through cortex quassialate (QA)-preferring glutamate receptors in cortex. Parallel studies have demonstrated that BMMA-induced acute excitotoxicity might be mediated through the N-methyl-D-aspartate receptor-channel complex. The broader importance of these observations lies in the demonstration that two chemically related amino acids of exogenous origin are linked to motor-system diseases. Elucidation of the molecular and cellular mechanisms underlying cycad and lathyrus neurotoxicity may be pertinent to an understanding of the etiology and pathogenesis of lookalike neurodegenerative disorders worldwide. Supported by NS-19611.
Eighty 2-, 3- and 4-position monosubstituted phenols representing various substituents were evaluated for relative toxicity (log BR) with a short-term static protocol in the Tetrahymena population growth inhibition bioassay. Quantitative structure-activity relationships were examined using the 1-octanol/water partition coefficient (log Kow) and ionization constant (pKa) as independent variables. The model log BR = 0.6655 (log Kow) - 0.1464 (pKo) + 0.2206, n = 67, r² = 0.909, s = 0.212 was found to be an excellent predictor of activity of phenols which elicit their toxic response by the polar narcosis mode of action. For the most part the tested derivatives showed little abiotic loss over the duration of the bioassay. Four derivatives did not elicit the measured response at saturation. Five derivatives exhibited altered HPLC spectrum and were not included in the Q SAR. In addition, the three carboxyl derivatives were also not included in the QSAR because of the buffer capacity of the medium probably altered ionization. Lastly, the 4-nitroso derivative was deleted. It probably acts as an electrophile.

The cytochrome P450s are a diverse group of monooxygenases that catalyze pivotal biotransformation reactions. The present study was undertaken to define developmental and tissue-specific profiles, and response to chemical pretreatments of 10 different rat P450 mRNAs. Specific oligomer probes were used in Northern blot experiments to evaluate the P450 gene products: IIA1, IIA2, IIB1, IIB2, IIC6, IIC7, IIE1, IIE2, IIIA1, and IIIB1. Marked differences in expression patterns, dependent on the inducing chemicals utilized, were noted among the P450s examined. Select P450 mRNAs were detectable in fetal livers (IIA1, IIB1, IIB2, IIIB1, IIIA1, IIIB1); while remaining P450s were first detected in neonatal animals. With the exception of form IIE1, expressed at substantial levels in 1 week neonates, the levels of all mRNAs increased in liver as a function of developmental age. In untreated animals, low level extraplastic expression was observed for forms IIB1 and IIE1; and in 3-MC treated rats, form IIA1. These approaches should provide a clearer conception of factors responsible for developmental, tissue-specific, and chemically-induced toxicities. Supported by NIH grants GM-32281 and ES-0496.

Although tumor development is believed to begin with covalent binding of the carcinogen to DNA, the events following initiation that lead to the appearance of tumors probably involve loss of gene control at the chromatin level. For several genes it is has been shown that activity or inactivity of the gene may be correlated with changes in chromatin and it is possible that adducts may change gene expression by altering chromatin structure. Because of this, we have used simple in vitro systems to determine the influence of bulky adducts on chromatin structure at the nucleosome level of organization. The pXP-14 plasmid which contains the 5S rRNA gene and SP-6 promoter was modified with anti-benzene(α)pyrene dioleoxide to give an average of one adduct per 500 bp. Digestion of the plasmid with restriction enzymes resulted in double stranded DNA fragments that contained the 5S rRNA gene and SP-6 promoter regions. Adduct location on the gene and promoter fragments will be determined after 32P-5’-end labeling of the transcribed and nontranscribed strands followed by incubation with T4 polymerase, which will digest the DNA in a 3'→5' direction until a bulky adduct is reached. Results from this work will contribute to our understanding of the influence of bulky adducts on nucleosome positioning. Supported by the U.S. Department of Energy Contract DE-AC05-76RL0 1830.

Cytocromes P450 are a superfamily of enzymes which are active in converting chemicals to intermediates associated with cell and organ-specific toxicities. Using synthetic oligodeoxynucleotide probes to discriminate specific mRNA target sequences, we have employed in situ hybridization techniques to explore the hepatic lobule distribution of 4 phenobarbital (PB)-inducible P450 gene products, members of 3 distinct subfamilies: P450IIB1 and P450IIB2; P450IIC6, and P450IIIA1. Constitutively expressed P450 mRNAs were distributed uniformly throughout the rat liver lobule. However, rats treated with PB show distinct regional profiles for induction of these P450 mRNAs. P450IIB1 and P450IIB2 mRNAs, the major PB-responsive forms, are induced throughout the lobule, with the notable exception of hepatocytes in the perportal region. P450IIC6 mRNA is induced modestly above constitutive levels following PB exposure, with a relatively uniform distribution pattern across the liver acinus. In contrast to the other P450s, a distinct increase of P450IIIA1 mRNA is seen in hepatocytes surrounding the central vein area in PB-treated rats. Elucidating the patterns of constitutive as well as induced expression of various cytochrome P450 isozymes should have predictive value for region-specific target organ toxicities. Supported by NIH grants GM-32281 and ES-0496.
RAT LIVER CARBOXYLASES: TISSUE DISTRIBUTION AND EVIDENCE FOR A MULTIGENE FAMILY. R. M. Long, H. Satoh, and L. R. Pohl. Laboratory of Chemical Pharmacology, NHLBI, NIH, Bethesda, MD.

A rat liver microsomal carboxylesterase (59 kDa) is one of the proteins covalently modified by the trifluoroacetyl (TFA) halide metabolite of halothane. The 59 kDa-TFA protein is recognized by antibodies in sera from halothane hepatitis patients. Previously, antibodies raised to purified 59 kDa-TFA were used to screen rat liver lambla gti DNA libraries, and a clone (1793 bp) was obtained that encodes a protein (58,086 Da) with structural features of a carboxylesterase. In the present study, this cDNA was used to probe Northern blots of RNA prepared from various rat tissues. Tissue distribution of carboxylesterase-related mRNA was liver > lung > testis > fat > kidney > heart. The size of the mRNA was 1.8-2 kb in all tissues; an additional minor band was found in liver at 3.6 kb. No detectable message was found in brain, spleen, or thymus. Western blot analysis of microsomal proteins from these tissues using 59 kDa-TFA antibodies indicated that amounts of expressed protein correlated with mRNA levels. When the cDNA was used to probe Southern blots of liver genomic DNA digested with several restriction enzymes, multiple hybridizing bands were found. These results suggest that carboxylesterases comprise a multigene family of isoenzymes that are expressed in several tissues. (NHLBI is supported by a PRAT Fellowship from NIH/ODS.)

S-(1,2,3,4,4-PENTACHLORO-1,3-BUTADIENYL)-L-CYSTEINE (PCBC) UNCOPLES RABBIT RENAL CORTICAL MITOCHONDRIAL (RCM) OXIDATIVE PHOSPHORYLATION (OX PHOS) BY DISSIPATING THE PROTON GRADIENT. R. G. Schnellmann and E. A. Lock, Univ. Georgia, Athens, GA, and ICI, Cheshire, United Kingdom.

A very early effect of PCBC on rabbit renal proximal tubules is uncoupling of OX PHOS (Schnellmann et al., Toxicol. 5: 176, 1986; TAP 50:513, 1987). The goal of this study was to determine the mechanism by which PCBC uncouples OX PHOS. PCBC increased state 4 respiration of RCM respiring on pyruvate/malate or succinate in a concentration- (10-100 uM) and time- (1 or 5 min) dependent manner. PCBC also increased state 4 respiration in the presence of oligomycin, an inhibitor of F1F0-ATPase. The effect of PCBC on proton permeability of RCM was determined by measuring passive RCM swelling. After a 2 min exposure to PCBC (100 uM), RCM swelled when placed in NH4Cl or NaCl, but not in KCl or sucrose. The protonophore FCCP (1 uM) produced similar effects. After 5 min, RCM swelled when placed in NH4Cl, NaCl, or KCl, but not in sucrose. Aminoacetic acid, an inhibitor of cysteine conjugate 8-lyase, blocked PCBC effects on respiration, indicating that PCBC can and must be metabolized to produce RCM toxicity. These results show that PCBC initially uncouples OX PHOS by dissipating the RCM proton gradient. Subsequently, additional ion permeabilities occurred. (Supported by NIH ES-04410 and F32 GM.)

STRUCTURE-ACTIVITY STUDIES ON THE ANTI-OXIDATION POTENTIAL OF INDOLE COMPOUNDS. M. Tabor, E. Coats, M. Sainsbury*, and HG. Sherzer, University of Cincinnati Medical Center, Cincinnati, OH; *University of Bath, Bath, England.

Certain indole compounds, including dietary indoles, may sequester reactive electrophiles or radicals, and are chemoprotective in toxicity and carcinogenicity bioassays. To understand the chemical structural basis for radical scavenging and to develop novel indole compounds with enhanced potency, we examined 28 structurally related compounds for antioxidation potential using in vitro lipid peroxidation assays (Biochem Pharm 37:333, 1988). Relationships between indole antioxidant effects and physicochemical properties were computed via multiparameter linear regression analysis with the Hansch structure-activity program. Physicochemical properties determined were: hydrophobic properties; electronic effects; atomic charges/electron densities. Indoles were compared to common antioxidants, butylated hydroxyaniline and tocopherol. Results indicated that partitioning equilibria influence antioxidant activity to some degree, but electron donation to the 3-methylene indole position increases antioxidant potency. QSAR calculations also indicated the importance of the 3-methylene indole position for antioxidant activity. These findings in relationship to the entire data set provide a basis to develop better chemoprotective indole compounds. (NIH ES-03373; UC Cancer Research Campaign)

INHIBITION OF INTERCELLULAR COMMUNICATION BY TOXIC XENOBIOTIC CHEMICALS IN VITRO IN A HUMAN EPITHELIAL CELL CULTURE SYSTEM. B. V. Machuca, S. Y. Oh, E. DeFeyter and J. E. Pieceko. Dept. of Pediatrics/Human Development, Michigan State University, E. Lansing, MI.

Gap junctional intercellular communication (GJIC) has been shown to be modulated by diverse exogenous chemicals which include neurotoxins, and tumor promoters etc. In this study we have investigated the inhibitory effects of a number of xenobiotic chemicals on GJIC in vitro in a human kidney epithelial cell line (HKE), which has extensive intercellular communication. GJIC was measured using two techniques, FRAP analysis and a dye transfer technique. The results showed that GJIC was blocked by the tumor promoter, TPA, in a dose and time dependent manner. Inhibition of GJIC by TPA was abolished by prior treatment of cells with a protein kinase C (PKC) inhibitor, TMB-8. GJIC was also inhibited by dieldrin, DDT and heptachlor epoxide at non-cytotoxic doses. GJIC down regulated by dieldrin was reversed 6 hours after treatment and was unaffected by TMB-8. The data showed that while TPA blocked GJIC through activation of PKC, the other chemicals act via other mechanism(s). The results also suggest that the HKE cell culture system can be a useful in vitro model to study the actions of toxic environmental chemicals on GJIC. (Supported by a U.S. Air Force grant USAFOSR-86-0084 and a gift from R.J. Reynolds Company).
REGENERATION MEDIATES REPAIR OF S-(1,2-DICHLORO-VINYL)-L-CYSTEINE (DCVC) NEPHROTOXICITY: STUDIES IN VIVO AND IN VITRO. J L Stevens, P B Hatzinger, and W T Hsieh. W Alton Jones Cell Science Center, Lake Placid, NY Sponsor: T W Jones.

The cellular biology of organ repair from nephrotoxic damage is poorly understood. Necrosis of rat kidney proximal tubular cells (RPTC) is seen 3-5 days after DCVC (30 mg/kg). At 7-13 days the tubules are lined with cells with low β-glutamyltransferase (GGT) and increased levels of the intermediate filament protein vimentin (VM), determined cytologically. At 20 days the tubules were normal. By western blot analysis, VM expression increases in the damaged tissue between 3 and 13 days, but returns to normal by 20 days. Cultured RPTC also have decreased levels of GGT and increased VM. The cells stain positive for VM and cytokeratin by immunofluorescence using monoclonal antibodies, showing that VM increases in the RPTC. GGT activity increases in the RPTC as the cells reach confluence, indicating differentiation. Therefore, the cultured cells model the repair response in vitro. Growth of the cells was regulated by specific growth factors, insulin and EGF, and required increased cAMP. Continued growth required extracellular matrix.

The studies show that during repair, tubules are lined with regenerating cells derived from RPTC. The process is regulated by polypeptide growth factors, changes in second messenger levels and extracellular matrix.

TRIFLUOROACETYLATED PROTEIN DISULFIDE ISOMERASE IS A HALOTHANE-INDUCED NEOANTIGEN. J L Martin, J G Kenna, B M Martin and L R Pohl. The Laboratory of Chemical Pharmacology, NIH, Bethesda, MD, Dept. of Anesthesiology, The Johns Hopkins Medical Institutions, Baltimore, MD, and Clinical Neurosciences Branch, NIMH, Bethesda, MD.

The sera of patients with halothane hepatitis contain antibodies directed against halothane-induced hepatocyte microsomal neoantigens (100kDa, 76kDa, 59kDa, 57kDa and 54kDa), that are covalently modified by the reactive trifluoroacetyl halide (TFA-X) metabolite of halothane. Previous studies indicate that these neoantigens may play a role in the development of the patients' liver injury. In the present study a TFA-57kDa neoantigen recognized by the patients' antibodies was purified from liver microsomes of halothane treated rats by DEAE sepharose and hydroxylapatite HPLC. Based upon its apparent molecular weight, isoelectric point and NH2-terminal amino acid sequence, the protein has been identified as protein disulfide isomerase. (J L Martin is supported by a PRAT fellowship from NIGMS).

EFFECTS OF CHLORDEcone (KEPone) ON THE RAT ADRENAL AND PITUITARY GLANDS. L W Chang, University of Arkansas for Medical Sciences, Little Rock, AR and J S Hong, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Our previous study indicated that hyperfunction of the pituitary adrenal axis was induced in rats exposed to chlordecone. Our present report is to present information on morphological alterations in rat adrenal and pituitary glands following a single injection of chlordecone. Male Fischer 344 rats were injected (i.p.) with 75 mg/kg chlordecone and were sacrificed 24 hours, 4 days, and 7 days following exposure. Hypertrophy of the adrenal glands were apparent 24 hours post-injection. Disruption of the cytoarchitecture of the adrenal cortex with hypertrophic changes of the cortical cells, especially those in the zona reticulata, were observed. This change was most prominent 4 days following injection. Electron microscopic examination revealed enlargement of the mitochondria and cytoplasmic degeneration of cells in the zona reticulata. Hypertrophy of the pituitary cortitrophs were also evident 4 days after chlordecone injection. Increased cytoplasmic volume and number of organelles (mitochondria and endoplasmic reticulum) were also noted. The hypertrophic changes in the adrenal cortical and pituitary cells suggest an increased activity of these endocrine systems which supports our previous biochemical findings.

ALLERGIC CONTACT SENSITIZATION POTENTIAL OF HYDROXYCITRONELLAL IN HUMANS. R A Ford, A M Api, and *R R Suskind, Research Institute for Fragrance Materials, Inc., Englewood Cliffs, NJ and * Institute of Environmental Health, University of Cincinnati Medical Center, Cincinnati, OH. Sponsor: O D Easterday

Hydroxycitronellal (HC), an important ingredient in fragrances, was studied for its sensitizing potential in human skin. 15 human maximization tests were conducted with HC obtained from 4 sources at induction concs. from 5% to 12%. No rxns. were induced in 5% in 2 panels; 10% sensitized 2/25 in one test but none in a second. 12% produced sensitization in 8/11 panels. In an initial modified HRIPT 2 positive rxns. to challenge were observed among 197 panelists, 1 at a concn. of 5% & the other at 7.5%. When 100 of the nonreacting panelists were reexposed in the same way, allergic sensitization rxns. appeared during the induction period with concns. as low as 2.5%. When 28 sensitized panelists were exposed to 14 concns. in a simulated use test, there were 3 reactors. A NOEL for sensitization has not been determined although the lowest concns. tested were in the product usage range.
Predictive assays for non-immunologic contact urticaria (NICU) in the guinea pig were performed on 47 cosmetic ingredients not previously reported to produce urticaria. One ear of groups of ten Hartley strain guinea pigs was dosed with 0.1 ml of test material; the opposite ear was dosed with a suitable control. Evaluation was by change in ear thickness measured with a micrometer as evidence of inflammatory edema and by visual examination for discrete lesions and vasoconstriction. Ear thickness measurements and visual examinations were performed 15, 30, 45, and 60 minutes and 24 hours after application of the test material. No significant change in ear thickness was produced by any of the materials at the dose initially tested. Erythema or vasoconstriction was observed after application of 10 materials. Each animal which developed a response was retested with the same material applied to the vehicle control ear at 48-96 hours or another group of animals was tested to confirm the observation. Inflammatory responses to 6 materials were reproduced. The materials tested, concentration, vehicle and results for each assay will be presented.

Topical application of the irritants tetradeacetylphorbol acetate (TPA) or ethyl phenylpropionate (EPP) stimulate prostaglandins (PGs) in animal models. In order to investigate the mechanisms for these effects and anti-inflammatory drug effects, we have performed a series of experiments using human skin organ and cell culture systems. TPA was approximately 1000 fold more potent than EPP for the stimulation of PGE2 production. Dexamethasone (DEX) pretreatment blocked the effects of EPP and blunted the effects of TPA on PG production. EPP and TPA also altered the synthesis of specific proteins in keratinocytes evaluated by SDS-gel electrophoresis. TPA stimulated a cellular protein of 45 KDa while EPP inhibited the synthesis of a protein of 50 KDa; striking effects were also observed on secreted proteins. The relationships between induced proteins and the generation of inflammatory mediators (including PGs) are currently under investigation. This work was supported by the Colgate-Palmolive Fellowship for In Vitro Toxicology in the Laboratory of Cellular Pharmacology.

The combination of psoralens and ultraviolet light (UVA) is a potent cell growth inhibitor. Using mouse sarcoma 180 cells, we investigated the manner by which UVA and several clinically important psoralen analogs induce their biological effects. We found that inhibition of cell growth was dependent on the concentration of psoralen as well as the dose of UVA light. The potent phototoxin 4,5',8-trimethylpsoralen was the most potent psoralen analog tested followed by 5-methoxypsoralen, 8-methoxypsoralen and 5-methylisopsoralen. Analysis of growth inhibition curves revealed that when the cells were treated with increasing concentrations of the psoralens, less UVA light was required to inhibit cell growth than expected by an additive relationship. Multivariate analysis of growth inhibition curves revealed a synergism between psoralens and UVA light. Our results suggest that synergism may be an important property of the psoralens that contributes to the toxicity of this group of phototoxins. Supported by NIH grant ES 03647.
Psoralens in combination with ultraviolet light (PUVA) are potent phototoxins. In the present studies we analyzed the biological effects of PUVA on the cell surface membranes of A431 epidermal cells. We found that PUVA was a potent modulator of epidermal growth factor (EGF) receptor function in these cells. This receptor is a 170 kD transmembrane glycoprotein with intrinsic tyrosine kinase activity. PUVA produced a dose-dependent inhibition of the EGF stimulated tyrosine kinase activity in A431 cells. This inhibition occurred within 45 sec of PUVA treatment and was apparent in both intact cells and in membranes prepared from the cells pretreated with PUVA. In contrast, PUVA failed to alter EGF receptor kinase activity directly in purified plasma membrane fractions of A431 cells. These results confirm that the cell membrane is a target for the psoralens and that intact cells are required for PUVA induced phototoxicity. Supported by NIEHS grant ES 03647.

The Draize test is an in vivo assay used to evaluate the potential ocular toxicity of compounds such as benzalkonium chloride, thimerosal and the phorbol ester, TPA. Due to concerns of interlaboratory variability of Draize test results, we were interested in developing an in vitro system to assess corneal toxicity. Eyes of CD-1 mice were treated with 100 ng/ml phorbolester, TPA. After 4 or 24 hrs, corneal cells were isolated and characterized morphologically. Flow cytometry was used to identify subpopulations of corneal cells based on size and density/granularity differences. Using specific antibodies to identify cytokeratin proteins and the class II MHC histocompatibility antigen, Ia, we identified corneal epithelial cells and Langerhans cells. Using specific probes, we also detected reactive oxygen intermediate production by corneal subpopulations. This method allows in vivo treatment of corneal cells with compounds of ocular toxic potential and provides a sensitive means of assessing morphological alterations and functional changes after ocular exposure.
The administration of high dosages of HMG CoA reductase inhibitors (HMGRI) resulted in the development of subcapsular lenticular opacities in dogs. Similar doses were without effect in other species tested. While dogs experienced profound decreases in serum cholesterol, a causal relationship between serum cholesterol lowering and cataract formation was not described. A strong relationship existed however between systemic exposure to inhibitor and the cataractogenic potential of these compounds. Analysis of lenses from dogs chronically treated with these agents revealed no correlation between the amount of drug associated with the lens and the presence of cataracts and no changes in cholesterol content or steroid composition. The kinetics of drug appearance in the aqueous and lens cortex was assessed and suggested slightly higher peak concentrations of drug were achieved by inhibitors which produced a higher incidence of cataracts. These data suggest that high doses of HMGRI may increase lenticular exposure to drug via a more viscous humor by producing a substantial systemic exposure to drug. This may result in increased concentrations of drug in the outer cortical regions where cholesterol synthesis is most critical. These data also predict a negligible risk of cataract development in man since the low doses employed clinically result in a very low systemic exposure to drug substance.

The metabolism of trans, trans-muconaldehyde (MUC), a hematotoxic microsomal metabolite of benzene, was studied. Purified yeast aldehyde dehydrogenase (ALDH) in the presence of Na\(^+\) metabolizes MUC at a maximal velocity of 862 nmol min\(^{-1}\) mg\(^{-1}\) protein with a Km of 0.95 μM based on the production of NADH measured spectrophotometrically at 340 nm. Enzyme inhibition was observed at MUC concentrations greater than 10 μM. Using TLC and HPLC techniques, a product with intermediate polarity compared with that of the dialdehyde (MUC) and the dicarboxylic acids was detected in both the ALDH and DBA/J mouse liver cytosol incubation mixtures. Mass spectral analysis supports the structural assignment of OCR-C\(^=\)CH-C\(^=\)CH-COOH for this intermediate. In the presence of liver cytosol supplemented with Na\(^+\) this intermediate was metabolized to MA. These findings indicate that MUC is metabolized by aldehyde dehydrogenase to a monoa-}

**EFFECT OF INHALATION ANESTHETICS ON THE UDP-GLUCURONIC ACID PATHWAY IN MOUSE LIVER.** J.B. Watkins III and D.R. Engles, Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN.

Large, rapid decreases in hepatic UDP-glucuronic acid concentrations, which occur following exposure to myriad chemicals, affect the subsequent glucuronidation of many drugs. To elucidate the mechanisms responsible for these changes, the effect of anesthesia on the substrates and enzymes for the synthesis and degradation of UDP-glucuronic acid was determined in male and female Swiss-Webster mice. Inhalation of 2.5% enflurane, 3.3% halothane, 3.5% isoflurane and 3.5% sevoflurane decreased the concentrations of UDP-glucuronic acid by 30-57%. UDP-glucose levels were decreased by 20% in male mice exposed to enflurane and sevoflurane, and by 60% in female mice anesthetized with halothane and sevoflurane. Activities of UDP-glucose dehydrogenase and UDP-glucuronosyltransferase were unaffected by exposure to volatile anesthetics. Nucleotide pyrophosphatase activity was increased by 67-69% in female mice after inhalation of halothane, isoflurane and sevoflurane. Kinetic studies indicated that the V\(_{\text{max}}\) for hydrolysis of 4-nitrophenol-thymidine 5'-phosphate ester by nucleotide pyrophosphatase was increased by 55-65% in female mice exposed to halothane, isoflurane and sevoflurane, whereas the Km for this reaction was unchanged. Thus, the alterations in nucleotide pyrophosphatase kinetics may be partly responsible for the decreased hepatic UDP-glucuronic acid concentrations observed in mice exposed to volatile anesthetics. (Supported by the PMF Fdn and AMA-Ed & Res Fdn).

**SPECIES DIFFERENCES IN THE IN VITRO GLUCURONIDATION OF DIGITOXIN (DT\(_3\)) AND DIGITOXIGENIN MONODIGITOXOSIDE (DT\(_1\)).** D.C. Eberhart, M.R. Halvorson and A. Parkinson, Kansas University Medical Center, Kansas City, KS.

We have shown previously that liver microsomes from various mammalian species catalyze markedly different pathways of DT\(_3\) oxidation. The purpose of the present study was to determine whether such marked species differences also apply to the glucuronidation of DT\(_3\) and DT\(_1\) (which is a metabolite of DT\(_3\) formed by rat liver microsomal cytochrome P-450). In the presence of the zwiterionic detergent CHAPS, liver microsomes glucuronidated DT\(_3\) in the rank order mouse > dog > rat > cynomolgus monkey > rabbit > hamster > human > guinea pig > cat. There was no relationship between the ability of the different microsomal samples to convert DT\(_3\) to DT\(_1\) and their ability to glucuronidate DT\(_1\). In the presence of CHAPS, liver microsomes glucuronidated DT\(_3\) in the rank order hamster >> dog >> rabbit >> monkey > rat > mouse >> human >> guinea pig. With both substrates, lower rates of glucuronidation and a slightly different rank order were observed when CHAPS was omitted from the incubation mixture. The ability of hamster liver microsomes to glucuronidate DT\(_3\) 100 times faster than rat liver microsomes explains in part why hamsters are more resistant than rats to the toxic effects of DT\(_3\). Similarly, the inability of cat liver microsomes to catalyze either the oxidation or glucuronidation of DT\(_3\) may be responsible in part for the marked sensitivity of cats to DT\(_3\) toxicity. Supported by NIH grants GM 37044, ES 00166 and ES 07079.
33 METABOLISM OF DIGITOXIN (DT₃) IN RATS AND HAMSTERS AND ITS RELATIONSHIP TO DIFFERENCES IN DT₃ TOXICITY. C Dorian, M R Halvorson, and A Parkinson. Kansas University Medical Center, Kansas City, KS. 66103

Whereas 10 mg/kg DT₃ is lethal to rats, hamsters tolerate dosages over 500 mg/kg. To determine why hamsters are essentially resistant to the toxic effects of DT₃, we have identified by HPLC the oxidative pathways of DT₃ biotransformation catalyzed by liver microsomes from rats and hamsters. Rat liver microsomes converted DT₃ primarily to digitoxigenin bidigitoxoside (DT₃G). A similar reaction converted DT₃ to DT₂ and DT₁. Unlike DT₃G, DT₂ was glucuronidated by rat liver microsomes. In contrast, hamster liver microsomes converted DT₃ primarily to 16- and 17-hydroxy-DT₃. Unlike the rat, liver microsomes from the hamster glucuronidated DT₃ directly. To assess the significance of these differences in digitoxin biotransformation, we attempted to measure the plasma half-life of DT₃ in rats and hamsters. This experiment was complicated by the fact that surgical implantation of arterial and venous catheters in hamsters caused a prolonged suppression (7 days) of liver microsomal cytochrome P-450. Consequently, rats and hamsters were administered [³H]-DT₃ (10 mg/kg) intraperitoneally, and blood was collected from 15 min to 4 h. The peak concentration of DT₃ was much greater in rat than hamster plasma, and the rate of elimination of DT₃ was greater from hamster plasma. These differences in the biotransformation and pharmacokinetics of DT₃ explain in part the marked difference in the susceptibility of rats and hamsters to the toxic effects of DT₃. Supported by NIH grants GM 37044 and ES 00166.

34 SPECIES DIFFERENCES IN TESTOSTERONE HYDROXYLATION BY LIVER MICROSONES: STUDIES WITH ANTIBODY AGAINST RAT CYTOCHROME P-450p. M R Halvorson, A J Sonderman and A Parkinson. Kansas University Medical Center, Kansas City, KS.

We have shown previously that, with few exceptions, 6β-hydroxytestosterone is the major pathway of testosterone oxidation catalyzed by liver microsomes from rat, mouse, hamster, guinea pig, rabbit, dog, cat, cynomolgus monkey and human. We have also shown that 6β-hydroxylation is closely associated with the 2β- and 15β-hydroxylation of testosterone, and have proposed that, in rat liver microsomes, all 3 reactions are catalyzed by cytochrome P-450p. The present study shows that antibody raised against cytochrome P-450p inhibited >75% of the 6β-hydroxylation of testosterone catalyzed by liver microsomes from all species tested, except rabbit (~30% inhibition). In most cases, the antibody also inhibited >80% of the 2β- and 15β-hydroxylation of testosterone, indicating that cytochrome P-450p (or related isozymes) is a major contributor to these pathways of testosterone oxidation. Antibody against cytochrome P-450p recognized 2 proteins in rat liver microsomes, both of which were inducible by treatment of rats with PCN, dexamethasone or tremolamycin. However, the 2 proteins differed in their developmental expression, and only one of them was inducible by treatment of rats with phenobarbital. It remains to be determined whether one or both forms of cytochrome P-450 p convert testosterone to 2β-, 6β- and 15β-hydroxytestosterone. Supported by NIH grants GM 37044, ES 00166 and ES 00709.

35 REACTION OF GLUTATHIONYL RADICAL (GS') WITH HYDROXYECOSANOIC ACIDS: A NEW MECHANISM FOR FORMATION OF LEUKOTRIENE C-LIKE COMPOUNDS. G L Foureman, J Curtis, and T E Eling. Laboratory of Molecular Biophysics, NIH, RTP, NC.

Oxidation of glutathione (GSH) by prostaglandin H synthase peroxidase results in formation of GS', which forms GSH conjugates independent of epoxide formation and glutathione transferase activity. A structural requirement for compounds to undergo GS' addition is the presence of a conjugated alkene. As 5-hydroxyeicosatetraenoic acid (5-HETE), a precursor of leukotriene (LT) formation, has such a structure we reasoned that 5-HETE may support the addition of GS' thereby forming LTC-like compounds. To test this hypothesis, [³H]-5-HETE (60 μM final) was added to GSH generating systems (either 0.08 mg/ml horseradish peroxidase [HRP] with 0.1 mM H₂O₂ or 0.5 mg/ml ram seminal vesicle microsomes [RSV] with 0.1 mM arachidonic acid [AA], 0.12 mM phenol, and 2.6 mM GSH), and incubated for 15 min. HPLC analysis of the mixtures with the HRP system showed 94% & the RSV system 47% of [³H]-5-HETE eluting not as 5-HETE but as polar, LTC-like material. Formation of this LTC-like material was dependent upon components of the generating systems: -HRP, 43%, boiled RSV, 12%; -GSH, 15% in HRP, 10% in RSV; -H₂O₂ in RSV, 47%; -AA in RSV, 41%; -phenol, 41% in HRP, 21% in RSV. This reaction may represent a new pathway for synthesis of LTC-like compounds in peroxidase-rich tissues.

36 GAS CHROMATOGRAPHY/MASS SPECTROMETRY OF LSD IN HUMAN PLASMA. D J Papac and R L Foltz, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT. Sponsor: G S Yost

The pharmacologic potency of lysergic acid diethylamide (LSD) combined with its rapid and extensive metabolism has made the detection of LSD in biological fluids extremely difficult. A GC/MS method has been developed which involves the formation of the N-trifluoroacetyl derivative and utilizes a fused-silica, dimethylsilicone capillary column, 12.5 meters in length with a film thickness of 0.33 microns. The temperature is programmed from 180 to 300 ⁰C at 1 ⁰C/min, and under these conditions the LSD-TFA elutes at approximately 6.5 minutes. Quantitation is performed based upon the ion ratio of the peaks at m/z 419 and 429 that are formed by electron capture ionization, corresponding to the molecular ions for the LSD-TFA and the deuterated analog. LSD taken orally at 1 μg/kg by a volunteer produced a peak plasma concentration of 1.9 ng/ml at 3 hours with an estimated half life of 5 hours. The described method can now be used to quantitate LSD reliably in plasma to 0.1 ng/ml. Supported by the Office of Naval Research, Contract N00014-85-C-0336.
RECENT DEVELOPMENTS IN THE ANALYSIS OF URINARY MERCAPTURIC ACID CONJUGATES USING TANDEM MASS SPECTROMETRY. C K Winter, A D Jones*, and T M Dinoff. Department of Entomology, University of California, Riverside, CA and *Facility for Advanced Instrumentation, University of California, Davis, CA.

Techniques applicable for the screening and quantitative analysis of urinary mercapturic acid conjugates of xenobiotics have been developed using a double-focusing mass spectrometer of reverse geometry. Conjugates are identified using tandem mass spectrometry linked scanning techniques monitoring the constant neutral loss of 129 daltons. This fragmentation is selective and characteristic for mercapturic acid conjugates. Direct analysis of urine extracts using solid probe techniques yields detection limits in the low nanogram range, while derivatization of the urine extracts with pentafluorobenzyl bromide allows for sensitive and selective GM/MS/MS analysis of the conjugates using electron capture ionization with detection limits in the sub-picogram range. Accurate quantitation is achieved through the use of stable isotopically-labelled internal standards. These techniques have been successful in the analysis of urine samples obtained from animal metabolism studies and from urine samples spiked with standards of synthetic mercapturic acid conjugates.

DEVELOPMENTAL CHANGES IN THE LEVELS OF A RAT LIVER MICROSMAL CARBOXYSTERASE (HYDROLASE A). E W Morgan, G Wood, D J Greenway and A Parkinson. University of Kansas Medical Center, Kansas City, KS.

Rat liver microsomes contain multiple forms of carboxysterases. These hydrolytic enzymes play an important role in the detoxication of xenobiotics in general, and of organophosphate insecticides in particular. Two carboxysterases, designated hydrolases A and B, were purified from rat liver microsomes, and shown by N-terminal amino acid sequence analysis to be distinct but structurally related isozymes. Antibody raised in rabbits against hydrolase A cross-reacted with hydrolase B. The cross-reacting antibodies were removed by immunoadsorption against hydrolase B bound covalently to Sepharose 4B. The immunoadsorbed antibody was used to study the developmental expression of hydrolase A in male and female Sprague Dawley rats, as determined by Western immunoblot. Hydrolase A was barely detectable in liver microsomes from 1- and 2-week-old rats, but was expressed at "adult" levels in rats 3 weeks of age or older. The ontogenic and absolute levels of hydrolase A were the same in male and female rats. The developmental expression of hydrolase A coincided with weaning, and, hence, coincides with an increase in exposure to xenobiotics in the diet. Interestingly, the developmental expression of this carboxysterase coincides with an age-dependent decrease in organophosphate toxicity. However, it remains to be established whether the expression of hydrolase A is causally related to the decrease in organophosphate toxicity in post-weaning rats.

COMPARATIVE PHARMACOKINETICS OF RETINOIC ACID DERIVATIVES IN PREGNANT HAMSTERS. R P Sharma, W B Howard, C C Willhite, and S T Omaye. Utah State University, Logan UT, California Dept. of Health Services, Berkeley CA, and Letterman Army Institute of Research, San Francisco CA.

Plasma uptake and disappearance of 8 retinoic acid congeners was investigated after oral administration of 35 nMol/Kg. Blood samples were collected at various time intervals and chemicals analyzed in plasma by HPLC and scintillation counting. All-trans-retinoic acid (RA) elimination half-life (t1/2) was 0.5 h and for 13-cis-retinoic acid (cRA) the t1/2 was 4.4 h. cis/trans isomerization of RA and cRA occurred in vivo, and both were oxidized to their respective 4-oxo derivatives. The t1/2 for 4-oxo-all-trans-retinoic acid was 5.7 h. For 9-cis-retinal and retinyl acetate, no parent chemicals were discerned by HPLC, the radioactivity persisted for 96 h. For N-ethyl-retinamide (NERA) and its 13-cis-congener (cHERA), the t1/2 values were 2 and 3 h, respectively; the former showed a biphasic decline. cis/trans isomerization of the retinamides accounted for the main biotransformation. Bioavailability of retinamides was 0.1x of free acids. For Ro 13-7410 at 100 µg/kg dose, the biphasic t1/2 were 1.5 and 2.5 h, respectively. Pharmacokinetics appeared to have a limited effect on retinol teratogenic potency. (Supported in part by HD 21399 and March of Dimes Foundation.)


Misonidazole (MISO) is a radiosensitizer which undergoes extensive reductive biotransformation in hypoxic tissue to form reactive metabolites. The purpose of this study was to determine if methyl substitution on position 4 of the imidazole ring of MISO would decrease metabolite reactivity. Biotransformation of tritiated 4-methylmisonidazole was examined by analysis of perfusion medium and bile. Protein covalent binding and hepatic glutathione concentrations were determined. 4-Methylmisonidazole undergoes limited biotransformation and produces less covalent bonding than MISO. However, 50 umol perfusions of 4-methylmisonidazole depleted hepatic glutathione by 53% while similar perfusions of MISO produced a 10% depletion. Thus, 4-methylmisonidazole represents a novel 2-nitroimidazolide which depletes hepatic glutathione without extensive biotransformation or protein covalent binding in the isolated perfused hypoxic rat liver. (Supported in part by Grant # CA 40284).

Japanese medaka is a species being considered in the development of alternative, short-term, low-cost carcinogen screening assays. An understanding of the metabolic capabilities of medaka for various classes of carcinogens is essential to characterize their potential use in carcinogen bioassays. Primary aromatic amines were selected for this study, with particular interest in N-oxidized metabolites. A method using reverse-phase gradient elution HPLC and liquid scintillation techniques was developed. Metabolic formation of phenylhydroxylamine was confirmed by matching the HPLC retention times for the radioactivity elution with the u.v. spectra for the spiked standard. Aniline was converted into phenylhydroxylamine at rates of 86 or 238 pmole/min per nmole P450 by rainbow trout or medaka, respectively. Trout and medaka microsomes N-hydroxylated p-chloroaniline at rates of 51 or 86 pmole/min/nmole P450, respectively. Some non-hydroxylated products or other N-oxidized metabolites such as nitrobenzene, nitrosobenzene, azobenzene or azoxybenzene were not detected. Phenylhydroxylamine formation was not evident in controls with boiled microsomes or in the absence of NADPH.

BIOTRANSFORMATION OF CYANIDE BY RHODANASE LOADED MURINE CARRIER ERTHROCYTES. P Leung, E Cannon, D M Sylvester, and J L Way, College of Medicine, Texas A & M University, College Station, TX.

Resealed carrier erythrocytes (RBC) containing rhodanese (1.6 IU) and thiocyanate were prepared by hypotonic dialysis. The loaded RBC were administered iv 24 hours prior to being challenged with a sub lethal dose of potassium cyanide (KCN, 5 mg/kg, sc) to evaluate its ability to metabolize cyanide. In control studies, apparent blood levels of cyanide in mice treated with KCN rose rapidly to maximal level within 5 minutes and declined exponentially. Similar blood levels of cyanide were obtained with mice pretreated with normal RBC. In contrast, mice treated with the concomitantly rhodanese and thiocyanate prior to cyanide administration produce a significant decrease in blood levels of cyanide with a comitant elevation in plasma levels of thiocyanate. More important, there is very little if any endogenous detectable plasma thiocyanate obtained from mice treated with normal RBC. These results suggest that carrier RBC represent a viable approach for the detoxification of cyanide. (Supported by funds from NIEHS, NICHS, NSF, USAMRC and Texas A & M University).

METABOLISM OF PHENAZOPYRIDINE (PAP) BY PRIMARY RAT HEPATOCEYES. B H Thomas, L W Whitehouse, G Solomonraj, and A Paktus. Drug Toxicology Division, Health and Welfare Canada, Ottawa, Ont., Canada.

The metabolism of the urinary tract analgesic PAP by male Wistar rat hepatocytes was studied in order to determine the role of the liver in the in vivo fate of PAP, and the enzyme systems involved. The metabolites of PAP were measured by HPLC using a method capable of measuring all the azo and non-azo metabolites. All the azo metabolites were seen in the urine of PAP-dosed rats after incubation of PAP (4 X 10^-4M) with hepatocytes for up to 1h, but formation of the 5,4'-dihydroxy metabolite was erratic. No cleavage of the azo bond was observed contrary to the in vivo situation, which suggests the involvement of gut bacteria. We have shown that the azo bond of PAP is readily cleaved by the contents of the rat intestinal tract. Addition of SKF-525A (2.5 X 10^-4M) inhibited both 5 and 4'-hydroxylation, but metyrapone and o-naphtho- flavone (1.0 X 10^-4M) both failed to produce any significant inhibition. Pretreatment of the rats with phenobarbital had no effect on metabolism, but Arochlor 1254 increased both 5 and 4'-hydroxylation. 8-Naphtho flavone pretreatment increased only 4'-hydroxylation. These data show that PAP hydroxylation is not due to the classical phenobarbital or 3-methylcholanthrene inducible P-450 isoenzymes.


The proposed ultimate mutagenic metabolite of acrylonitrile, 2-cyanoethylene oxide (ANO), induces a 10-fold increase above the spontaneous (spon.) thymidine kinase- (-tk-), and hypoxanthine-guanine phosphoribosyltransferase- (-hprt-) mutant fraction in TK6 human lymphoblastoid cells (TK6 cells), at a 10-fold lower concentration than the parent compound. Several spon. and ANO-induced tk-/- and hprt- mutants were isolated and molecular analysis of the mutations was performed by Southern blot hybridization studies of the tk gene and deoxy DNA sequencing of the coding regions of hprt. Two phenotypic classes of tk-/- mutants that differ in their growth rates were analyzed. One class (tkr) has a normal growth rate relative to TK6 cells while the second class of mutants (tks) grows at a slower rate. For Southern analysis of the tk gene, a 14.8kb band corresponding to the active tk allele was analyzed for alterations. 22% of the spon. and 8% of the ANO-induced tk mutants analyzed had lost the 14.8kb band. In contrast, 96% of the tks mutants analyzed (spon. and ANO-induced) had lost the 14.8kb band. Since ANO-induced tks mutants are a minor fraction of the total ANO-induced mutants, a collection of ANO-induced hprt- mutants were isolated to determine the hprt DNA sequence alterations. ANO induced exon loss in the hprt gene in 64% of the mutants examined (9/14), and point mutations at both G:C and A:T base pairs (5/14). These data indicate that ANO can induce a diverse class of mutations in human cells.
FORMATION AND PERSISTENCE OF 7-(2'-OXOETHYL)GUANINE AND N2,3-ETHENOGUANINE IN RAT TISSUE DNA AFTER VINYL CHLORIDE EXPOSURE.

N Fedtke, J A Boucheron, M J Turner Jr, J A Swenberg, CIIT, Research Triangle Park, NC.

The DNA adducts N2-ethenoguanine (eG) and 7-(2'-oxoethyl)guanine (OEG) are formed in experimental animals after vinyl chloride exposure. Highly sensitive methods were developed for the quantitative determination of these adducts in DNA hydrolysates (deproteinization by mild acid hydrolysis). For analysis of OEG, a combined reversed phase/cation exchange (RP/SCX) HPLC column with fluorescence detection (excitation 255 nm, 340 nm emission filter) was used. The limit of detection was 15 pmol OEG per µmol unmodified guanine. For analysis of eG, isotope dilution mass spectrometry was used; eG was isolated from DNA hydrolysates by low pressure strong cation exchange chromatography and subsequent C-18 sorbent extraction. The purified eG was derivatized with pentafluorobenzyl bromide (electrophore labeling). The resulting dibenzylated derivative was quantitated by monitoring the characteristic fragment ion m/z 354 in negative ion chemical ionization GC-MS. The internal standard was eG labeled with 13C by four positions. The limit of detection was 60 fmol eG per µmol unmodified guanine. The formation of OEG and eG was determined by these methods in liver DNA of pre-weaning Sprague-Dawley rats exposed to 600 ppm vinyl chloride by inhalation (4 hrs/day; 5 days). Concentrations found immediately following the last exposure were 247 ± 23 pmol OEG and 1.7 ± 0.2 pmol eG per µmol guanine, respectively (n=6). Current studies are quantitating these adducts in other tissues and determining their persistence after exposure.

DETERMINATION OF DNA DAMAGE MECHANISMS IN VITRO BY THE USE OF RAPID GEL ASSAYS.


The acidine half-mustard ICR-170 induces revertants in Salmonella strains containing a frameshift mutation. On that basis, as well as its similarity to the known DNA intercalator proflavine, it is assumed to be a DNA intercalator. Using ICR-170 as a model compound, we report here the application of simple assays that can rapidly and unambiguously determine in vitro mechanisms by which a chemical causes DNA damage: 1) supercoiled DNA treated with ICR-170 and then the AP endonuclease associated with E. coli exonuclease III is converted to nicked circular DNA, indicating that ICR-170 induces AP sites; 2) DNA ligase converts nicked circular DNA into closed circular DNA with a small number of supercoils; inclusion of ICR-170 in the ligase reaction increases the number of resulting supercoils due to the ICR-170-DNA intercalation; 3) linear DNA treated with bifunctional quinacrine mustard (QM), denatured and electrophoresed on non-denaturing gels contains interstrand crosslinks, evidenced by the appearance of double-stranded DNA; crosslinks are not observed in ICR-170-treated DNA; 4) ICR-170 treatment of supercoiled DNA under standard conditions does not produce nicked circular DNA. Thus ICR-170 intercalates into DNA, but also induces AP sites. ICR-170 does not in and of itself cause DNA strand breaks or interstrand crosslinking. In all cases the assays use at the most µg amounts of chemical, and since the reaction products are separated by simple, low-cost gel electrophoresis these assays are also extremely rapid. They are ideally suited to structure/activity relationships and can serve as a valuable adjunct to standard short-term genotoxicity tests.

EVALUATION OF THREE POTENTIAL MECHANISMS FOR THE INDUCTION OF CHROMOSOME ABERRATIONS BY BROPIRIMINE (U-54,461).


Broprimine (ABPP; U-54,461) at high concentrations, in the presence of a rat liver activation system (S9), induces chromosome aberrations in Chinese Hamster Ovary (CHO) cells. We have evaluated three potential mechanisms for this effect. In the first, bioactivation of broprimine to reactive species was assessed by quantifying covalent binding to protein and DNA. No evidence of enzyme-catalyzed covalent binding of ABPP to either macromolecule was observed. In a second series of experiments, the lysosomal toxicity of ABPP was assessed by following the loss of lucifer yellow from lysosomes of CHO cells by fluorescence microscopy. Minor alterations in lysosomal morphology were observed, but no leakage of lucifer yellow was detectable. A third hypothesis, enhanced uptake of ABPP in the presence of S9, was examined by following the uptake of 14C-ABPP into CHO cells vs time. Under the conditions of the assay, no evidence of enhanced uptake, as measured by post-wash radioactivity, was observed. These data indicate that additional experiments, including those examining alternate mechanisms, must be conducted to explain ABPP-induced chromosome aberrations.

EFFECT OF NALIDIXIC ACID ON DNA REPAIR IN RAT HEPATOCYTES.

A McQueen, R R Rosado and G M Williams. American Health Foundation, Valhalla, NY.

Several quinolones have been synthesized and found to be effective antibiotics. The first of these compounds to be used was nalidixic acid (NA). Unlike many of the newer quinolones, NA did not induce unscheduled DNA synthesis in hepatocytes following in vitro exposure. Since there have been reports that NA inhibits DNA repair, the effect of NA on DNA repair in hepatocytes was investigated as a basis for this negative result. Rat hepatocytes were isolated by collagenase perfusion and monolayer cultures established. DNA was damaged by ultraviolet (UV) light or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). Cultures were incubated with 3H-thymidine in the presence and absence of NA or aphidicolin, a DNA polymerase inhibitor. DNA repair was determined by autoradiography. Cells exposed to 50 J UV/m2 had grain counts of 61.6±12.5 compared to 58.8±16.7 in cultures incubated with NA following UV exposure. Similar results were observed when damage was induced with MNNG. In contrast, the addition of aphidicolin prevented repair synthesis. Thus, NA did not inhibit DNA repair. Consequently, the negative results previously observed were not due to interference with the test endpoint; rather, they indicate that NA does not damage DNA.
IN VITRO TESTING USING THE SISTER CHROMATID EXCHANGE ASSAY: CORRELATION WITH IN VITRO CHROMOSOMAL ABERRATION ASSAY AND SALMONELLA ASSAY. KS Loveday, Arthur D. Little, Inc. Acorn Park, Cambridge, MA 02140. Sponsor: A. Silvek

62 coded chemicals were tested for induction of SCEs and chromosomal aberrations in CHO cells. These chemicals were also tested in other laboratories for Salmonella mutagenesis. The SCE results were compared with the other two assays. The goal was to obtain an experimentally defined "potency" level for percent increase in SCEs which would correlate with other genetic endpoints. The 62 chemicals were ranked according to the percent increase of induced SCEs. 19 chemicals induced at least a 100% increase in SCEs, and 16 of these induced either chromosomal damage or mutations (positive correlation of 84%). As the "potency" level was decreased, the positive correlation also decreased. Only 59% of the chemicals which induced at least a 20% increase in SCEs also induced aberrations or mutations. The negative correlation showed a reverse trend. 74% of the chemicals which had less than a 100% increase in SCEs were negative in the other two tests. For the chemicals which had less than a 20% increase in SCEs 94% were negative in the other two tests. Although an SCE increase of 50% seemed the best compromise for correlating SCE with other genetic endpoints, the SCE test seems most useful for defining chemicals which are non-genotoxic.

METHYLENE BLUE PLUS LIGHT CAUSES FORMATION OF 8-HYDROXY-2'-DEOXYGUANOSINE FORMATION IN DNA AND 8-HYDROXY-GUANOSINE FORMATION IN RNA. R A Floyd, M S West, K Euff, J E Schneider, and X Zhu. Oklahoma Medical Research Foundation, Molecular Toxicology Research Group, Oklahoma City, OK.

We have discovered that methylene blue plus light (MBh+L) causes formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in DNA and 8-hydroxyguanosine (8-OHG) in RNA. The amount of 8-OHdG formed in DNA by MBh increases as a function of time in white light and as the MB concentration increases. When the nucleoside of adenine, cytosine, thymine and guanine are subjected to MBh treatment, only deoxyguanosine reacts based on the recovery of the nucleosides. Using HPLC-electrochemical detection, 8-OHdG is effectively formed in the system. Using the same approach we have demonstrated that 8-OHG is formed from guanosine either as a part of RNA or as the free nucleoside. This research was supported in part by NIH Grant No. CA42854.

OXIDATIVE DNA DAMAGE IN LIVERS OF SPRAGUE-DAWLEY RATS TREATED WITH 2-NITROPROPANE. C C Conaway, E S Fiala, and J E Mathis. American Health Foundation, Valhalla, NY.

2-Nitropropane (2-NP) is a potent hepatocarcinogen in Sprague-Dawley rats; the mechanism of its carcinogenicity has not been heretofore examined. In support of our hypothesis that the genotoxicity of 2-NP may be due to reactions of oxygen generated during its metabolism we report that one-electron oxidation of 2-NP anion by horseradish peroxidase-H_2O_2 causes oxidation of guanine (G) in calf thymus DNA to 8-hydroxyguanine (8-OH-G) in vitro. Treatment of male Sprague-Dawley rats p.o. with 100 mg/kg 2-NP resulted in 2.4 fold (p = 0.07) and 3.4 fold (p < 0.001) increases in 8-OH-G in liver DNA isolated from rats sacrificed 3 hr and 6 hr, respectively, after dosing. In contrast, no significant changes in 8-OH-G were observed in liver DNA from rats similarly treated with non-carcinogenic and nonmutagenic 1-nitropropane. Supported by NIH Grant ES03257 and NCI Grant CA17613.
Increased frequencies of both aneuploidy and chromosomal aberrations have been reported in workers occupationally exposed to benzene. We have therefore studied the aneuploidy-inducing and clastogenic properties of benzene metabolites using the induction of micronuclei (MN) in cytokinesis-blocked human lymphocytes. An anti-kinetochore antibody was employed to distinguish chromosome containing MN from those containing acentric fragments. In vitro treatments with the benzene metabolites, hydroquinone (HQ) and 1,4-benzoquinone (BQ), resulted in significant increases in MN. HQ induced a dose-dependent increase in MN with 150 μM HQ producing a 10-fold increase. An elevated level of both kinetochore-positive and negative MN was also observed, indicating that HQ has both aneuploidy-inducing and clastogenic effects. BQ was acutely toxic at low concentrations (2-5 μM). Surviving cells exhibited a minor increase (∼2-fold) in MN but no dose-response was observed. The frequencies of cells containing kinetochore-positive MN were similar for both BQ and control cells.

Supported by NIH grant P42 ES04705. Work performed in part under the auspices of US DOE by the LLNL under contract no. W-7405-ENG 48.
MODULATION OF CHEMICALLY-INDUCED HEPATOXICITY BY ALTERING LIVER MACROPHAGE FUNCTION. D L Lessin, A J Wasserman, C R Gardner. Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ.

Treatment of female Sprague-Dawley rats with hepatotoxicants such as acetaminophen (AA) (1.2 g/kg) results in a rapid (within 24 hr) accumulation of activated macrophages in the liver in the absence of necrosis. These macrophages display altered morphology, enhanced phagocytosis, cytotoxicity and production of reactive oxygen intermediates. In the present studies we determined if alterations in macrophage function modified the hepatotoxicity of AA. We found that pretreatment of rats with agents that activate liver macrophages, such as lipopolysaccharide (5 mg/kg), enhanced AA hepatotoxicity as evidenced by the early development of necrosis. In contrast, accumulation of macrophages in the liver as well as subsequent toxicity following AA exposure were blocked by treatment of rats with dextran sulfate (5 mg/kg), an agent known to inhibit macrophage function. These results provide additional support for our model that activated macrophages contribute to hepatotoxicity. Supported by NIH grant GM34310.

DELTA-9-TETRAHYDROCANNABINOL INHIBITS MACROPHAGE PROTEIN EXPRESSION ELICITED BY IMMUNOMODULATORS. G A Cabral, F Strudbeck, and L Pringle. Department of Microbiology and Immunology, Virginia Commonwealth University, Richmond, VA. Sponsor: S G Bradley

We have shown that Delta-9-tetrahydrocannabinol (THC), the major psychoactive component of marijuana, inhibits macrophage "full activation" as exemplified by decreased contact-dependent tumoricidal activity. THC did not alter attachment of macrophages to target cells, suggesting that the drug suppressed the synthesis and/or expression of effector molecules. Thus, the objective of this study was to determine the effect of THC on protein expression elicited by modulators which prime and/or trigger macrophages in the activation process. Peritoneal macrophages of (B6C3F1 mice receiving Propionibacterium acnes (P. acnes) exhibited a novel protein profile when compared to resident macrophages. In contrast, macrophages from mice treated with P. acnes in concert with THC exhibited profiles which reverted to those of resident macrophages. Similar inhibition in the expression of restricted protein subsets was obtained when the murine macrophage line P388D1 (DBA/2) was treated in vitro with gamma interferon and/or bacterial lipopolysaccharide in concert with drug. These results suggest that THC alters macrophage competence by suppressing the expression of effector molecules.

ALTERATION OF ENDOOTOXIN SENSITIVITY BY T-2 TOXIN. M J Taylor and C Frayssinet. CNRS, Institut de Recherches Scientifiques sur le Cancer, Villejuif, France and NIH, NIEHS, NTP, Research Triangle Park, NC. Sponsor: M I Luster

T-2 toxin (T-2) is a trichothece mycotoxin produced by several Fusarium species, a feed contaminant, and immunotoxin. An immunotoxic relationship between T-2 and endotoxin (E coli O55:B5, a cell wall component of gram negative bacteria, was characterized. Endotoxins share many of their diverse, immunomodulatory activities with T-2. Endotoxemia and hypothermia (a manifestation of endotoxemia) were observed in male, swiss mice following T-2 exposure (po, 8 mg/kg). Endotoxin-induced (ip, 50μg) hypothermia decreased dramatically in endotoxin pretreated (5 μg/day/5 days) mice. However, T-2 induced hypothermia was not reduced by endotoxin pretreatment, indicating that T-2 induced hypothermia was not directly linked to endotoxemia. This phenomenon was demonstrated similarly by use of endotoxin responsive and nonresponsive mouse strains. Both hypothermia and mortality increased, following an apparent synergy of T-2 and endotoxin in mice treated simultaneously with T-2 and endotoxin. The magnitudes of these responses depended upon the dose of T-2 toxin. In mice, pretreated with endotoxin, and then simultaneously treated with T-2 and endotoxin, both mortality and hypothermia decreased significantly. Endotoxin resistance was compromised by acute exposure to T-2; however, endotoxin pretreatment reduced this effect. The effect of T-2 on endotoxin sensitivity may be an important factor in the evaluation of host resistance in T-2 treated animals.

IMMUNOTOXIC EFFECTS OF A DRINKING WATER CHEMICAL MIXTURE IN MICE. D R Germaine, R S H Yang, M F Ackermann, G J Rosenthal and M I Luster. DTRT, NTP, NIEHS, Research Triangle Park, NC

Immune function studies were conducted in B6C3F1 mice which were exposed for either 14 or 90 days via drinking water to a chemical mixture. The mixture consisted of the 25 most common ground water contaminants found near toxic waste dumps, as determined by EPA surveys. At the maximum concentration used in this study, 5 chemicals were >10-fold higher, 1 each was 10-fold higher, and 8 were <1 times the average EPA survey level. None of the animals developed overt signs of toxicity such as body or liver weight changes. Mice exposed to the maximum concentration of this mixture for 14 days or a 50 % dilution for 90 days showed immune function changes which were related to rapidly proliferating cells, including suppression of hematopoietic stem cells and antibody synthesis. Some of these effects, i.e., CFU-GM colony formation, were also suppressed at lower concentrations of the chemical mixture. There were no effects on T cell function or T and B cell numbers in any of the treatment groups. Altered resistance to challenge with infectious agents also occurred in mice given the highest concentration, which correlated with the immune function changes. Paired water studies indicated that the immune effects were due to a direct effect by the chemicals and not decreased water intake. Additional immune function studies were performed in CD1 mice pre- and postnatally exposed to the chemical mixture. These offspring showed immune function changes nearly identical to those in the 14 and 90 day exposed B6C3F1 mice. No additional effects were attributable to perinatal exposure.
DETECTION OF EFFECTS OF TBTO, HCB, AND O₃ ON NATURAL KILLER (NK) ACTIVITY IN THE RAT LUNG. H Van Loveren, F A Blommaert, B I Krajcic, F J A Rombout, and J G Vos. National Institute of Public Health and Environmental Protection, P.O. Box 1, Bilthoven, the Netherlands.

The respiratory tract is a major route of exposure to noxious agents and pathogenic particles such as viruses. NK activity is an important first line of defense to viruses as well as neoplasms, and testing the effects of exposure to toxic compounds on this activity is of importance for the understanding of the immunotoxic potential of the compounds. Lymphoid cell suspensions, obtained after enzymatic dispersion of rat lungs and purification over nylon wool columns showed in vitro NK activity towards YAC lymphoma cells. Validation of the test with well known NK stimulators such as Bacillus Calmette Guerin (BCG), interferon-γ (IFN-γ) and inhibitors like anti-asialo GM1 antibody confirmed the reliability of the test as an assay for detecting NK activity in rat lungs. Using this assay, we tested the effects of HCB, TBTO, and O₃. Oral exposure to HCB in concentrations of 150 and 450 mg/kg food for six weeks suppressed the NK activity in rat lungs. This was also true for six weeks of oral exposure of rats to 20 and 80 mg TBTO/kg food, but to a lesser extent. Inhalatory exposure to O₃ for 7 days at 0.4 - 0.8 mg/m³ resulted in splenic lymphocytes and deoxyuridine suppression tests on their bone marrow cells. Serum corticosterone and adrenocorticotropic hormone levels also determined. Mitogen responsiveness decreased following low O₃ exposure and increased at the two higher doses. All three dosing groups revealed equally depressed plaque forming cell activity, and circulating anti-sheep red blood cell IgM levels were depressed in Nₐ₀-dosed animals. The bone marrow activity was depressed in a dose-dependent manner. Total leukocytes in circulating blood were depressed at all three dosing levels. There were no differences observed in serum hormone levels. Nitrous oxide appears to have mixed immunomodulatory effects on lymphocytes, however, the effects were not always related to bone marrow progenitor cell depression.

MODULATION OF MACROPHAGE FUNCTION BY IN VIVO TREATMENT WITH MALATHION. K E Rodgers, D D Ellefson. Livingston Research Center University of Southern California, Los Angeles, CA.

Malathion is an organophosphate pesticide which is widely used in public health situations due to its low mammalian toxicity. Malathion was previously shown to elevate immune response to antigen and mitogenic responses. Mixing experiments indicated that macrophages from malathion-treated mice elevated the mitogenic responses of control lymphocytes. Peritoneal macrophages (PM) were harvested from mice treated orally with noncholinergergic doses of purified malathion and their phagocytic (Pg) and respiratory burst (Rb) activities ascertained. Within 4 hours after malathion administration, the PM were larger in size and had increased Rb (2 fold) and Pg (approximately 25%) activities. At day 1 following treatment, Rb activity was elevated 5 fold and Pg activity was increased by 50%. By 7 days after treatment, the PM from treated mice were more quiescent. These studies indicated that acute administration of noncholinergergic doses of purified malathion stimulates PM function at early time points after treatment. Supported by PHS ES 04337.

IMMUNOLOGIC, HEMATOLOGIC, AND ENDOCRINE RESPONSES TO SUBCHRONIC, GRADED LEVELS OF NITROUS OXIDE IN CD-1 MICE. C E Healy, D B Drown, and R P Sharma. Toxicology Program, Utah State University, Logan, UT.

Occupational exposures to subanesthetic levels of nitrous oxide (N₂O) have been documented, and may result in altered proliferative cell activities. Male CD-1 mice were exposed to 0, 50, 500 and 5000 ppm of N₂O for 6 hr/day, 5 days/week for 13 weeks. Modified tier I immunotoxicologic assays were performed on their splenic lymphocytes and deoxyuridine suppression tests on their bone marrow cells. Serum corticosterone and adrenocorticotropic hormone levels also determined. Mitogen responsiveness decreased following low N₂O exposure and increased at the two higher doses. All three dosing groups revealed equally depressed plaque forming cell activity, and circulating anti-sheep red blood cell IgM levels were depressed in N₂O-dosed animals. The bone marrow activity was depressed in a dose-dependent manner. Total leukocytes in circulating blood were depressed at all three dosing levels. There were no differences observed in serum hormone levels. Nitrous oxide appears to have mixed immunomodulatory effects on lymphocytes, however, the effects were not always related to bone marrow progenitor cell depression.

DIFFERENTIAL ROLE OF COMPLEMENT ACTIVATION IN LUNG PARTICLE-INDUCED INFLAMMATORY RESPONSES. D B Warheit and MA Hartley, Du Pont-Haskell Lab., Newark, DE.

Previously we demonstrated that complement-mediated mechanisms play a role in lung particle clearance. In this regard, pulmonary macrophage clearance was reduced in complement-deficient rats and mice exposed to a variety of dusts. In the present studies we have tested the hypothesis that carbonyl iron (CI) and quartz-induced inflammation is complement-mediated. CD⁺, C₅⁺, and C₅⁻ mice as well as normal and complement-depleted (CVF-treated) rats were exposed to CI or quartz aerosols for 6 hrs at 100 mg/m³. Subsequently, fluids and cells from sham and exposed animals were recovered by lavage (BAL) at 0, 24, 48, and 172 hrs, and 1 month postexposure, and measured for cellular and biochemical indices. Our results showed that lung inflammation was observed in normal (CD⁺, C₅⁺) mice but absent in C₅⁻ mice exposed to CI (p<0.05). In contrast, quartz inhalation produced permanent inflammation, along with increases in LDH, protein, and alkaline phosphatase, compared to controls. CVF treatment in rats and C₅ deficiency in mice had no effect upon the quartz-induced parameters, suggesting that complement does not play a major role in the acute response to quartz. Our results indicate that different mechanisms may account for CI and quartz-induced pulmonary inflammation.
MODULATION OF NATURAL KILLER ACTIVITY BY 12-O-TETRADECANOLPHORBOL-13-ACETATE IN PHORBOL ESTER-SENSITIVE (SENCAR) AND RESISTANT (B6C3F1) MICE. RW Preifer, LW Updyke, A Chuthaputti, HL Yoon, and CKW Yim. Dept. of Pharmacol. and Toxicol., Sch. of Pharmacy and Pharmaceut Sci., Purdue Univ., West Lafayette, IN.

This laboratory has been investigating the systemic immunoinflammatory alterations induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) in phorbol ester-sensitive (SENCAR) and resistant (B6C3F1) mice as a basis for strain-dependent differences in the sensitivity of mouse skin to tumor promotion. Following two weeks of topical application of TPA at 2, 4, and 8 μg/mouse, SENCAR mice demonstrated a suppression of natural killer (NK) activity (50% of control) in the spleen (no significant change in lymph nodes); in addition, substantial dose-dependent increases in cell numbers occurred in the spleen (2- to 3-fold relative to controls) and lymph nodes (30-fold). Alternatively, NK activity was enhanced in the spleens and lymph nodes of B6C3F1 mice with an increase in cell number (4-7 fold) occurring in the lymph nodes only. Therefore, alterations in NK activity may, in part, account for the resistance of particular strains of mice to TPA-induced tumor promotion in two-stage protocols. Preliminary findings implicate the macrophage in these TPA-induced strain-dependent changes. (NIH S07 RR05586/PMA).


Ammonium metavanadate has been shown to have a broad immunomodulating effect in mice after subchronic exposure; host resistance to pathogenic Listeria monocytogenes and in vitro phagocytic activity of harvested peritoneal macrophages (PEM) were strongly affected by vanadium treatment. The effect of vanadium on the functional role of resident PEM in active listeriosis was investigated in mice intraperitoneally dosed with 2.5 or 10 mg V/Kg every 3d for 6 wks. Vanadium treatment results in altered patterns of clearance of the microorganisms from the peritoneal cavity, liver and spleen. The total in vitro phagocytic uptake of Listeria by PEM was consistently decreased as a function of infection period. Similarly, intracellular killing of Listeria was decreased although the PEM from the vanadium-treated and control mice were more bacteriostatic than bactericidal. Population distributions of Listeria within infected PEM were not affected by host-potentiation with vanadium. Thus, vanadium exposure interferes with both the uptake and ultimate intraphagolysosomal killing of Listeria.

EFFECT OF FUSARIN C ON PROLIFERATION OF INTERLEUKIN 2 (IL-2) DEPENDENT CELL LINE AND ANTIGEN SPECIFIC T CELL HYBRIDOMAS. D. Marjanović, P. Holt, W P Norred, *M Merčeć, C W. Bacon and R T Riley. USDA/ARS, Athens, GA and *Immunology Branch, NIH, Bethesda, MD.

Fusarin C is a highly mutagenic compound produced by several strains of Fusarium monili-forme. Señor which has been reported to cause leuconeoencephalomalacia in horses, hepatocarcinoma in rats and possibly plays a role in the etiology of human oesophageal cancer. The mutagenic activity of this compound is comparable with that of aflatoxin B1, a very well known immunosuppressive agent for different animal species. The effect of Fusarin C on IL-2 driven proliferation of CTL-L2 cells on proliferation of cytochrome C specific T cell hybridomas (2B4.11) and mouse spleenocytes was investigated. It was shown that 6 different doses of Fusarin C (10⁻⁶ to 10⁻¹⁰M) significantly inhibited IL-2 mediated proliferation of CTL-L2 cells. T cell hybridomas were slightly more sensitive to Fusarin C (10⁻¹¹ to 10⁻¹⁰M) than the IL-2 dependent cell line. Inhibition of [³H]thymidine incorporation was dose dependent. These results indicate that Fusarin C is a potent inhibitor of lymphoid cell proliferation. We are currently investigating the role of Fusarin C in immunosuppression and the relationship to carcinogenesis.

CYTOMEGALOVIRUS-COMPLEMENTARY HOST RESISTANCE MODELS FOR IMMUNOTOXICITY TESTING IN MICE AND RATS. M J K Selgrade, D M Starnes, and M J Daniels, Inhalation Toxicology Division, HERL, US EPA, RTP, NC.

Cytomegaloviruses (CMV) are useful host resistance models because they are animal models for a well-known human opportunistic infection, they are species specific (hence animal CMV's are not human pathogens), and they are not readily transmitted from cage to cage in an animal colony. Mouse cytomegalovirus (MCMV) has been extensively characterized. Exposure to a number of different chemicals enhances susceptibility to MCMV which correlates with suppression of natural killer cell activity (NKC). Much less is known about rat cytomegalovirus (RCMV). In comparing the two models we have found that mortality is more difficult to achieve in untreated adult rats, but does occur when rats are immunosuppressed. The course of infection in key target organs is similar although virus titers in rat organs are somewhat lower. Augmentation of NKC activity following infection is more prolonged in rats as compared to mice, but suppression of NKC with cyclophosphamide correlates with increased mortality in rats in a manner similar to that observed in mice. Both Fischer-344 and Lewis rats appear to be suitable hosts. The data suggest that RCMV would be a useful model to include in a rat immunotoxicity testing tier. With this model comparisons between mice and rats are feasible. This abstract does not necessarily reflect EPA policy.
INDUCTION OF SERUM COLONY STIMULATING ACTIVITY (CSA) FOLLOWING DIMETHYLNITROSAMINE (DMN) EXPOSURE. J F Lockwood, M J Myers, and L B Schook. Lab of Molecular Immunology, Dept. of Animal Sciences, Univ. of Illinois, Urbana, IL.

Previous studies have shown DMN exposure in vivo affects granulocyte and macrophage hematopoiesis. The purpose of this study was to determine the presence of hematopoietic growth activity in serum from DMN exposed animals. Normal bone marrow stem cells were cultured in soft agar supplemented with recombinant GM-CSF or IL-1 as a source of CSF-1, and the number of colonies enumerated on day 10. Similarly, serum CSA was assessed in this assay system with the addition of pooled serum from animals treated with DMN for 14 days. Serum obtained from DMN exposed animals supported colony formation in normal bone marrow stem cells whereas naive or vehicle exposed animals failed to support colony formation. Differential staining demonstrated the presence of cells of the monocyctic and granulocytic lineages. Treatment of serum with anti-CSF-1 antibodies did not affect cell phenotype, number or CSA. Adoptive transfer of serum from DMN exposed mice to naive recipients produced blood cell profiles similar to that observed in DMN mice. These results suggest that the alteration of macrophage and granulocyte hematopoiesis observed in DMN exposed mice may be due to enhanced GM-CSF activity. (Supported by ES-04348)


A human lymphoblastoid cell line with higher levels of native cytochrome P450IA1 activity was isolated. By DNA transfection, human cDNAs for cytochrome P450IA1 and epoxide hydrolase were introduced. The resultant cell line, MCL-I, was substantially more sensitive to the mutagenicity of dimethylnitrosamine and benz(a)pyrene than the parent AhR-1 cell line. The increase in native cytochrome P450IA1 activity was achieved by mutation and selection based on resistance of mutant AhR-1 cells to the phototoxicity of benz(ghi)perylene. One resultant clone 13, was used for DNA transfection. The complete cDNAs for cytochrome P450IA2 and the microsomal epoxide hydrolase were isolated from a human liver cDNA library. A vector containing both cDNAs was then constructed and transfected into L3 cells to produce MCL-1 cells. Gene expression was detected at the level of enzyme activity and increased sensitivity to benzo(a)pyrene and dimethylnitrosamine cytotoxicity/mutagenicity. The potential usefulness of drug metabolizing gene transfection into mammalian cells for in vitro genetic toxicity testing will be discussed.


Chemical inhibitors are valuable tools for probing and modulating cytochrome P-450 function in vivo. In general, however, the compounds currently available lack the specificity necessary to target individual cyttochrome P-450 forms. We have therefore begun to elaborate both a rational and an empirical approach for designing specific irreversible cytochrome P-450 inhibitors, based on the concept of mechanism-based inactivation. Our rational approach has involved the synthesis of specific inactivators of the rat and rabbit hepatic cytochromes P-450 responsible for 21-hydroxylation of progesterone by the replacement of the 17β-methylketone side chain in progesterone or pregnenolone with a vinyl or chlorofluoromethylketone group. Our empirical approach has involved the synthesis of a large number of chloroarene analogs bearing modifications in the dichloromethyl, p-nitrophenyl, or propionediol moieties. Through this approach, one compound, N-(2-p-nitrophenoxy) chlorofluorooacetamide, has been identified that appears to specifically inactivate the major phenobarbital-inducible form of rat liver cytochrome P-450 both in vitro and in vivo. Through the use of these specific probes, the roles of individual cytochromes P-450 forms in catalyzing bioactivation and detoxification reactions in vivo can now be evaluated. Supported by NIH grants ES03619 and ES00151 (J.H), ES03516 (L.K), and GM31001 (E.J).

EXCRETION OF THE MERCAPTURIC ACID S-[2-(N7-GUANYL)ETHYL]-N-ACETLYCYSTEINE IN RAT URINE FOLLOWING ADMINISTRATION OF ETHYlene DIBROMIDE. D H Kim and P P Guengerich. Vanderbilt University, Nashville, TN.

Administration of the carcinogen ethylene dibromide (EDB) to rats resulted in the urinary excretion of S-[2-(N7-guanyl)ethyl]-N-acetylcyysteine, derived from the DNA adduct S-[2-(N7-guanyl)ethyl]glutathione. This mercapturic acid was isolated from urine by reverse phase and propylylene HPLC and was quantitated by measurement of fluorescence intensity. The urinary mercapturic acid was identified as S-[2-(N7-guanyl)ethyl]-N-acetylcyysteine. The HPLC retention time was identical to that of the authentic mercapturic acid and the radioactivity co-eluted when 14C-EDB was administered, and 1H NMR and UV spectra were identical to those of the authentic mercapturic acid. Excretion of this mercapturic acid into urine of rats given injection of various doses of EDB occurred in a dose-dependent, linear manner over the range of 0.5-37 mg/kg. The amount of mercapturic acid excreted into urine 48 hr after EDB injection was higher than the level of the DNA adduct in liver, indicating that extrahepatic DNA adducents also contribute to mercapturic acid production. The excretion of the mercapturic acid may provide a means of non-invasive estimation of DNA adducts derived from EDB exposure. (Supported in part by USPHS grants CA 44353 and ES 00267.)
A physiologically-based model for DFP pharmacokinetics and dynamics was developed and validated for the simulation of AChE inhibition in mammals. Tissue partition coefficients, enzyme hydrolysis constants, enzyme synthesis rates, and AChE/DFP bimolecular inhibition rate constants which dictate DFP kinetic and dynamic behavior were obtained from the literature or determined in laboratory studies. Model predictions of DFP kinetics in mice after a single intravenous infusion (1 mg/kg) or inhalation exposure (60 ppm 5 min) were in agreement with those determined experimentally. The model also successfully predicted AChE inhibition and enzyme resynthesis in the brain, blood, and diaphragm of these animals. The effects of repeated subcutaneous dosing (0.7 mg/kg, 23 d) with DFP on AChE inhibition and resynthesis in the brain and plasma of rats was simulated, and model predictions of the effects of this repeated dosing regime on AChE inhibition patterns were quite good. These studies show that a well validated physiologically-based model can successfully simulate pharmacodynamic consequences of acute and subchronic DFP exposures. (Supported by DOD Contract No. F33615-83-C-0532).

The male reproductive system and the nervous system are the primary biological targets of a group of important toxicants, including the acylamides, DFP, carbon disulfide, the hexacarbons, and chronically administered organophosphorus esters. In the tests, the developing germ cell depends upon the support and nourishment provided by the Sertoli cell. In the nervous system, the axon represents the conduit for information and organelle movement between the cell body and the periphery. The cytoskeletons of Sertoli cell processes and axons share a unique morphological and biochemical features with the Sertoli cell containing an abundant high molecular weight microtubule associated protein, called HMW-2, which is analogous both structurally and functionally to MAP-1C, a putative microtubule-dependent retrograde translocator in the nervous system. Both HMW-2 and MAP-1C are cytoplasmic dynes expressing ATPase activity. The effect of acrylamide or disopropylfluorophosphate (DFP) on the ATPase activity of sucrose density gradient purified HMW-2 was assessed. After 30 minutes in vitro pre-incubation at 37°C, acrylamide (up to 3 mM) had little effect while DFP decreased HMW-2 ATPase activity at high concentration (1 mM DFP, 83% of control ATPase activity; 5 mM DFP, 29%). Additional in vitro and in vivo experiments will continue to explore the general hypothesis that the action of selective nervous system and testicular toxicants results from alterations in a shared cytoskeletal organization.
THE EFFECT OF LEAD ON OSTEOCALCIN LEVELS IN RAT OSTEOSARCOMA 17/2.8 (ROS) CELLS. C J Long. University of Arkansas for Medical Sciences, Little Rock, AR. J F Rosen. Albert Einstein College of Medicine, Bronx, NY. J R Pounds. Brookhaven National Laboratory, Upton, NY.

The serum level of osteocalcin (OC), a bone specific protein produced by osteoblasts, is decreased in lead intoxicated children, and the serum level of OC is positively correlated with bone formation. To investigate the effect of lead on the hormonal regulation of OC production, ROS cells were treated with 0.05, 0.10, or 25 μM lead acetate for 24 hr, followed by an additional 24 hr treatment with lead or with without 1.25(OH)2D3 (100 pg/ml media). At the end of this period a radioimmunoassay was conducted to determine the amount of OC in the cells and secreted in the media. 1,25(OH)2D3 caused an increase in OC secreted into the media in cultures containing 0 μM lead, but this increase was inhibited by lead in a dose dependent manner, to the extent that OC secretion by 10 and 25 μM lead treated groups was less than cultures without 1,25(OH)2D3 treatment. After 24 hr of treatment with 1,25(OH)2D3, intracellular levels of OC were slightly elevated, but there was no lead effect on intracellular OC levels. These data suggest that lead attenuates 1,25(OH)2D3 induction of OC in ROS cells. The physiological role of osteocalcin has yet to be unambiguously discerned, but given the probability that OC plays a central role in bone mineralization, lead may have significant toxic effects on bone cell metabolism. (Supported by NIH grants ES01000 and ES04040).


Crewmembers of armored vehicles inhale lead-containing aerosols produced during weapons firing. Because significantly more lead is present in new, long-range charges and a crew ballistic shelter, which confines exhaust gases, is present on vehicles being tested, a lead exposure/response study was conducted on 45 artillery crewmembers during weapons firing at Fort Sill, OK. Physical and chemical characteristics of the aerosol were determined, as well as lead exposures, for each crewmember during three or four 96-h firing exercises. Results showed a striking dependence of air lead (PbA) concentrations on wind direction and velocity. SEM/EDAX analysis revealed lead-containing particles to be 0.5 to 5 um in diameter. A significant relationship between total PbA exposure and rise in blood lead (PbB) was established (R²=0.85). In addition, on the highest exposure day, the relationship between rise in PbB (μg Pb/dl blood) and 24-h time-weighted-average PbA (μg Pb/m³) was PbB = 3.55 + 0.10 PbA (R²=0.25). No relationship was found between PbB and change in nerve conduction velocity for three motor and three sensory nerves. Work supported by the U.S. Army under an interagency agreement with Hqqs. U.S. Army Med. R&D Command, Project Order 66P68021.

78 EFFECTS OF LEAD ON THE KINETICS OF ADENOSINE TRIPHOSPHATASE SYSTEM AND PROTECTION BY THIOL REAGENTS. C S Chetty, S Rajanna, and B Rajanna, Selma University, Selma, AL. Sponsor: Prasada Rao S Kodavanti.

The mechanisms of inhibition of rat brain P2 fraction Na⁺, K⁺-ATPase and associated component parameters by lead acetate were studied in vitro. Lead inhibited Na⁺, K⁺-ATPase (IC₅₀=2.0×10⁻⁵M, K⁺-stimulated para-nitrophenolphosphate (K⁺-PNPase: IC₅₀=3.5×10⁻⁶M) and 3H-ouabain binding (IC₅₀=2.0×10⁻⁵M) in a concentration dependent manner. Altered pH (6.0 to 9.0) or temperature (17 to 37°C) demonstrated increased inhibition of Na⁺-K⁺ ATPase. Kinetic studies of the substrate activation of Na⁺, K⁺-ATPase at different pH (6.5, 7.5 and 8.5) and temperatures (17, 27 and 37°C) indicated significant changes in kinetic constants such as Kₘ and Vₘₐₓ. Results also indicated significant changes in substrate dependent kinetics of K⁺-PNPase. Sulphhydryl agents such as dithiothreitol (DTT) and cysteine but not glutathione protected lead inhibition of Na⁺, K⁺-ATPase at pH 7.5 and 8.5 to different extents. These results suggest that lead inhibited Na⁺, K⁺-ATPase by interacting with dephosphorylation of the enzyme-phosphoryl complex. (Supported by NIH/NIBRS #08169-10).
81 EXPOSURE TO LEAD IN FOOD: SOURCES AND ESTIMATED RISK. M. Sills and E.K. Silberfeld, Environmental Defense Fund, Washington DC.

Exposure to lead (Pb) remains a national public health problem in the US. We have analyzed recent data from the US FDA to determine the sources and significance of Pb in the processed food supply. While a voluntary industry program has reduced use of lead solder in canning, about 20% of domestically produced cans still use lead in solder and imported cans are not tested. Another major source of lead in food is Pb inks in fuel about 20% of gasoline sold in the US in 1987-8 is leaded. The FDA data indicate that Pb solder and airborne Pb contribute Pb to food and that these sources can result in significant increases in Pb intake. When food is compared on the basis of method of processing, Pb levels in canned samples were on average 29% higher than those in fresh/frozen samples of the same food (range 8 to 136). Impacts of airborne Pb were responsible for a 4.3-fold elevation in leafy vegetables as compared to protected produce. These levels of Pb are sufficient to result in excessive intake. If a consumer always chose canned instead of fresh/frozen foods, total intake could be as high as 114 mcg/day. Using FDA estimates of food consumption, average intake was 24 mcg/day. These intakes are estimated to cause elevations in blood Pb of 5.8 and 4.2 mcg/dl respectively.

We conclude that Pb contamination of food is a preventable source of Pb exposure and a significant source of overall Pb levels in the US.


We are investigating the common earthworm (Lumbricus terrestris) as a possible substitute for rodents to obtain preliminary information on the toxicity of metals. LD-50 values of selenite and selenate (Serda and Forst, Proc. West. Pharmacol. Soc. 30:227 (1987)) and of cadmium and zinc (Nguyen and Forst, Biol. Trace Element Res. "in press") have been obtained. Like in rodents, two isoforms of Cd-metallothionein have been found. (Nguyen and Forst, Biol. Trace Element Res. "in press")

The carcinogen detoxifying enzyme GSH S-transferase is present in these worms (Stenerson et al, Biochem J. 181:47 (1979)). Using these authors techniques the enzyme as assayed for activity by measuring at 380nm the rate of formation in 1-5min of the GSH-complex from the substrate, 1-Cl-2,4-dinitrobenzene.

Using appropriate blanks, both selenite and selenate at concentrations from 10^-6 to 10^-4M were tested as possible antagonists. Only the selenite was active; inhibition was noted between 10^-4 and 10^-3M. The latter had no effect throughout the entire range.

83 PROTECTIVE EFFECT OF SELENIUM ON IMPAIRED GLUCOSE OUTPUT FROM HEPATOCYTES BY CADMIUM. R. Bell, J. L. Barle, and V. N. Okuninukare, Florida A&M University, College of Pharmacy, Tallahassee, FL. Sponsor: R. C. Schnell

The protective effect of selenium(Se) on certain hepatotoxic and pancreatotoxic manifestations of cadmium(Cd) has been established by Merlai and Singhal 1975. Male, albino Sprague-Dawley derived rats (210-260 g) received sodium acetate (NaAc, 1.23 mg/kg), Cd (0.84 mg/kg), Se (1.6 mg/kg) or simultaneous injections of Cd and Se, 0.84 and 1.6 mg/kg, respectively via the intraperitoneal route, 72 hr prior to experimentation. Rats were fasted for 24 hr, hepatocytes isolated and incubated (50mcg cells wet wt in 1 ml volume) for 60 min at 37°C with or without lactic acid (10μM). Glucose output was measured using the glucose analyzer. Selenium increased (p<0.05), while Cd decreased hepatic glucose output (p<0.05), as compared to NaAc treated controls. Glucose output from hepatocytes of rats treated with Se and Cd simultaneously did not differ significantly from NaAc treated controls. Results indicate that Se ameliorates Cd-affected hepatic glucose output. Supported by NIH/DRR/MBRS RR08111 and NTR/DDR/RBMI RR03020.

84 ANTICARCINOGENIC EFFECTS OF CADMIUM IN B6C3F1 MOUSE LIVER. M.P. Winkle, B Diwan, R.M. Bae, and J.M. Rice. NCI and BCDP, PR1, Frederick, MD.

Studies were designed to investigate the tumor-initiating and/or promoting ability of cadmium (Cd) in various tissues of B6C3F1 mice. In an initiation study, 5 wk old male mice received a single sc injection of either 20 or 22.5 μmol CdCl2/kg. Beginning 2 wk later, they were given drinking water containing 500 ppm of barbitral (BB), a known multitissue promoter, for 92 wks. No tumors were observed in animals given Cd alone. Mice given BB alone developed a significantly higher incidence of hepatocellular adenomas than the control group (7.44 vs 2.24 adenomas/liver). Cd given 2 wks earlier inhibited BB-promoted spontaneous liver tumors in a dose dependent manner (20 Cd + BB, 3.93 tumors/liver; 22.5 Cd + BB, 1.87 tumors/liver). In a promotion study, male mice received a single ip injection of N-nitrosodiethylamine (DEN, 90 mg/kg) at 5 wks of age and 2 wks later were fed Cd (500 or 1000 ppm, in diet) for 50 wks. DEN alone induced liver tumors in 13/45 mice (29%). Subsequent feeding of Cd inhibited DEN-induced liver carcinogenesis (DEN + Cd 500, 3/42; DEN + Cd 1000, 0/47). The lung tumor rate (DEN, 15/45) was also reduced by Cd (DEN + Cd 500, 11/42; DEN + Cd 1000, 1/47). Thus, Cd failed to initiate any tumors promotable by BB in B6C3F1 mice; however, Cd did exhibit a marked inhibitory effect on the development of liver and lung tumors in these mice.
DEGRADATION AND METAL COMPOSITION OF HEPATIC ISOMETALLOTHIONEINS DURING CONDITIONS OF INDUCTION. W.C. Kershaw and C.D. Knauss. Univ. of Kansas Med. Ctr., Kansas City, KS.

The hypothesis that metals bound to isometallothioneins (MT-I & MT-2) stabilize each protein with respect to intracellular degradation was evaluated by measuring the half-lives of hepatic MT-I and MT-2, as well as the metal composition of each isoprotein during various conditions of induction. These conditions included developing neonatal rats and single dose administration of ethanol (109 mmol/kg, po) or Zn (1 mmol/kg, sc) to adult male rats. Half-lives were estimated by pulse-labeling MT with $^{35}$S-cysteine in vivo and measuring the isotopic decay in MT-I and MT-2 isolated by anion-exchange chromatography. Metal (Zn and Cu) and protein contents of iso-MT fractions were measured by atomic absorption spectrometry and the CD/hemoglobin affinity assay, respectively, 24 hr after administration of inducing agent or parturition. Half-lives of MT-I (1/3 of MT-2) in livers of ethanol- and Zn-treated rats were 9(9) and 21(33) hr, respectively, while the half-life of hepatic MT-I (MT-2) in 1-day-old rats was 49(73) hr. During all conditions of induction, both isoforms of MT contained approximately 6 atoms Zn/mole MT and were essentially void of Cu. In conclusion, markedly different half-lives were noted among iso-MTs that were nearly identical with respect to metal composition. Consequently, degradation of iso-MTs does not appear to be influenced solely by their metal content. These observations suggest that proteolytic reactions that participate in the catabolism of iso-MTs are altered following chemical treatments and during neonatal development. (Supported by USPHS Grants ES-01142 and ES-07079).

ALTERED NICKEL BINDING PROTEINS IN NICKEL RESISTANT MOUSE CELLS. X-W Wang, R J Imbra. New York University Medical Center, Institute of Environmental Medicine, New York, NY. Sponsor: Max Costa.

Nickel (II) is a potent carcinogen and, at high concentrations, is toxic to mammalian cells. Indirect evidence also suggests that nickel is a required trace element for normal growth and development. However, the metabolic fate of nickel in cells has not yet been clearly described. To understand potential mechanisms of nickel action in cells, we have selected a mouse Balb/c-3T3 fibroblast cell line that grows and divides in the presence of concentrations of nickel chloride that are toxic to the parental cell line. Using gel electrophoresis, three nickel binding proteins (67 kd, 55 kd and 49 kd) are observed in extracts from the parental Balb/c-3T3 cells. In contrast, only the 67 kd (probably albumen from the medium) protein binds nickel in extracts from the nickel resistant cells. This suggests either that the 55 kd and 48 kd proteins are absent in nickel resistant cells or have lost the ability to bind nickel. We are currently attempting to isolate these proteins from normal Balb/c-3T3 cells, using column chromatography and nickel affinity chromatography, in order to obtain antibodies to these proteins. These antibodies can be used to examine nickel resistant cells for the presence and distribution of the 55 kd and 48 kd proteins. These studies may allow us to determine a mechanism of nickel toxicity.

IDENTIFICATION OF BI-INDUCED METAL-BINDING PROTEIN IN MOUSE KIDNEY. K. Kobayashi, H. Toyoda, N. Sato, T. Kagami, A. Naganuma and N. Imura. Dep. of Public Health, S-h. of Pharmaceutical Sciences, Kita-sato University, Shirokanedai, Minato-ku, Tokyo, Japan

Recently we have demonstrated that induction of a metal-binding protein in appropriate organs by preadministration of bismuth(Bi) markedly decreased the toxicity of anticancer drugs such as cisplatin and Adriamycin in mice. Meanwhile, the metal-binding protein induced by Bi in rat kidney was reported to be different from metallothionein(MT). However, the identity of this protein has not been investigated in detail thereafter. The purpose of our present study is to identify the metal-binding protein predominantly induced by Bi in mouse kidney using molecular biological techniques. Renal concentration of the protein measured by $^{115}$In-g binding assay was increased by the administration of Bi(NO$_3$)$_3$ (100mmol/kg, sc) and reached the maximum at 24hr after the administration. Northern blot analysis of total RNA extracted from the kidney using mouse MT-1 cDNA probe showed that the level of MT-1 mRNA was increased markedly by Bi administration. Further, we have confirmed a single copy of MT-1 gene in mouse DNA by southern blot analysis. These results strongly suggest that the metal-binding protein induced by Bi in the mouse kidney is MT. The identity of the metal-binding protein induced by Bi in rat kidney will also be discussed.

IMMUNOPRECIPITATION OF ETHANOL-INDUCED RABBIT UDP-GLUCORONOSYLTRANSFERASE (GT) BY $p$-NITROPHENOL GT ANTIBODIES. R M Hutabarat, M D Green*, T R Tephy*, and G S Yost, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT and *Department of Pharmacology, University of Iowa, Iowa City, IA.

Chronic administration of ethanol to male New Zealand white rabbits results in the induction of a GT isozyme in hepatic microsomes. Characterization of the protein shows an apparent catalytic and structural difference from the constitutive isozyme isolated from untreated male rabbits. Polyclonal sheep antibodies raised against female rabbit liver $p$-nitrophenol GT were used to further differentiate the two isozymes. The antibodies inhibited the 1-naphthol GT activity of both the ethanol-induced and control isozymes by approximately 40%; however, immunoprecipitation of 125 $\mu$g of the ethanol-induced and constitutive GT isozymes required 12.5 $\mu$g and 12.2 $\mu$g of IgG, respectively. This 1000-fold increase in specificity of the IgG for immunoprecipitation of the control GT indicates that the proteins may be distinct isozymes. Supported by USPHS grants AA06555 and GM 26221. GSY is a USPHS Research Career Development Awardee (HL02119).
EFFECT OF DOSE CHANGES AND ETHANOL COADMINISTRATION ON TRICHLOROETHYLENE METABOLISM. J L Larson and R J Bull. Pharmacology/Toxicology Graduate Program, College of Pharmacy, Washington State University, Pullman, WA.

The redox state of the hepatocyte affects the amounts of trichloroethanol (TOH) and trichloroacetic acid (TCA) formed in the metabolism of chloralhydrate. We attempted to alter the metabolism of trichloroethylene (TCE) by ethanol (EtOH) coadministration. All rats were dosed with either 0.2, 0.6, or 3.0 g/kg TCE, with the experimental groups receiving 0.2, 0.6, or 3.0 g/kg EtOH, respectively. Blood and urine samples were collected over 72 hours. The metabolism of TCE may be saturated at 0.6 g/kg and is certainly so at 3.0 g/kg, as evidenced by prolonged residence times for TCE and metabolites in the body. The amount of TOH and TCA formed, however, continued to increase even past the point of saturation of TCE metabolism, albeit at a less than linear rate. As predicted, EtOH decreased blood levels of TCA, but only at early times at the high dose. EtOH did increase the urinary TOH/TCA ratio at all dose levels. This result is consistent with the hypothesis of a more reduced state in the hepatocyte caused by the generation of reducing equivalents by EtOH metabolism. The metabolism of TCE is shifted preferentially towards reduction to TOH away from oxidation to TCA. (This work was funded by U.S. Air Force Grant #AFOSR-86-0284).

PURIFICATION OF TWO ISOZYMES OF RAT LIVER MICROSMAL CYTOCHROME P-450 WITH TESTOSTERONE 7α-HYDROXYLASE ACTIVITY. M P Arlott1 and A Parkinson2. 1NCTR, Division of Genetic Toxicology, Jefferson, AR, and 2University of Kansas Medical Center, Kansas City, KS

Antibody against cytochrome P-450a, which catalyzes the 7α-hydroxylation of testosterone, recognized three proteins in rat liver microsomes. One of these proteins corresponded to P-450a. The other two proteins did not correspond to P-450a, P-450o, P-450d, P-451a, P-450f, P-450g, P-450h, P-450i, P-450j, P-450k or P-450p. These proteins were designated P-450m and P-450n. Like P-450a, P-450m was present in liver microsomes from both male and female rats. However, whereas as P-450a was detectable in liver microsomes from one-week-old rats, P-450m was barely detectable until the rats were at least 3 weeks old. P-450m was detectable only in liver microsomes from post-puberal (>4 week-old) male rats. Purified P-450m catalyzed the 15α- and 7α-hydroxylation of testosterone, whereas P-450n exhibited little testosterone hydroxylase activity. The ability of P-450m to catalyze the 7α-hydroxylation of testosterone was not due to contamination with P-450a. With one exception, P-450a and P-450b had identical N-terminal amino acid sequences for the first 20 residues. This high degree of homology explains in part the immunochemical relatedness of P-450a and P-450m. In summary, P-450a, P-450m and P-450n are independently regulated, immunochemically related proteins present in rat liver microsomes. Furthermore, P-450a and P-450m are isozymes that both catalyze the 7α-hydroxylation of testosterone. Supported by NIH grants GM 37044, ES 00166 and ES 07079.

FUNCTIONAL CHARACTERIZATION OF A PHENOBARBITAL-INDUCIBLE DOG LIVER CYTOCHROME P-450 STRUCTURALLY RELATED TO RAT AND HUMAN ENZYMES OF THE P450IIH (STERIOD-INDUCIBLE) GENE FAMILY. P J Ciancio and J R Halpern. University of Arizona, Tucson, AZ.

A cytochrome P-450 called PBD-1 isolated from liver microsomes of an adult male Beagle dog treated with phenobarbital (PB) possesses structural and functional similarities to members of the P450IIH gene family in rat and human liver microsomes. Like rat and human P450IIH forms, PBD-1 loses catalytic activity upon purification. Steroid 6β-hydroxylase activity, a marker for P450IIH forms, increases 2.4-fold with PBTreatment, and in microsomes from PB-treated dogs androstenedione 6β- and testosterone 6β-hydroxylases are selectively inhibited by 10 mg anti-PBD-1 IgG/nmol P-450. NADPH-dependent tri- acetyletoxoademecynin (TAO) complex formation and erythromycin (ERY) demethylase activity, also markers for P450IIH forms, increase 4- and 5-fold, respectively, in dog liver microsomes upon PBTreatment, whereas immunochemically determined PBD-1 increases 2.3-fold. In microsomes from PB-treated dogs, 3 mg anti-PBD-1 IgG/nmol P-450 inhibits greater than 75% and 50% of TAO complex formation and ERY demethylase activity. These data suggest that PBD-1 is responsible for a major portion of merozoide antibiotic metabolism and for steroid 6β-hydroxylase activities in microsomes from PB-treated dogs. Differences in titer for immunoblot and fold-induction for the various enzyme activities suggest functional heterogeneity in this family of dog liver cytochromes P-450. (Supported by NIH grants ES 00151, ES 03619 and SOTR 05665).

EXTRAHEPATIC INDUCTION OF CYTOCHROME P-450 BY N-SUBSTITUTED IMIDAZOLES. W L Hopkins and M R Frankin, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.

Of many investigated, two N-substituted imidazole compounds, clotrimazole (CtOZ) and N-benzylimidazole (NBI), are high magnitude inducers of cyt P-450 in rat liver. Cyt P-450 is also present in extrahepatic organs, especially in kidney, lung and intestine. Since organ-specific metabolism of xenobiotics can be responsible for tissue-selective toxicities, the possibility that N-substituted imidazoles may also induce extrahepatic cyt P-450 was investigated. The induction of cyt P-450 and the activities of several cyt P-450 isozymes were determined in lunge, kidneys and intestines of male Sprague-Dawley rats which had been treated with several different N-substituted imidazoles (three daily doses of 75 mg/kg, ig). The rats were sacrificed 48 hrs after the last dose to ensure clearance of residual compound, since N-substituted imidazoles are potent inhibitors of cyt P-450 in the extrahepatic organs, neither NBI nor CtOZ induced total cyt P-450 concentrations to the same extent as in the liver. NBI was a good inducer of cyt P-450 in the kidney (250% of control) whereas CtOZ did not induce in this organ. Induction in the intestine by NBI was highly variable but, in some cases, was as great as 350% of control, while CtOZ only induced intestinal cyt P-450 to a slight degree. Lung cyt P-450 was induced only slightly by either compound. NBI induced 7-ethoxyresorufin deE activity to varying extents in all three organs, indicative of P450-type induction. CtOZ slightly induced ethynorphine demethylation activity in intestinal and benzphetamine demE activity in lung microsomes, indicative of P450-type induction in these tissues. Other N-substituted imidazoles examined induced cyt P-450 only slightly in extrahepatic tissues, with the possible exception of nalidixone, which induced kidney cyt P-450 200% of control and in a P450-type manner. Supported by USPHS grant #GM39335.
ROLE OF THE 4S BINDING PROTEIN IN THE INDUCTION OF ARYL HYDROCARBON HYDROXYLASE IN MAMMALIAN CELLS. C Kampe and S Safe.
Departments of Biochemistry and Biophysics and Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.

Both the aryl hydrocarbon (Ah) receptor (9S) and the 4S binding protein have been implicated in the regulation of cytochrome P-450A1 gene expression by halogenated aryl hydrocarbons and polynuclear aromatic hydrocarbons, respectively. Using [3H]-2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and [3H]-benzo[a]pyrene (B[a]P) as radioligands for the 9S and 4S binding protein, it was shown that the 9S receptor was present in both rat hepatoma H-4-II E cell and wild type mouse Hepa 1c1c7 cells whereas the 4S binding protein was detected only in the Hepa 1c1c7 cells. Dose-response studies with 2,3,7,8-TCDD and benzo[a]anthracene demonstrated that both compounds induced aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin O-deethylase (EROD) in both mouse Hepa 1c1c7 and rat hepatoma H-4-II E cells in culture and increased the transcription of cytochrome P-450A1 mRNA. These results suggest that the 4S binding protein is not necessary for the induction of cytochrome P-450A1 gene transcription in certain mammalian cells in culture.

DISPOSITION OF BENZ(a)ACRIDINE IN THE RAT. N Nan, W F Mueller, New Mexico State University Toxicology Program, Las Cruces, NM, and W C Weimer, Battelle Northwest, Richland, WA. Sponsor: D L Springer.

Benz(a)acridine (BaAc) is a suspected carcinogen present in solvent-refined coal liquid. As part of the evaluation of the hazard it represents for exposed workers, its metabolism, body distribution and excretion were studied in rats. C-14-labeled BaAc was synthesized by condensation of 2-naphthol-1-C-14 with 2-amino benzyl alcohol at 220°C. BaAc was incubated for 30 minutes with the 10,000 x g supernatant of liver homogenates from rats that had either received no pretreatment or had been induced with Aroclor 1254 or the nitrogen-containing polycyclic hydrocarbon (NPAC) fraction of the 800-850°C distillation cut of a solvent-refined coal liquid by intraperitoneal injection 5 days before sacrifice. HPLC analysis of the ethyl acetate incubation extracts showed that larger amounts of polar metabolites were formed after induction with PCB or NPAC as compared to uninduced liver preparations. Phenolic and other oxygenated species were identified as BaAc metabolites by GC-MS. After dermal application of 10 μg/kg BaAc to adult rats, an average of 34% of the applied radioactivity was excrated within 48 hours; 18% in urine and 22% in feces. Highest residual activity after 48 hours was found in intestines, liver, and kidney.

NICOTINE (N) METABOLISM BY P-450s AND FLAVIN-CONTAINING MONOOXYGENASE (FMO) IN RABBIT LUNG (RL). D E Williams, M R Shigenaga and N Castegnoli. Oregon State Univ., Corvallis, OR and Univ. of California, San Francisco, CA.

RL microsomes metabolize N primarily by P-450-dependent oxidation at the C-5' position and by N'-oxidation. Little is known about the relative role of P-450 isozymes or the involvement of FMO in the latter reaction. The contribution of these various monooxygenases to N metabolism in RL microsomes was determined utilizing specific inhibitors, immunotitration with antibodies, and reconstitution of P-450s 2 and 5 and FMO. Incubation of RL microsomes with inhibitors or with anti-P-450 reductase IgG, indicated that formation of the major metabolite, N-5'-aziridino ion (NII) was entirely dependent upon P-450, and that FMO may contribute to N'-oxide (NO) production. Studies with reconstituted P-450s 2 and 5 showed that only P-450 2 metabolized N, producing predominantly NII with a small amount of NO. The Km of NII production by P-450 2 was 67-138 μM with a Vmax of 1.5 min⁻¹. RL microsomal production of NII and benzphetamine-N-demethylation were inhibited in parallel by anti-P-450 2 IgG. N is a poor substrate for RL FMO (Km, 1.1 μM; Vmax, 0.5 min⁻¹) compared to pig liver FMO (Km, 380 μM; Vmax, 57 min⁻¹). Therefore, NII, the major metabolite of N in RL, is formed by P-450 2. FMO may be responsible for 30-50% of the NO produced. Supported by NIH HL38650 and the American Lung Association of Oregon.
BIOCHEMICAL CORRELATES OF CHLORDECONE (CD)-
INDUCED PRETREATMENT DISPOSITION RESPONSE (PDR)
IN MICE. H M Carpenter, Z W Cai, and L R Curtis.
Oak Creek Laboratory of Biology, Oregon State
University, Corvallis, OR.

Our previous work showed that pretreatment of mice with low doses of CD results in an altered disposition of a subsequent dose of 14C-CD (Carpenter and Curtis, Drug Metabol. Disposition, 1988, in press). Additional experiments using a pretreatment dose of 0, 5 or 40 mg CD/kg three days prior to a tracer dose of 5 mg 14C-CD/kg in male C57BL/6J mice were conducted. These studies showed that the subcellular distribution of 14C-CD was altered. There was decreased label in the nuclear fraction of liver, but higher amounts in microsomes and cytosol. In the kidney, amounts of label in the nuclear fraction were unchanged, but the amount of label in the microsomes was decreased. CD did not change liver to body weight ratios or amounts of microsomal protein, but increased cytochrome P-450 and ethoxyresorufin and ethoxyoxcarcin-O-deethylase activities. There were no effects on total lipids in the liver, but the amounts of the 16:0, 16:1 and 18:0 fatty acids decreased while 18:1n-9 and 20:4n-6 were increased. CD-induced PDR is apparently not related to changes in total lipid, but CD does alter the amounts of several fatty acids. (Supported by AFOSR grant 87-0185).

TRICHLOROMETHYL RADICAL BINDING TO MICROSONAL
LIPIDS OF RAT LIVER IN CARBON TETRACHLORIDE
METABOLISM. B S Kaphalia and C J S Anagwi.
Department of Pathology, The University of Texas
Medical Branch, Galveston, TX.

Formation of trichloromethyl radical (CCl3) and its binding to lipids of endoplasmic reticulum may be responsible for acute hepatotoxicity of carbon tetrachloride (CCl4). To investigate lipid adducts of CCl3, rats pretreated with phenobarbital were administered 1 mmole/kg 14C-CCl4 (500 μCi) by gavage and killed after 1 hr. Hepatic microsomes were prepared and microsomal lipids were extracted and separated into neutral- and phospholipids by sepharose carboxylate. In vivo studies with CCl4 were incubated anaerobically with hepatic microsomes of phenobarbital pretreated rats in a NADPH generating system. After incubation, lipids were extracted and separated as described above. Neutral lipids obtained from in vivo and in vitro systems were separated by column chromatography and the major radioactive fractions were methylated with BF3/MEOH. The fatty acid esters were extracted in hexane and were separated by reverse phase high performance liquid chromatography (HPLC). The radioactive fractions were analyzed by ammonia chemical ionization mass spectrometry (CIMS). Two pseudomolecular ions m/z 430/432/434 and 454/456/458, with three chlorine pattern (100:98:32) showed the presence of trichloromethyloctadecenoic and trichloromethyldecosatrienoic acids respectively as CCl3 adducts of linoleic and arachidonic acids. Two major radioactive phospholipid fractions from in vivo and in vitro treated lipids when hydrolysed by phospholipase A2 showed that most of the radioactivity was associated with fatty acids. The fatty acids were identified by GC and the radioactive fractions were identified by CIMS. The covalent binding of CCl3 radical to linoleic and arachidonic acids moiety of neutral and phospholipids of endoplasmic reticulum may be a contributing factor in CCl4 induced hepatotoxicity. (Supported by ES04813 and DK27125).

EFFECTS OF CARBON TETRACHLORIDE, ETHANOL AND
METHYL ETHYL KETONE ON THE OXIDATION OF 2-
BUTANOL BY RAT LUNG AND LIVER. G P Carlson,
Department of Pharmacology and Toxicology,
Purdue University, West Lafayette, IN.

The microsomal alcohol oxidizing system has been well studied in the liver but not in the lung. To examine the effects of inducers and inhibitors of xenobiotic metabolism on this system in the lung, 2-butanol metabolism was measured and comparisons made with the liver. Rats were treated with CCl4, ethanol or methyl ethyl ketone (MEK), and microsomes were prepared from liver and lung. MEK production from 2-butanol was determined by gas chromatography. Enzyme activity in control rats was 6 to 8 times higher in liver than in lung. CCl4 (1.0 ml/kg, ip) decreased activity in both tissues but especially in the lung. Low doses of ethanol (0.5 ml/kg or 1.0 ml/kg ip for 7 days) were inhibitory in the lung but not in the liver whereas a high dose (3.0 ml/kg) decreased microsomal alcohol oxidizing system activity in both tissues. The effects of the two inhibitors were not additive. MEK induced 2-butanol oxidation in both tissues. The lung, like the liver, does have microsomal alcohol oxidizing activity which is alterable by both inducers and inhibitors. Supported in part by NIEHS Grant ES04362.

IN VITRO BENZENE HEPATOTOXICITY. A Dimitriadis, K H Jung, R Snyder and S JI. Joint Graduate Program in Toxicology, Rutgers University, Piscataway, N.J.

The liver is not known to be a target site for benzene toxicity. However, we have recently observed that the isolated perfused rat liver can be injured by the infusion of 120 μM benzene as indicated by (1) the inhibition of hepatic O2 uptake appearing at 15 - 60 min after benzene infusion and proceeding at a rate of 10 -30% of the basal respiratory inhibition/hr, and (2) a leakage of lactate dehydrogenase in the effluent at a rate of 10 -40 μg/liver/hr. The liver of Sprague-Dawley or Fisher 334 female rats was removed under pentobarbital anesthesia and perfused through the portal vein with the Krebs-Henseleit buffer at a constant flow of 5 -6 ml/g/min at 37° C. The rate of hepatic O2 uptake was continuously measured using a Clark-type O2 electrode positioned in the outflow line. A slowly emerging respiratory inhibition was also observed within 20 -40 min of inducing 40 but not 20 μM p-benzoquinone. The detailed mechanisms of the benzene- and benzquinone-induced respiratory inhibition of the perfused liver remain to be elucidated; but the intriguing possibility exists that these inhibitions are mediated by a benzene metabolite-induced collapse of microcirculation confined in small areas of the liver as indicated by inhomogeneous staining with a trypan blue solution (0.1%). Supported in part by EOHSI, N.J.

Our laboratory has reported that while phenobarbital (PB) pretreatment increases NADPH cytochrome P-450 reductase (NR), it does not lead to an increase in menadione-mediated cytotoxicity. We hypothesized that PB pretreatment induces cytoprotective mechanisms capable of mitigating the consequences of the increase in NR. In support of this, we have found that PB pretreatment increases many of the factors involved in the glutathione cycle as well as DT-Diaphorase (DT), the enzyme responsible for the cytoprotective 2e reduction of menadione. The purpose of this investigation was to explore the role of DT in overcoming the PB-induced NR during menadione metabolism in isolated hepatocytes. Hepatocytes were isolated from naive and PB pretreated male S.D. rats and the menadione-stimulated superoxide dismutase (SOD)-sensitive reduction of acetylated cytochrome c was measured in the presence and absence of dicumarol. Despite the increased NR in the PB pretreated hepatocytes, there was no increase in menadione-stimulated generation of O2-. The percentage of menadione metabolized by DT was found to be inversely proportional to the concentration of the menadione employed. Furthermore, PB pretreatment was found to increase the proportion of menadione metabolized by DT, a finding that is consistent with our proposed hypothesis. (Supported by AFOSR-88-0009 and ES-07045)

103 TOXICITY OF HALOTHANE IN GUINEA PIG LIVER SLICES. BN Chantors, J Fernando, AJ Gandolfi, R Breedel, Dept Anesthesiology, University of Arizona, Tucson, AZ.

Guinea pigs have proven to be a reliable model of halothane associated hepatotoxicity. An in vitro system with Hartley male guinea pig liver tissue was designed to assess the toxicity of halothane in the target organ. Precision-cut liver slices (250-300 μm) were incubated in sealed roller vials containing Krebs-Henseleit buffer (plus vitamins, amino acids, glutamine, gencamycin) at 37°C, under 21% (pO2 = 150 mm Hg in medium) and 95% (pO2 = 603 in media) O2 environments. Halothane (5-50 μl) was injected through a Teflon septa cap on a filter paper wick and vaporized. Viability of the slices was monitored by intracellular K+ at 3, 6, 9, 12, 24 hr. Under the 21% O2 environment 10 μl (1.9 mm in medium) of halothane was non-toxic through 6 hr of incubation. Above 2.7 mm halothane a dramatic decrease in intracellular K+ was observed between 2-4 hr. When a higher O2 environment (95%) was used to maintain slice viability at longer incubations (12 hr), 1.9 mm halothane again remained nontoxic. However, 2.1 μl halothane caused a 50% reduction in liver slice K+ content by 12 hr and 2.7 μl caused an 80% decrease by 9 hr. Under these conditions only a very small quantity of reductive biotransformation occurs. This suggests that halothane hepatotoxicity in the guinea pig liver slices could be attributed to its oxidative biotransformation. (NIH DR 16715)

102 BIOCHEMICAL ALTERATIONS IN MOUSE LIVER INDUCED BY NITROGEN MUSTARD. Nabil M. Elsayed, Phuong Ta, and Don Korte, Jr. Division of Toxicology, Letterman Army Institute of Research Presidio of San Francisco, CA.

We have reported previously that systemic administration of the vesicant butyl 2-chloroethyl sulfide (BCS), a nonfunctional sulfur mustard analog, results in biochemical alterations in mouse lung, brain, kidney, and eyes consistent with oxidative stress. In this study, we examined the systemic effect of a bifunctional vesicant, nitrogen mustard (NM), on mouse liver. We injected Swiss Webster mice, ip, either with NM (1.75 mg/kg body weight) or saline. After 24 hr, the mice were sacrificed and the liver analyzed for biochemical markers of oxidative stress. In general, the response was similar to that obtained after BCS treatment. The results, expressed per mg protein, showed a significant increase in the activities of glucose-6-phosphate dehydrogenase 56%, isocitrate dehydrogenase 45%, and glutathione peroxidase 182%. Superoxide dismutase activity and total glutathione content were 27%, and 23% lower, but these changes were not statistically significant. Total DNA content increased significantly, 60%, but protein content remained unchanged (-7%, NS). It is possible that NM can act as a free radical in the initial phase of the reaction in both the toxicity of the molecule and the reactive aziridinium moiety is formed. Increased glutathione peroxidase activity and the lack of a similar increase in superoxide dismutase activity support this concept. It is also possible that NM favors free radical formation by binding cellular glutathione, thus impairing its antioxidant and detoxification functions.


Cholestasis was induced in the perfused rat liver by addition of E17G (17.5 μM) during infusion of taurocholate (TC), tauroursodeoxycholate (TUDC) or dehydrocholate (DHC) at 20 nmol/min/g liver. Increasing the infusion rate of TC, TUDC or DHC caused a dose-dependent reversal in the cholestatic effects of E17G. At higher infusion rates, both TC and TUDC caused a marked removal of E17G from the liver. At lower TC infusion rates, the removal of E17G was effected by bile excretion, but at the highest infusion rate (180 nmol/min/g liver), there was a marked regurgitation of E17G into the perfusate. With TUDC, only a slight back efflux was seen. In contrast, DHC, at higher infusion rates, did not enhance E17G biliary excretion or regurgitation. The relative effects of TC, TUDC and DHC paralleled their relative hydrophobicity and detergent effects. The reversal of E17G-induced cholestasis by TC was accompanied by a reversal of the adverse effects of E17G on glucose clearance. While TC was the most effective in reversing E17G cholestasis, it had some toxicity. TUDC was less effective, but displayed no toxicity. Accordingly, TUDC has a higher therapeutic index which might be of relevance in efforts to treat estrogen-in-ducked cholestasis with bile salts.
When acetaminophen (AA) (25 mM) was introduced into the perfused rat liver, the hepatic O2 uptake was inhibited first rapidly and then more slowly. The rapid inhibition was found to be due to mitochondrial blockade, whereas the slow inhibition was associated with microcirculatory aberrations as demonstrated by inhomogeneous staining by trypan blue (TB) infusion (0.1%). NaCN (0.5 mM) also caused rapid and slow respiratory inhibitions and heterogeneous TB staining. Similarly inhomogeneous TB staining was noted when the liver was perfused with an anoxic medium. Accompanying the slow inhibition or heterogeneous TB staining was a release of lactate dehydrogenase (LDH) in the effluent at the rate of 110.2 ± 12.2 (mean ± SEM; n = 33) U/g liver/hr. In the AA-induced respiratory inhibition, monitoring the cumulative amounts of LDH released into the effluent against the cumulative inhibition of O2 uptake gave a series of straight lines, indicating that LDH release and slow inhibition are causally related. Either the slow inhibition preceded LDH release or the reverse order was found, indicating that either one of these processes can act as the cause of the other. Supported by EHSI, N.J.

Experiments were conducted to examine the effect of selenium on allyl alcohol induced hepatotoxicity in the rat. Male Sprague-Dawley rats received sodium selenite (12.5 µmol/kg, ip) either 24 or 48 hr prior to receiving allyl alcohol (0.8 µmol/kg, ip). Toxicity was assessed at varying intervals (2 - 24 hr) thereafter by measurement of plasma alanine and aspartate aminotransferases (ALT, AST) and sorbitol dehydrogenase (SDH) and by histological examination. Selenite treatment produced a marked amelioration in the hepatotoxicity induced by allyl alcohol as assessed by the three enzymatic measures and histological examination both at 2 and 24 hrs after the alcohol administration. Selenite administered 48 hr prior to allyl alcohol also prevented hepatotoxicity (24 hr). The effect of allyl alcohol on hepatic glutathione levels was more complex with a marked increase (81.5%) at 2 hr but a return to control levels at 8 hr post alcohol treatment. Selenite, in some instances, produced a statistical (but not physiologically real) change in the allyl alcohol alteration of hepatic GSH levels. (Supported by a Burroughs-Wellcome Toxicology Award).

Measurement of protein and DNA synthesis, intracellular enzyme leakage, and intracellular potassium (K+) levels were used to document the effects of the following anti-ulcer drugs on cultures of postnatal rat hepatocytes: cimetidine, ranitidine, oxmetidine, tiotidine, and ORF 17578, a potent H-2 antagonist (Katz et al., J. Pharm. Exp. Ther. 237: 404, 1986). Oxmetidine, a known hepatotoxin in vivo and in vitro, was cytotoxic at a concentration of 100 µM. Protein and DNA synthesis were decreased approximately 50%, whereas K+ and enzyme leakage were unaffected. At a concentration of 1000 µM, however, K+ was reduced nearly 50% and enzyme leakage was increased four-fold over controls. In a descending order of toxicity, defined by the above parameters, the compounds were ranked as follows: oxmetidine > tiotidine > cimetidine > ranitidine > ORF 17578. ORF 17578 showed only decreases in protein and DNA synthesis of 44% at the highest concentration tested, 2000 µM. These results demonstrate the sensitivity of protein and DNA synthesis relative to other standard parameters for measuring cytotoxicity, as well as the ability of this system to rank the toxicity of different drugs within a specific class. Further studies will be necessary to compare the in vitro ranking with that obtained by in vivo studies.

Obesity is a serious health problem that shortens life expectancy and intensifies a spectrum of human illnesses. Abnormal liver histology is customary (>90%). Periportal and portal fibrosis are common severe injuries (25%). This study explores whether obesity predisposes the liver to periportal damage by drugs and chemicals. When doses of 5, 10, and 25 mg/kg allyl alcohol (AA) were administered to age-matched obese and control Sprague-Dawley rats, the dose-toxicity (ALT) curve for obese animals was shifted to the left (p < 0.05 (*)). A fixed 13.4 mg dose of AA produced greater mortality (44% vs 0%, *). ALT elevation (5170 ± 2290 vs 1850 ± 907 U/ml, X ± SD, n=5,4) and liver necrosis (*) in obese rats than in much smaller control animals (721 ± 22g vs 537 ± 36g, *). Bioactivation of AA by hepatic cytosol was similar for obese and control rats (0.60 ± 0.10 vs 0.64 ± 0.06 umol/min/mg). However, hepatic concentration of glutathione, a key detoxification cofactor, was below normal in obese animals (4.26 ± 0.46 vs 5.65 ± 0.61 umol/g, *). In conclusion, periportal AA injury was exacerbated in obese rats. This may relate to diminished hepatic glutathione concentration and a resulting decrease in acrolein detoxification (Supported by NIH Grants GM 20852 and GM 41564).
Biliary excretion of organic anions was impaired in rats fed Cd in the diet. Plasma clearance of these compounds indicated hepatic uptake was normal, suggesting transport from liver to bile as the site of toxic action (J. Pharmacol. Exp. Ther. 231:495). In this study, biliary excretion of phenolphthalein glucuronide (PG), a marker for hepatocellular active transport, was impaired in rats treated with 60 mg Cd/kg by gavage. At 24 hr following Cd, 43% of a bolus of 10 umol PG/kg was excreted within 45 min as compared to 55% in control rats. These rats recovered PG excretory function 72 hr following Cd. Glutamate uptake into BCV has been used to investigate active transport specifically within the canalicular domain of the hepatic cell membrane (J. Biol. Chem. 261:6216). Our study system for glutamate transport was isolated BCV incubated with 1.5 umol glutamate and stopped by rapid filtration. Peak Na*-stimulated glutamate uptake was 1.36 fmol/ug protein BCV after 5 min compared to Na*-free (K+*) accumulation of 0.95 fmol/ug protein. In vitro treatment of the BCV with 2.0 moles CD/ug protein reduced the Na*-stimulated accumulation by at least 50%. These results suggest active transport at the bile canalicus is sensitive to the toxic action of Cd. (Supported by NIEHS grant ES-07060 and AFOSR grant 87-0185.)

The cyclic heptapeptide hepatotoxin, MCLR (>95% pure) from the cyanobacterium Microcystis aeruginosa was administered iv to anesthetized (chloralose + ketamine) crossbred female swine at 0.0 (vehicle control), 25 µg/kg (sublethal dose) or 72 µg/kg (lethal dose). Prior to dosing, noncompliant catheters were placed in the cranial vena cava, ascending aorta, and portal vein; and temperature pulse decay probes in the liver and kidney. Surviving gils were killed 5 hr postdosing. In high dose gils, reductions in perfusion to 50% of predose occurred in liver and kidney in less than 30 and 40 min, respectively. Central venous pressure in these pigs declined even faster than reductions in hepatic perfusion. Aortic mean pressure (AOM) was 50% of the predose value at approximately 55 min postdosing. Concurrent with the decrease in AOM was a 2 fold increase in portal venous pressure suggesting partial obstruction of hepatic blood flow. The 1.8 fold increase in liver weight (% body weight) and 3.65 fold in liver hemoglobin in the lethal vs. control group were compatible with severe intrahepatic necrosis and hemorrhage in the high dose pigs. Terminal hypokalemia and hypoglycemia were probably secondary to shock and liver damage.

The hepatotoxic, microcystin-LR (MCLR), produced by the cyanobacterium Microcystis aeruginosa, causes progressive centriflobular hepatocyte disassociation and necrosis. Ultrastructurally, progressive hepatocyte disassociation, membrane blebbing and invagination and loss of microvilli begin at 10 min. To determine the target cell and if the cytoskeleton is affected, rat hepatocytes (HPS) and non-hepatocytes ((NHPS) endothelial and kupper cells) were isolated and evaluated. In suspension, MCLR caused plasma membrane blebbing in HPS, but not in NHPS. Cultured HPS were treated with MCLR, fixed, and stained with rhodamine-phalloidin (RP). One hr. after 100 µg MCLR/ml, scattered cells had small blebs with actin at their bases. By 6 hrs. most were affected with blebs and centrally aggregated actin, some with actin rays extending to the surface. To compare with effects in vivo, rats were killed at 5, 10, 20, 30, 45 or 60 min. after PBS or a lethal dose of MCLR and liver sections stained with RP. At 20 min. small actin aggregations were present in centriflobular HPS. With time, these aggregations coalesced and this lesion progressed peripherally. The progression and distribution of actin changes in vivo correlated with light microscopic lesions and were comparable to those seen in vitro. Therefore, MCLR induces cytoskeletal changes in vivo and in vitro.

Volatile alka(e)nnes are exhaled continuously by men and animals. They are scission products of polyunsaturated fatty acids and amino acids if O₂ radicals attack lipids or proteins. Bacterial or endogenous, but because of their rapid oxidation by cyt. P450 isocynes the minute amounts expired unmetabolized have been overlooked. The production of one or several of these gases is considerably increased following the action of many toxic compounds. In vitro experiments with red blood cells, lipid membranes or proteins indicated that alka(e)nnes can be viewed as almost specific for oxidative processes affecting different components of lipids and proteins. Substances which are believed to be responsible for lipid peroxidation promoted an attack of O₂ radicals on proteins predominantly or exclusively. A typical example is ethanol which induces the formation of O₂ radicals which oxidize amino acids, peptides and proteins, but not lipids. This non-invasive procedure which is easy to handle can be successfully used for differentiating primary lesions on macromolecules without any harm for the animal.
Polyclonal antibodies raised against the adult form of rat hepatic cytochrome P450A1 were used to immunologically detect an analogous isozymic form(s) in various tissues of the rat conceptus during the progression of organogenesis. Tissue investigated were the embryo proper, the visceral yolk sac and the epiblastal cone. Studies were performed on conceptuses from day 10 (day of conception - day 0) to day 14 of gestation. Ethoxyresorufin deethylation, benzo(a)pyrene (BaP) hydroxylation and 7- and 9-hydroxylation of 2-acetylaminofluorene (AAF) were utilized in assessments of P450A1 activities during the same gestational period. In untreated conceptuses, P450A1 could not be detected immunologically in any of the 3 tissues at any stage of gestation investigated. The deethylation reaction was quantifiable in embryos and yolk sacs of untreated conceptuses, but was not inhibited by anti-P450A1 antibodies, α-naphthoflavone or metyrapone. Treatment of pregnant rats with 40 mg/kg of 3'-methylcholanthrene 48 hr prior to removal of the conceptuses resulted in marked increases in measured enzymatic activities as well as in readily immunoreactive P450A1. Inducibility for the deethylation was greatest in the visceral yolk sac (3-5X), was evident in the embryo proper (2-3X) but was minimal in the epiblastal cone (1.5X). Much greater induction (up to 70X) was observed with BaP and AAF as substrates. Induced activities were effectively inhibited by anti-P450A1 antibodies and by α-naphthoflavone but not by metyrapone. The results indicated that P450A1, or a very closely related isoform(s), is both inducible and functional in tissues of the conceptus throughout organogenesis. (Supported by NIH grants ES-04041 and ES-04342).

Studies designed to evaluate effects of glutathione (GSH) depletion by BSO on the ability of chemical embroyotoxins to elicit dysmorphogenesis in whole embryo culture showed that BSO-treatment significantly increased embryonic protein content. No changes in embryonic protein occurred within BSO cultured cultures were routinely gassed with 95% O2, 5% CO2 at 35°C. BSO cultures were incubated for 3-4 hr, reaching levels 30% greater than controls. HPLC analysis of GSH and select amino acids were done (data not shown). It was found that BSO depleted GSH to 16% and 10% of controls (at 20 hr) for embryos and VVS, respectively, and were accompanied by increases of 25% and 100% for glutathione and asp., respectively, in the VVS. Following 96 hr exposure, GSH levels declined further and free amino acids increased in both embryo and VVS to levels as high as 10X control. Induction of conceptuses with P450A1-harvesting serum albumin for the final 5 hr of culture resulted in a 20X increase in TCA soluble radioactivity with BSO, confirming an increase in lysosomal proteinolysis. Addition of the proteolytic enzyme inhibitors, leupeptin (50 μg/ml), leupeptin (50 μg/ml), eliminated the increase in amino acids otherwise elicited by BSO. Leupeptin also resulted in a decrease in embryonic and VVS GSH by 16% and 38%, respectively, independent of BSO. These data suggest a link between detoxification of embroyotoxins involving GSH intracellular redox status and regulation of histolofic proteinolysis in the developing rodent conceptus. (Supported by NIH ES-00404 and ES-03137).

To examine the importance of glutathione (GSH) in the embryo and the role of other intermediates generated in the embryo N-acetoxy-2-acetylaminofluorene (AAAF) and acetoaminophen (AAP), the effect of GSH depletion on the embroyotoxicity, dysmorphogenesis and covalent binding of these agents was examined. Both AAAF (90 μM) and AAP (500 μM) produced concentration-dependent and significant decreases in embryonic length and protein content during days 10 to 11 of gestation. The predominant malformations were prosopencephalic hypoplasia (AAAF) and abnormal neutralization (AAP). Exposure of conceptuses to 3H-AAP followed by density gradient centrifugation resulted in detectable binding to protein but not DNA or RNA, indicating that the rat conceptus is capable of bioactivating AAP to a soft electrophile that selectively arylates protein. In contrast, conceptuses exposed to 3H-AAAF exhibited readily detectable binding to RNA, DNA and protein, indicating the generation of a hard electrophile. After depletion of GSH with BSO, exposure to AAP resulted in significant potentiation (relative to AAP alone) of the observed embroyotoxicity, abnormal neutralization and covalent binding. In contrast, pretreatment with BSO did not potentiate the AAP-elicted embroyotoxicity or prosopencephalic hypoplasia, although a slight increase in binding of 3H-AAAF to DNA was observed. These data are consistent with the concept that abnormal neutralization elicited by AAP results from the generation of a soft electrophile species, whereas elicitation of prosopencephalic hypoplasia by AAP is a consequence of conversion to a relatively hard electrophile. Supported by NIH grants ES-04041 and ES-03157.

Cyclophosphamide (CP) destroys ovarian follicles in a time- and dose-dependent fashion. Glutathione (GSH) is essential in CP detoxification such that, changes in its concentration can alter general CP toxicity. It is also known that prior treatment with a low dose of CP increases hepatic GSH, decreasing the toxicity of subsequent doses. Therefore, we postulated that a fractionated CP dosing regimen would elevate GSH and diminish ovarian toxicity. Female C57BL/6N mice were treated I.p. with either a single dose of 0, 75, 200 or 500 mg/kg CP or the same total dose fractionated over five days. Hepatic and ovarian GSH levels, and differential follicle counts were determined 2, 6, 24, 72 and 180 hours post treatment. A single dose of 75 mg/kg caused an increase in hepatic GSH to 125% of control at 24 hrs while fractionation of 75, 200, and 500 mg/kg increased GSH to 118, 111, and 126% of control, respectively. A single dose of 75, 200, 500 reduced primordial follicle counts to 67, 14, and 5% of control (ED50 = 122 mg/kg) while the fractionated dose reduced them to 105%, 70, and 5% of control (ED50 = 257 mg/kg), respectively. Fractionation of CP shifted the follicle destruction dose-response curve to the right, significantly reducing the toxicity of CP. This may be a direct result of the elevated GSH levels produced by the previous fractions of the dose.


VCH is present in gasses discharged during synthetic rubber production. Chronic treatment of B6C3F1 mice and F-344 rats with VCH by gavage will induce ovarian tumors in mice but not rats. Our objective was to determine the mechanism of the species difference in VCH induced ovarian tumors. A critical step in the induction of ovarian tumors is the destruction of the small oocyte. Therefore, small oocyte counts obtained from serially sectioned ovaries were used as an index of toxicity. VCH or its epoxide metabolites (VCH-1,2-epoxide, VCH-7,8-epoxide, and VCH diepoxide) were given to 28 day old mice I.p. in corn oil daily at doses ranging from 0.07 to 7.4 mmole/kg for 30d. All compounds reduced the number of small oocytes compared to corn oil controls, however, the dose which reduced the small oocyte count to 50% of control was 5 fold lower for the monooxepoxides and 18 fold lower for the diepoxide. Rats 28 days of age were treated with VCH (7.4 mmole/kg/d X 30d) I.p., a regime which reduced the small oocyte count to 10% of control in mice. Interestingly, this treatment had no effect on small oocyte counts in rats. The 1,2-epoxide was present in the blood of mice with the highest concentration at 2 hr (50 mmole/ml). The blood concentration of the 1,2-epoxide in rats was less (0.5 mmole/ml) at all times examined. Thus it appears that metabolism of VCH to epoxides and their subsequent destruction of oocytes are critical steps in VCH induced ovarian tumors. Rats may be resistant to ovarian tumor induction by VCH because the amount of VCH coconverted to epoxides is insufficient to produce oocyte destruction. Supported by NIH/NIGMS Training Grant T32 GM07039.


Numerous metals can be found in milk at levels that cause neonatal toxicity, but little is known about the excretion of Ni into milk, or the toxicity of Ni to lactating or suckling animals. Therefore, single or multiple s.c. doses of NiCl2 were given to lactating rats, and Ni concentrations were determined in plasma and milk. Plasma and milk [Ni] increased linearly after single doses of 10, 50, or 100 μmole NiCl2/kg. Peak plasma [Ni] was reached 4 hr after a single dose of 100 μmole/kg, while milk [Ni] increased until at least 6 hr. After 4 daily doses of 100 μmole NiCl2/kg, plasma [Ni] was 10.2 ± 1.0 mg/l (Mean±SB), similar to that following a single dose, while milk [Ni] was 1.03 ± 0.66 mg/l, 5.5 X greater than after a single dose. Pup plasma [Ni] was 50±4 μg/ml. Multiple doses of 100 μmole NiCl2/kg caused decreased maternal thymus weight, increased milk solid and lipid, no change in milk protein, decreased milk lactose, and decreased pup liver weight independent of decreased maternal food consumption. Hepatic lipid peroxidation was not affected by Ni in the dams or the suckling pups. The results show that multiple doses of NiCl2 lead to milk/plasma [Ni] of 0.10, changes in milk quality, decreased thymus weight in lactating rats, and decreased liver weight in pups.

PLACENTAL AND LACTATIONAL TRANSFER OF AMIODARONE (AD) AND DEETHYLAMIODARONE (DAD) IN FISCHER 344 RATS. D A Hill and M J Reasor. Department of Pharmacology and Toxicology, West Virginia Univ., Health Science Center, Morgantown, WV.

AD, a cardiac antiarrhythmic drug, causes numerous side effects including pulmonary and hepatic toxicity. Our objectives were to evaluate placental and lactational transfer of AD and its principal metabolite, DAD, and determine tissue specificity of the transferred drugs. Fischer 344 females were treated in two ways: 1) dams were given AD 35mg/kg/day, p.o., for the last 7 days of pregnancy. The newborns were analyzed for tissue drug levels before suckling. 2) previously untreated dams were given AD 35mg/kg/day, p.o., for the first 14 days of lactation while their pups suckled. At the end of treatment period the pups' tissues were analyzed for drug levels. The results showed that AD and DAD crossed the fetal/placental barrier and were concentrated in fetal lung and liver. Similar levels of AD and DAD were found in newborn liver as well as in newborn lung: [liver: 20μg/g AD, 27μg/g DAD; lung: 56μg/g AD, 63μg/g DAD]. AD and DAD were also transferable through lactation. In neonatal lung and liver, DAD levels were 2-3X higher than AD levels. Our study showed that AD and DAD were transferable across the placenta and through lactation, and the lung and liver appeared to be the targets for accumulation. Supported by the American Heart Association and NIH/NIGMS Training Grant T32 GM07039.
Secalonic acid D (SAD) is a teratogenic mycotoxin, inducing cleft palate in offspring of treated mice. In order to investigate the role of SAD-induced effects on maternal corticosterone (C) levels in SAD-induced teratogenesis, maternal blood samples were collected 24, 48, 72, and 96 hr following treatment of pregnant CDI mice on day 11 of pregnancy with 5% NaHCO₃ (control), 20% Dimethylsulfoxide (DMSO), 30 mg/kg SAD in NaHCO₃, or in 20% DMSO, intraperitoneally. Plasma corticosterone levels were assayed using a commercial radioimmunoassay kit. Secalonic acid D significantly (p<0.01) increased circulating maternal C levels at 24 and 48 hr following dosing in NaHCO₃, which returned to control levels by 72 hr. Inclusion of DMSO in the solvent resulted in a significant blunting of SAD effect on maternal C levels at both 24 and 48 hr time points. In males, on the other hand, SAD failed to alter circulating C levels following single doses of up to 45 mg/kg in NaHCO₃. Multiple treatments with 15 or 30 mg/kg of SAD, however, increased C in males which was again significantly inhibited (p<0.01) by DMSO. These results demonstrate for the first time the effect of SAD on mammalian endocrine system and provide indirect evidence for the role of corticosterone involvement in SAD teratogenicity. Supported by NIH grant DE07107.

The developmental toxicity of SAD 93944, a histamine H₁ antagonist, was evaluated when administered alone (A) or combined (B) with P, a sympathomimetic amine. In A, pregnant Wistar rats received SAD 93944 by gavage at doses of 33.3, 100 or 300 mg/kg on days 6-15. No maternal or developmental toxicity was evident. In B, pregnant Wistar rats received a combination (5/4 w/w) of SAD 93944 and P at doses of 33/27, 100/80 or 300/2/10 mg/kg on days 6-15. Controls received water (CM), SAD 93944 (300 mg/kg) only or P (240 mg/kg) only. Again SAD 93944 alone had no effect on dams or fetuses. P alone or when SAD 93944, produced a variety of dose-related toxic effects: decreases in dam body weight (to 85% of CM) and food consumption (to 46% CM) and deficits in fetal weight (85% of CM) and ossification. Effects were more severe in the high dose combination group than in dams treated with P alone. Maternal toxicity was slight in mid- and low-dose groups. Fetal effects were slight in mid- and absent in low-dose groups. No conclusive evidence of embryothalality nor teratogenicity was obtained. These observations suggest that developmental effects were due to maternal toxicity, indicating that SAD 93944, alone or combined with P, has no specific liability as a developmental toxicant.

Experimental evidence exists to indicate that retinoids may act as detergents to disrupt biological membranes. Taurine, an amino sulfonic acid, has been shown to possess membrane-stabilizing and cytoprotective properties. This may have been the basis of a recent patent which claims that "a therapeutically effective amount of isotretinoin ... co-administered to patients suffering from severe cystic acne with a protective amount of taurine [would] reduce the side effects [including teratogenicity] of isotretinoin." (U.S. Patent # 4545977, inventor G. E. Gaul) In order to test the protective influence of taurine, we treated pregnant rats with taurine alone, isotretinoin alone, and combinations of isotretinoin and taurine. No embryotoxic effects, considered to be treatment related, were observed in the litters of rats treated with up to 4000 mg/kg/day of taurine on gestation days 7-15. In order to investigate the possible protective effect of taurine, rats were pretreated with 1000, 2000, or 4000 mg/kg/day of taurine on gestation day 7, treated with isotretinoin on days 8, 9, and 10 thirty minutes after they had been pretreated with taurine on these three days, and finally dosed with taurine alone on day 11. Two teratogenic doses of isotretinoin were tested: 75 and 150 mg/kg/day. Based on the combined resorption and malformation rates, we failed to find any support for a protective role for taurine under the conditions of our study.

Using NMRI mice, the perinatal effects (gestation period, litter size, stillbirth, neonatal mortality, fetal birthweights and sex ratio) of the pyrantel pamoate suspension (Combantrin) were investigated. The drug was administered to three groups of pregnant NMRI mice during each trimester of their pregnancy. Three doses of the drugs (10, 20 or 30 mg/kg/day) were used. When the drug was used during the first trimester of pregnancy, the following findings were obtained: the gestation period in the drug-treated animals was longer (p<0.01), a reduction in the number of offspring (litter size) (p<0.01), and an increase in fetal birth weight (p<0.01). However, when the drug was given during the second trimester of pregnancy, it showed no effect. On the other hand, when the drug was given during the third trimester of pregnancy, the percentage of stillborn was raised (p<0.01). Our research showed the need for further clinical investigations before the drug can be prescribed to pregnant mothers.
Arasine gas is a potent hemolytic agent but the effects of exposure to tolerated concentrations on pregnancy and prenatal development have not been previously reported. In the present evaluation, groups of dam-breeding mice and rats were exposed to arsine concentrations of 0.025, 0.5, or 2.5 ppm on gestation days (gd) 6 through 15. Animals were killed on gd 17 (mice) and gd 20 (rats) and endpoints of maternal and developmental toxicity were evaluated. In mice, maternal spleen size was significantly increased in the 2.5 ppm group. The number of live fetuses, mean fetal body weight, and percentages of resorptions or malformations per litter were not affected by arsine exposure. In rats, maternal spleens were enlarged in the 2.5 ppm group, and fetuses weighed more than in the control group. A slight increase in postimplantation loss was not statistically significant. Other endpoints of developmental toxicity were not affected by arsine treatment. In another experiment, the arsenic content of maternal blood and fetal livers increased with increasing atmospheric arsine concentrations, as assessed on gd 20. In conclusion, arsines at atmospheric concentrations that caused increased in maternal spleen size in rats and mice did not affect endpoints of developmental toxicity.

The hypothesis has been formulated that the ratio of a chemical's adult toxicity (A) to its developmental toxicity (D) is constant across animal species. If true, it has significant implications for the development of alternative test methods, and in interspecies extrapolation for risk assessment. The purpose of this study was to test the hypothesis by determining A/D for 14 chemicals in four species: mouse, frog, fish, fruit fly. Species were selected because of their extensive use in toxicology and because intact developing and adult individuals can be tested. Chemicals were selected which represent a range of teratogenic potencies and mechanisms. Mice were treated according to a standard Segment 2 design. For the other species, embryos/larvae and adults were exposed to test agents for comparable periods via the water or diet, and adult and developmental toxicity determined. A/D ratios for the four species were within the same order of magnitude for only 3 of 14 chemicals. Ratios of 3 or higher, suggested as an index of developmental hazard, were observed across species for only 4 of 14 chemicals. Our results demonstrate that A/D ratios are not constant across species and are probably not useful for interspecies comparisons and extrapolation.
DIRECT BINDING OF RADIOLabeled POLYCHLORINATED DIPHENYLALKYLARON AND DIBENZO-p-DIOXIN CONGENERS TO THE Ah RECEPTOR. R Rosenberg, J Safe and S Safe. Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.

The microcholorination of 1,6-[3H] dibenz-p-dioxin and 1,4,6-[3H] dibenzofuran gave mixtures from which the following congeners (sp. activity, 30-50 Ci/mmol) were purified by high pressure liquid chromatography: 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), 1,2,3,7,8-pentachlorodibenzo-p-dioxin (PeCDD), 1,2,3,7,8-pentachlorodibenzofuran (PeCDF), 2,3,7,8-TCDF and 2,3,7-trichlorodibenzo-p-dioxin (TCDD). Direct binding studies using rat hepatic cytosol showed that the Kd values for all six congeners were approximately 5-10 nM. The lack of structure-dependent binding affinities to the Ah receptor is in contrast to previous competitive receptor binding studies and the well-known structure-induction and structure-toxicity relationships for these compounds. Further studies on the rates of occupied and unoccupied cytosolic receptor degradation and the thermodynamics of these radioligand-receptor interactions have also been determined and the results will be discussed in terms of the proposed mechanism of action of these compounds (ES-03554).

TCDD ALTERS EMBRYONIC PALATAL CELL DIFFERENTIATION IN VITRO. J D Abbott, J J Diliberto, L S Birnbaum. NEHS, RTP, NC.

TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) is teratogenic in mice, inducing cleft palate and hydrencephaly. After exposure in vivo TCDD specifically alters differentiation of embryonic palatal medial epithelial cells. In this study embryonic C57BL/6J palatal shelves were placed in organ culture on gestation day (GD) 12 in DMEM:F12 medium (1:1) with 1% FBS. Shelves were cultured for 72 hours in control media or with TCDD from 1 x 10^-12 to 1 x 10^-7 M in 1% DMPSO. The in vitro exposures were based on levels shown to occur in the fetus after in vivo exposure. Responses to TCDD in vitro occur over a narrow range of concentrations; the most effective level was 5 x 10^-7 M and toxicity was observed at 1 x 10^-5 M. TCDD prevented programmed cell death of the medial peridermal cells. At a stage when control medial cells ceased proliferation and EGF receptors were not detected immunohistochemically, TCDD-exposed medial cells incorporated H-TDR and high levels of EGF receptors were detected. When examined by SEM and TEM, a TCDD-induced shift in the differentiation of medial cells toward an oral-like phenotype was observed. The measured responses to TCDD are indistinguishable after exposure in vitro and in vivo. The availability of an in vitro system will facilitate studies of TCDD toxicity which are difficult or impossible to perform in vivo, such as comparisons of TCDD effects between species, including human tissues.


TCDD has been shown to be antifertogenic in tissues (liver and uterus) containing estrogen receptors (ER). TCDD has also been reported to reduce the surge of ornithine decarboxylase (ODC) activity (a rate limiting enzyme in polyamine biosynthesis) that follows partial hepatectomy or hormone administration. To understand the role of polyamines in the mechanism of action of TCDD we conducted two series of experiments. In one, a polyamine biosynthetic inhibitor difluoromethylornithine (DFMO) was administered in 3 week old mice as 1% solution in drinking water for 6 weeks. We determined the ER levels of liver and uterine tissues by Scatchard analysis. Liver ER levels were 2724 and 1472 females/mg protein for control and DFMO treatment groups, respectively. There was no significant difference in the ER levels in the uterus. In another set of experiments mice were administered 1% DFMO, TCDD (200 ug/kg body weight), or DFMO + TCDD. Observation of these mice over 3 weeks showed that DFMO increased the toxicity of TCDD. Since DFMO at this dosage is non-toxic, inhibition of ODC seems to have a role in the toxicity of TCDD. Furthermore, it is possible that the inhibition of polyamine biosynthesis by TCDD could lead to a reduction of ER level. Supported by a grant from the National Cancer Institute (CA42439 to T.T)

MODULATION OF MALE GERM CELL (MGC) ADENYLATE CYCLASE BY TPA AND TCDD IN VITRO. L Beebe and D A Barsotti. Philadelphia College of Pharmacy & Science, Philadelphia, PA, and ATSDR, Atlanta, GA. Sponsor: R F Orzechowski.

The male germ cell plasma membrane exhibits adenylate cyclase (AC) activity which is similar to the somatic cell enzymes by its responsiveness to forskolin, sodium fluoride, and GTP. Experiments were conducted to investigate the interaction of two promotional agents, TPA and TCDD, with the regulation of adenylate cyclase in this cell population. AC was quantified in membranes isolated from MSC exposed in vitro to TPA or TCDD utilizing a two-step chromatographic separation of 32P-cAMP from 32P-ATP. Exposure of MSC to TPA or TCDD over time (1.7 nM, 15-120 min) and concentration (1 nM-1 uM, 60 min) demonstrated an inhibition of coupling between the guanine nucleotide stimulatory protein (Ga) and the catalytic unit (C), as measured by the concomitant stimulation of AC by forskolin (10 uM) and GTP (50 uM). TPA-mediated inhibition of Ga-C coupling was abolished following pretreatment with cytochalasin B, while the effect of TCDD was reversed by the addition of cycloheximide. Therefore, these data suggest that both TPA and TCDD elicit similar modulation of germ cell AC, although these responses are mediated by distinct cellular processes.
STRUCTURE-DEPENDENT INDUCTION OF ARYL HYDROCARBON HYDROXYLASE BY TCDD AND RELATED COMPOUNDS: MECHANISTIC STUDIES. T. Zacharewski, M. Harris, and S. Safe. Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.

The structure-induction relationships observed for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzofuran (PeCDD), 1,2,3,7,8-pentachlorodibenzofuran (PeCDD), 1,2,3,7,8-TCDF and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in rat hepatoma H-4-II-E cells confirmed that 2,3,7,8-substituted congeners were more active as inducers of aryl hydrocarbon hydroxylase (AHH) than compounds containing only three lateral chloro substituents (1,2,7,8-TCDF and 2,3,7-ECDD). However, using [3H]analogues (sp. activity 30-50 Ci/mmol) of these congeners, it was shown that the sedimentation coefficients of their respective cytosolic and nuclear receptor complexes were comparable; moreover, the rates of degradation of their respective occupied nuclear receptors were also not structure-dependent. However, the following structure-dependent effects correlated with the structure-induction relationships: namely, (i) higher levels of nuclear receptor complexes were observed for the 2,3,7,8-substituted compounds and (ii) at comparable levels of nuclear receptor, the induction responses were higher for the 2,3,7,8-substituted congeners (ES-03556).

TRANSPORT, METABOLISM AND CONTROL OF THYROID HORMONES TREATED WITH 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN. W. L. Roth and S. D. Aust. Dept. of Biochem., Michigan State Univ., E. Lansing, MI.

Male Fischer rats treated with 10 nmol TCDD/Kg body weight (3.23 ug/Kg) were injected with tracer doses (iv) of either T4 or T3 3 days later. Blood, liver, kidney, bile and urine samples were collected and extracted at time points ranging from 2 min to 15 hr post-injection. HPLC separation of these extracts yielded data for tissue uptake, excretion, deiodination, and glucuronidation of T4, T3, and major metabolites in each compartment sampled. Transport and metabolism rates for a five compartment model were estimated and then optimized via the CONSAM analysis and modeling program. As predicted from the induction curve for cytochrome P-450c and associated glucuronosyl transferases by TCDD, glucuronidation of T4 was maximal at this dose of TCDD. However, steady-state T4 levels decrease only 40%, compared with a 75% drop which occurs at 77 mol TCDD/Kg (25 ug/Kg). Tissue uptake and metabolism of T3 were not significantly changed. Data for T3 production from T4 in the liver suggest that deiodination is controlled by the flux of T4 through the plasma membrane high affinity uptake system, rather than by the concentration of T4 in the cytoplasm of hepatocytes. (Supported by NIH Grant No. ES03586.)

2,2',4,4',5,5'-HEXACHLOROBIPHENYL (HCB) AS A 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ANTAGONIST IN C57BL/6 MICE: L. Biegel, D. Davis, M. Harris, L. Safe and S. Safe. Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.

At dose levels as high as 750 to 1000 umol/Kg, HCB did not cause fetal cleft palate, suppress the splenic plaque-forming cell response to sheep red blood cells or induce hepatic microsomal ethoxyresorufin O-deethylase (EROD) in C57BL/6 mice. However, cotreatment with an effective dose of TCDD plus HCB (400-1000 umol/Kg) partially antagonized TCDD-mediated cleft palate, immunotoxicity (i.e., suppression of the splenic plaque-forming cell response to sheep red blood cells) and hepatic microsomal EROD induction. 4,4'-Diodo-2,3',5,5'-tetrachlorobiphenyl (12-TCPB) exhibited a greater partial antagonist activity and (131P)TCPB (2,3,5,6-Ci/mol) did not exhibit any specific binding activity to the cytosolic Ah receptor. These results contrasted with previous studies with Aroclor 1254 which suggested that this mixture acted as a competitive Ah receptor antagonist (ES-03843).


TCDD suppresses spontaneous breast tumors in the rat, exhibits antifertility activity in vivo and in vitro, and induces cytophromes P-450. The effect of TCDD on cell proliferation and induction of estrogen metabolism in MCF-7 cells was determined to establish an in vitro model of TCDD's action on estrogen-dependent human breast tumors. TCDD (10^-9M) suppresses postconfluent MCF-7 estrogen-dependent cell proliferation, characteristic of cancer cell growth in vitro, and induces a persistent 8- and 2-fold increase in 2 and 18-hydroxylation of 17b-estradiol (E2), respectively. This was accompanied by a concomitant increase in aryl hydrocarbon hydroxylase activity indicative of cytochromes P-450 induction. These results suggest that increased E2 hydroxylation may play a role in the antifertility activity of TCDD in MCF-7 cells and that TCDD's activity may furnish a prototype for the study and management of estrogen-dependent breast tumors. Supported in part by NIH ES03561 and NIH HD 19825 and CA 39734.
Susceptibility to dioxin hepatotoxicity is transmitted as an autosomal recessive in mice.
S.W. Jordan, D.C. Allison, K.K. Bose. Sponsor: W.M. Hadley. School of Medicine, University of New Mexico, Albuquerque, NM and School of Medicine, Johns Hopkins University, Baltimore, MD.

Livers of mice which are responsive or unresponsive to P450IAl enzyme induction by TCDD were histologically evaluated one month after administration of a single dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (75-150 mcg/kg body weight) in a coded study. Mouse strains used were C57Bl/6J, DBA/2J, the F1 cross and progeny of the F1 backcrosses to the two parental strains. Livers from 536 mice were examined. Histologic evidence of hepatocellular necrosis was found in susceptible mice treated with TCDD. Transmission of susceptibility to liver damage by TCDD was consistent with an autosomal mode of transmission for both sexes, although females showed significantly less damage than males. Autosomal recessive transmission was unexpected, since liver damage is hypothesized to be linked to the level of microsomal P450IAl, aryl hydrocarbon hydroxylase, inducibility of which is known to be transmitted as an autosomal dominant. Since susceptibility to hepatocellular necrosis is transmitted in a different manner than P450IAl inducibility, we conclude that the mechanism of TCDD hepatotoxicity is more complex than can be explained by P450IAl induction alone (Supported in part by DOE-USVA # DE-AC04-76EV01013).


DPX may result in mutations and are a measure of the HCHO concentration in target cells. The yield of DPX in the upper respiratory tract (URT) was determined after 6-h exposure of monkeys (MM) (0.7, 2.6 ppm) and rats (RN) (0.3, 0.7, 2.6, 10 ppm) to HCHO. Tissue samples were collected from several regions of the URT of MM and from the nasal respiratory mucosa of RN. DNA was isolated, enzymatically hydrolyzed, and HCHO-bound to DNA was analyzed by HPLC. Metabolic incorporation of 14C into DNA differed in the two species, with predominant labeling of thymidine in MM but with equivalent labeling of purines and thymidine in RN. Yields of DPX (pmol/mg DNA) in the anterior nasal mucosa and turbinates increased nonlinearly with concentration in MM: 0.7 ppm (0.38 ± 0.10), 2 ppm (2.6 ± 0.3), 6 ppm (18.2 ± 3.4), and in RN: 0.3 ppm (1.4 ± 0.6), 0.7 ppm (2.9 ± 0.4), 2 ppm (19.9 ± 3.7), 6 ppm (108 ± 6.0), 10 ppm (266 ± 30), suggesting that pathways leading to detoxification or removal of HCHO and more significantly more efficient at low than at high concentrations. Yields of DPX were lower in MM than in RN; the ratio (MM/RN) increased with concentration between 0.7 ppm (0.092 ± 0.027) and 6 ppm (0.172 ± 0.024). Low yields of DPX were detected in the nasopharynx and trachea of MM, but no DPX were found in the maxillary sinus, bronchioles, or proximal lung. These results demonstrate selective involvement of URT tissues with respective to DPX formation and that differences between monkeys and rats with respect to DPX formation are concentration dependent.

2,3,7,8-Tetrachlorodibenzo-p-dioxin as a porphyrinogen: Mechanistic studies. C. Yao and S. S. Safe. Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.

The comparative activities of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (75 μg/kg) and 6-methyl-1,3,7,8-tetrachlorodibenzo-furan (MCDF) (750 μmol/kg) as porphyrinogens and as inducers of hepatic aryl hydrocarbon hydroxylase (AHH) were determined in normal and ovariecetized female C57Bl/6 mice. Three weeks after treatment, TCDD caused a greater than 6- and 10-fold increase in the accumulation of hepta plus octa-carboxy porphyrins and AHH, respectively, in both groups of mice whereas MCDF did not significantly alter these hepatic responses. Cotreatment of both groups of mice with MCDF (750 μmol/kg) plus TCDD (75 μg/kg) resulted in significant decrease in TCDD-mediated porphyrinogenicity but not AHH induction. In contrast, MCDF did not antagonize TCDD-mediated porphyrinogenicity in male C57Bl/6 mice. The significance of one and other related results will be discussed in terms of the proposed mechanisms of TCDD-induced porphyria (ES-02404).


Previous studies have shown that subchronic exposure to O3 results in hyperplastic and metaplastic changes in nasal airway epithelium of monkeys. In addition, acute O3 exposure causes a transient influx of neutrophils (PMN) into the nasal surface epithelium. This study was designed to characterize the response of rat nasal airway epithelium to O3 and examine the relationship between PMN influx, cell proliferation, and alterations in nasal epithelial mucosubstances. Rats were exposed to 0, 0.12, or 0.4 ppm O3, Animals were killed immediately after 3 or 7 days of exposure, or 3 or 7 days after 7 days of exposure. Rats were injected with bromodeoxyuridine (BrDU) 2 hr before death to label cells in the S-phase of the cell cycle. Cuboidal epitetha covering maxillary turbinate were examined for changes in cell proliferation (total epithelial nuclei/mm basal lamina and BrDU labeling, numbers of intrapithelial PMN, and quantity of epithelial mucosubstances. Exposure to 0.12 ppm O3 did not affect any of the measured parameters. Rats exposed to 0.8 ppm O3 (3 or 7 days exposure) had a transient increase in cell proliferation concomitant with an increase in intrapithelial PMN. Marked surface epithelial hyperplasia, with increased stored mucosubstances was evident after 7 days of exposure to 0.8 ppm O3. These data suggest an interaction between PMN influx, cell proliferation, and the adaptive response of the nasal epithelium to O3. Further studies are needed to understand what triggers O3-induced nasal epithelial cell proliferation and secretory cell hyperplasia. (Research sponsored by NIH Grant ES04282 and by the US DOE/GEER under Contract No. DE-AC04-76EV01013.)
LOW-LEVEL OZONE EXPOSURE CAUSES INCREASES IN RAT NASAL EPITHELIAL MUCOSUBSTANCES. J A Hotchkiss, J R Harkema, R F Henderson.
Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

Previous studies have shown that ambient levels of O3 can induce secretory cell hyperplasia and increases in epithelial mucosubstances in the nasal airways of rats and monkeys. Quantitative changes were restricted to transitional (mucous) (TE) and respiratory (ciliated, pseudostratified) epithelia (RE) in the anterior aspect of the nasal cavity. The goal was to characterize changes in cell number and quantity of mucosubstances in surface epithelia of rat nasal airways after short-term O3 exposure. Rats were exposed for 7 days (5 hr/day) to 0.0, 0.12, or 0.8 ppm O3 and killed immediately or 7 days after the last exposure. Nasal cavities were processed for morphometric analysis of intrapapillary mucosubstances. Compared to controls, rats exposed to 0.12 ppm O3 had increased amounts of stored mucosubstances within epithelium lining the medial aspect of the nasal turbinate, but no change within epithelia of the nasopharynx. There was evidence of epithelial hyperplasia in TE lining nasal and maxilloturbinate respiratory mucosubstances in rats exposed to 0.8 ppm O3. These animals had increased quantities of stored mucosubstances within TE and RE lining the turbinates and lateral walls of the anterior nasal airway. Rats exposed to 0.8 ppm O3 had a significant decrease in stored mucosubstances within epithelium of the nasal septum at the end of exposure, but control levels by 7 days post-exposure. Results of this study demonstrate that exposure to O3, at or below the current U.S. NAAQS can induce alterations within an upper respiratory airway of the rat and that this persists for at least 7 days post-exposure. (Research sponsored by NIH Grant ES04282 and by the US DOE/OBER under Contract No. DE-AC04-76EV01013.)


Formaldehyde (HCHO) is a nasal carcinogen in rats but it remains to be determined what cancer risk this chemical poses for humans. The present studies were designed to provide data for a comparison of formaldehyde-induced responses in rats with previous findings in rhesus monkeys (Monticello et al, in press). Twenty-four male F-344 rats were divided into 4 groups of 6 animals/group. Groups 1 and 2 (controls), were sham exposed to 0 ppm HCHO for 6 hr/day for either 4 d, or 6 wks (5 d/wk). Groups 3 and 4, were exposed to 6 ppm HCHO for either 4 d, or 6 wks (5 d/wk), respectively. The respiratory tract was assessed using histopathology. HCHO-induced lesions in the rat were characterized by epithelial degeneration, hyperplasia and squamous metaplasia, and were present in the anterior portion of the nasal passages, primarily confined to the lining of the lateral and middle meatus. The nature and severity of HCHO-induced lesions in rats resembled those in monkeys similarly exposed to HCHO. However, in monkeys, nasal lesions were more widely distributed, extending to the nasopharynx. In addition, HCHO-induced lesions in the monkey continued distally to involve the trachea, carina and proximal portion of the major bronchi. These studies demonstrate clear differences in the distribution of formaldehyde induced lesions between rats and primates, which should be considered when attempting to assess human risks from data generated in rodent inhalation toxicology studies.

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Cigarette smoke and ozone, environmental agents known to be irritants to nasal epithelia, cause an inflammatory response and development of hyperplastic lesions in these tissues. Exposures to cigarette smoke and ozone have been conducted to determine which of the four types of nasal epithelium (cuboidal, respiratory, squamous and olfactory) are sensitive to these agents. Sensitivities of the epithelium have been monitored by assays of cell proliferation using BrdU incorporation. Rats were exposed to diluted mainstream cigarette smoke by nose-only or whole-body inhalation exposures or were sham-exposed to air. All animals received 1200 mg particulate-hr&m-3 daily for either 1 or 4 wks (preceded by one-half concentration for 1 wk). Significant increases in proliferation were detected in the cuboidal epithelium after both exposure periods. A proliferative response was not seen in the other types of epithelium. A hyperplastic response occurred within the cuboidal epithelium. Rats were also exposed to ozone (0.00, 0.12, 0.8 ppm 6 hr/day) for 3 or 7 days followed by a 3 or 7 day post exposure period. A proliferative response was noted in the cuboidal epithelium with 0.8 ppm exposure, which quickly diminished 7 days post exposure. These studies show that nasal epithelium is sensitive to irritation by cigarette smoke and ozone. (Research supported for the U.S. DOE/OBER under contract No. DE-AC04-76EV01013.)

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Hydrogen fluoride (HF), hydrogen bromide (HBr), and hydrogen chloride (HCl) can be generated as pyrolysis products of the fire retardant Halon 1301, as well as other materials. In this study, we examined the acute toxicities of these halides in the respiratory tract. Fischer-344 rats were exposed to HF, HBr, or HCl at concentrations ranging from 100 to 1000 ppm for 30 min and were sacrificed at 8 and 24 hr post-exposure for histopathologic analyses of the upper and lower respiratory tract and for lung gravimetric measurements. No evidence of direct injury to the lungs by the halides was detected in the histopathologic or gravimetric analyses. Tissue injury was confined to the nasal region only. Histopathologic findings included epithelial and submucosal necrosis, accumulations of inflammatory cells and exudates, and the extravasation of erythrocytes. The severity of injury increased with increasing exposure concentrations to the halides. The relative toxicities of the halides in the nasal compartment were found to be: HF>HCl>HBr. These results indicate that acute respiratory tract injury resulting from the nose breathing of HF, HCl, and HBr in the rat is restricted to the nasal region and that these halides are not equivalently toxic.
Glutaraldehyde, a 5-carbon bifunctional aldehyde, is a potent crosslinking agent, and in this respect resembles the rodent nasal carcinoma formaldehyde. As glutaraldehyde has not been tested in a rodent carcinogenicity bioassay, studies were carried out to compare glutaraldehyde with formaldehyde for selected end points. Like formaldehyde, glutaraldehyde did not deplete intracellular glutathione at sub-cytotoxic levels (5-10 µM) in cultured human lymphoblasts. Nasal instillation studies revealed glutaraldehyde to be at least 5 times more potent for the induction of nasal epithelial lesions than formaldehyde in male F-344 rats. Lesions induced by 40 mM glutaraldehyde were typical of acute aldehyde toxicity, and included respiratory epithelial hyperplasia, squamous metaplasia, and focal olfactory degeneration. A 5-bromo-2-deoxyuridine/immunogold technique revealed a 2-5-fold increase in cell proliferation over controls associated with the respiratory epithelial lesions 3 days after instillation, with considerable inter-animal variation. Because no lesions or increase in cell proliferation were noted following instillation of 10 mM glutaraldehyde, a steep concentration-response was revealed for this compound. Histology demonstrated that glutaraldehyde was a substrate for aldehyde dehydrogenase in the nasal epithelium of the rat, and in this respect glutaraldehyde resembles acetaldehyde, a nasal carcinogen. These findings suggest the need to perform a chronic inhalation study to assess the carcinogenic potential of glutaraldehyde.

Inhalation exposure to methyl bromide (MeBr) induces lesions in several tissues, including the brain and olfactory mucosa. However, the mechanisms responsible for the toxicity of MeBr and other monohalomethanes are unknown. Morphological studies of rats exposed to 200 ppm MeBr for 1-6 hours indicated that the primary lesion in the olfactory mucosa occurs in the sustentacular cell. Biochemical endpoints were evaluated in rats exposed to 200 ppm MeBr for 6 hours. The MeBr-induced histopathologic changes in the olfactory mucosa were associated with [1] decreased P-450-dependent alkyloxydealkylase (AOD) activities, [2] decreased reduced glutathione (GSH), GSH transferase, and GSH reductase activities, and [3] lower metabolism of arachidonic acid (AA) as determined by HPLC. BW755C, a dual prostaglandin H synthase/lipoxygenase inhibitor, protected rats from MeBr-mediated olfactory toxicity, prevented the MeBr-induced inhibition of GSH transferase, but not the decrease in GSH, and had no consistent effects on olfactory tissue AA-dependent leukotriene and prostaglandin formation as determined by RIA. BW755C was also observed to be a potent in vivo inhibitor of olfactory AOD activity. These data indicate that the mechanism of MeBr olfactory toxicity is unlikely to be production of cytotoxic AA metabolites, since the protective effects of BW755C are not associated with changes in AA metabolism. The potential role of the P-450-dependent monooxygenase system in the toxicity of MeBr is being further investigated.
149 EFFECTS OF EICOSAPENTAENOIC AND DECOSEAHEXAOENOIC ACIDS ON FETAL THYMUS ORGAN CULTURES.

Previous work in our laboratory has implicated arachidonic acid metabolites, particularly prostanoids, as mediators of thymocyte proliferation/differentiation in cultured fetal thymuses. Moreover, certain auto-immune diseases can be modulated by cyclooxygenase inhibitors and fish oil components, e.g., eicosapentaenoic and docosahexaenoic (EPA and DCHA) acids, two n-3 fatty acids. It is reported that cellular accumulations of EPA and DCHA in rat tissues relate to impaired arachidonic acid metabolism and production of novel eicosanoids. We have investigated effects of EPA and DCHA on organ-cultured fetal thymuses. Since this culture system is a proven model for study of fetal thymocyte development, it may prove useful in assessing potentially immunotoxic agents during in utero exposure. Day 14 thymuses were cultured in RPMl 1640 medium with or without EPA or DCHA. Both fatty acids at 300 and 400 μM proved toxic following a 5 day incubation. In contrast, when thymuses were pulsed with 250 μM EPA for 48, 72 or 96h, cell proliferation increased significantly over control values. Since EPA appears to potentiate fetal thymocyte growth in vitro, we are now defining cell populations in this experimental protocol to better understand the role(s) of eicosanoids in these developmental processes.

151 SUPPRESSION OF THE IN VITRO SRBC PFC RESPONSE BY BENZO(A)PYRENE IS INDEPENDENT OF Ah RECEPTOR PHENOTYPE. S P Mudzinski, Dept. of Microbiology and Immunology, Albany Medical College, Albany, NY. Sponsor: D A Lawrence

Benzo(a)pyrene (BaP) mediated immunosuppression and BaP conversion to the 7,8-dihydroxy-7,8-dihydro-BaP (7,8 dio) are quantitatively dependent on the high affinity Ah receptor (AhR) phenotype in the mouse. The objective of this study was to determine if 7,8 dio-mediated suppression of the in vitro sheep red blood cell (SRBC) plaque-forming cell (PFC) response is high affinity AhR dependent and/or free radical mediated. Chemicals and SRBC were added at culture initiation to splenocytes from C57BL/6 b/b (high affinity AhR) or from congenic C57BL/6 d/d (low affinity AhR) mice. After five days, anti-SRBC PFC per 10^6 recovered cells were enumerated. The 7,8 dio suppressed the PFC responses of the b/b splenocytes (IC50=15 ng/ml) and the responses of the d/d splenocytes (IC50=3 ng/ml) when compared to vehicle controls. None of the tested concentrations were cytotoxic. Suppression of the responses of b/b or d/d splenocytes was partially reversed by co-incubation with α-naphthoflavone (α-NF) but not with β-α-tocopherol, butylated hydroxyanisole (BHA) or α-tocopherol (α-T). These data suggest that 7,8 BaP dio suppresses the in vitro SRBC PFC response by an α-NF-inhibitable mechanism that appears not to be free radical mediated and that may be independent of the AhR phenotype. Supported by NIH grant ES-04020

150 SEPARATION OF DIMETHYLNITROSAMINE-INDUCED IMMUNOSUPPRESSION AND MUTAGENICITY. H G Haggerty, B S Kim, and M P Holsapple. Dept. of Pharmacology & Toxicology, Medical College of Virginia/VCU, Richmond, VA.

While both liver S9 homogenates and primary hepatocytes can metabolize DMN to its reactive intermediates, only the primary hepatocytes can suppress the IgM antibody response. However, when unstimulated murine splenocytes are cocultured with either metabolic activating system (MAS), alkaline elution analysis demonstrated the production of DNA single strand breaks (SSB). Hepatocytes produced a linear elution profile with detectable doses as low as 1μm, while S9 homogenates produced a convex elution profile with detectable doses as low as 5mM. Immunosuppression induced by DMN under splenocyte-hepatocyte coculture conditions can be reversed by the DMN demethylase inhibitor, aminooacetomitrile (AAN), exogenous calf thymus DNA, and rodrick during the culture period. The production of SSB with either MAS was reversed by AAN. The addition of exogenous calf thymus DNA to the hepatocyte-splenocyte coculture had no effect on the production of SSB. Rocking the hepatocyte-splenocyte coculture reversed the elution profile of SSB from linear to convex. Alpha-undecarboxylamine, a mutagen equally as potent as N-nitrosocetoxymethyl)ethylamine, had no suppressive effects on the IgM antibody response and even showed a hint of enhancement. These results suggest that the immunosuppression produced by exposure to DMN can be separated from its mutagenic effects. (Supported by NIH grant ES03564 and Training grant ES07087).

152 ROLE OF SULFATE CONJUGATION IN DMN-INDUCED IMMUNOSUPPRESSION. M P Holsapple, B S Kim*, J R Ha* and W D Stevens. Dept. of Pharmacol. & Toxicol., Medical College of Virginia/VCU, Richmond, VA and *KAIST, Seoul, Korea.

Dimethylnitrosamine (DMN) can be activated to produce immunosuppression when cultured with primary hepatocytes, but not with incubated with liver homogenates or purified microsomes. We have investigated the role by a Phase II-generated intermediate, specifically a sulfate conjugate. Addition of sodium sulfate to the hepatocyte culture system, at concentrations up to 10 mM, produced a dose-related enhancement in the DMN-induced immunosuppression. Addition of a sulfate conjugation inhibitor, salicylamide (SAM) or pentachlorophenol (PCP), produced a dose-related reversal of the DMN-induced immunosuppression. Both series of results are consistent with a role by a sulfate conjugate. Using p-nitrophenol-sulfate (PNPS) as a donor, the generation of PNP as an indicator of sulfate conjugation and sarfrole as a positive control, we have confirmed the sulfate conjugation capabilities of hepatocytes and liver homogenates. Surprisingly, both systems were capable of conjugating sarfrole, although the latter system was dependent on exogenous cofactors. Neither system was capable of conjugating either DMN or DEN. Subsequent results demonstrated that both SAM and CP inhibited the Phase I-mediated demethylation of DMN and aminopyrine. The enhancement by sarfrole is being studied. These results do not support a role for a sulfate conjugate in DMN-induced immunosuppression. (Supported by NIH ROI ES03564 and by a Korean Science and Engineering Foundation research grant.)
ROLE OF THE AH LOCUS IN TCDD IMMUNOTOXICITY: STUDIES IN C57BL/6 MICE CONGENIC AT THE AH LOCUS. N L Kerkvliet, L B Steppan, M C Henderson, and D R Buhiel. College of Veterinary Medicine, Department of Animal Sciences, and Environmental Health Science Center, Oregon State University, Corvallis, OR.

There are conflicting reports in the literature regarding the role of the Ah locus in TCDD immunotoxicity. The present study has utilized C57BL/6 mice congenic at the Ah locus to examine the influence of Ah phenotype on TCDD-induced suppression of the antibody response to SRBC. In addition, the relative importance of the Ah phenotype of host vs lymphocyte was examined in adoptive transfer experiments. A single dose of TCDD (0.5, 1.0, 2.0, 5.0 or 20.0 μg/kg) was administered orally two days prior to SRBC injection. Peak antibody responses were measured 5 days later in intact mice or 7 days later in adoptive hosts. Body and organ weights (spleen, thymus, liver) and AhH induction were also measured. The Ah phenotype of the animals significantly influenced their sensitivity to TCDD-induced thymic involution, liver hypertrophy, and suppression of the anti-SRBC response. Based on a biphase dose response curve in AhH mice, suppression of the SRBC response by TCDD appeared to reflect an Ah locus-dependent as well as an Ah locus-independent aspect. The Ah phenotype of both the host tissue and the lymphoid cells was important in conferring sensitivity to TCDD immunotoxicity. Supported by grants ES00210 and ES00040.

MECHANISMS OF IMMUNOTOXICITY OF PCB: FLOW CYTOMETRIC ANALYSIS OF LYMPHOCYTE SUBPOPULATIONS AND IL2R EXPRESSION DURING ALLOGENEIC TUMOR REJECTION. J A Brauer and N L Kerkvliet. College of Veterinary Medicine, Oregon State University, Corvallis, OR.

The in vivo generation of cytotoxic T lymphocytes (CTL) is significantly suppressed in C57BL/6 mice following an exposure to 3,4,5,3'-4',5'-HxCB, a toxic Ah-receptor binding PCB isomer. Spleen cells from mice exposed to 0 or 10 mg/kg HxCB were obtained at various times after allogeneic challenge and analyzed by flow cytometry for lymphocyte expression of Thy 1.2, Ig, L3T4, and Lyt2. In control mice, the percentage and total number of Lyt2 cells increased in a linear fashion over days 7-10, which correlated with the development of CTL activity, while L3T4 cells remained fairly constant. During the same time frame, no significant increase in the percentage or total number of Lyt2 or L3T4 cells was observed in HxCB-treated mice. Induction of IL2R expression on L3T4 cells by Con A was reduced by HxCB exposure while IL2R on Lyt2 cells was intact. Thus, HxCB-induced suppression of CTL activity is not due to lymphocytotoxicity, but, rather, to an abrogation of the normal activation and/or proliferation events that occur in response to allogeneic challenge. Interference with IL2R expression on L3T4 cells appears to be a possible mechanism in the immunotoxicity of HxCB in the activation of Lyt2 CTL. Supported by ES00210 and ES00906.
STRUCTURE-DEPENDENT ACTIVITIES OF POLYCHLORINATED BIPHENYL CONGENERS AS AH RECEPTOR AGONISTS AND PARTIAL ANTAGONISTS-IMMUNOTOXICITY. D Davis and S Safe. Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.

The commercial PCB mixture, Aroclor 1254, is a weak Ah receptor agonist and at subeffective doses (25-150 umol/kg), the mixture antagonizes the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)- mediated inhibition of the splenic plaque-forming cell response to sheep red blood cells in C57BL/6 mice. Structure-activity relationships for PCB as immuno-toxins (i.e., Ah receptor agonists) and as partial antagonists of TCDD-mediated immuno-toxicity were determined using individual PCB congeners. PCB congeners with two or more ortho-chloro substitutions were inactive as immunotoxins (e.g., 2,2',4,4',6,6'-, 2,3',4,4',5,5'-, and 2,2',4,4',5,6'-hexachlorobiphenyl, (HCB)); compounds with three or four ortho-chloro substituents did not exhibit partial antagonist activity, however, 2,2',4,4',5,5'-HCB was an effective TCDD antagonist at doses of 400 and 1000 umol/kg. 2,3',4,4',5,5'-HCB was the only PCB congener which was as active as Aroclor 1254 as a TCDD antagonist. These data and competitive Ah receptor binding affinities indicate that there may be multiple mechanisms of action for PCBs as TCDD antagonist using the murine model (ES-03843).


As part of a continuing study to identify and characterize immunomodulatory effects of organophosphorus compounds, we noted that 0-Tropyl-0-nitrophenyl phenylphosphate prevents mitogen (concanavalin A)-induced activation and proliferation of rat splenocytes with an IC50 of 1.2uM as indicated by counting activated cells with an electronic particle counter. However, replacement of the C4-H0-group of this compound with C4-H3-S resulted in a compound which inhibited lymphocyte activation when tested in the presence of the routine culture medium additive 2-mercaptoethanol (2-ME), but in the absence of 2-ME the compound enhanced activation and proliferation. Two of the phenylphosphonothioates tested were able to substitute for 2-ME and allow near maximal growth of a strictly 2-ME-dependent lymphocyte cell line (CTLL-2). These compounds exhibited essentially additive effects with 2-ME on CTLL-2 cells and rat splenocytes. These results suggest very similar modes of action for phosphonothioates and 2-ME. Finally, a phosphonothioate selected for low neurotoxic potential was examined and found to effectively support lymphocyte growth in vivo and to exhibit relatively low acute toxicity in mice. (Supported by NIH, R15 AI24932-01)

METABOLISM OF BENZO(A)PYRENE (BaP) BY SPLENIC MICROSONES OF BaP-PRETREATED MICE. T T Kawabata and K L White, Jr. Medical College of Virginia/VCU, Richmond, VA.

BaP administration to mice at a dose of 200 mg/kg for 7 days has been found to suppress splenic immune responses. Immunosuppression produced by BaP exposure is thought to be mediated by its reactive metabolites generated within the spleen. BaP exposure has been shown to result in the induction of hepatic cytochromes P-450 capable of generating reactive BaP metabolites. Therefore, it was hypothesized that BaP exposure may result in the induction of P-450 within splenocytes and increase the formation of immunosuppressive metabolites. Female B6C3F1 mice were administered BaP (200 mg/kg) or vehicle (corn oil) subcutaneously for 2 or 4 days. Splenic microsomes were incubated with [3H]BaP. The metabolites were extracted and analyzed by HPLC. The major metabolites generated by microsomes of vehicle exposed mice were [3H]BaP-7,8-diol, -9,10-diol, -9-hydroxy, -3-hydroxy, and -7-hydroxy. The minor metabolites detected were [3H]BaP-4,5-diol, -1,6-dione, -3,6-dione, and -6,12-dione. After 2 and 4 days of BaP exposure, BaP metabolism dramatically increased, however, the pattern of metabolites generated was not altered. The levels of [3H]BaP-7,8-diol generated increased 4- and 7-fold above control levels after 2 and 4 days of BaP exposure, respectively. Therefore, a greater amount of immunosuppressive BaP metabolites may be generated within splenocytes after BaP exposure. (Supported by PHS grant ES03343.)

LYMPHOCYTE IS THE IMMUNE CELL TARGETED BY DIDEOXYADENOSINE. W Tsao*, E E Sikorski*, B A Fuchs*, M L Stern*, M J Luster, and A E Munson*. Department of Pharmacology and Toxicology, Medical College of Virginia/ Virginia Commonwealth University, Richmond, VA and Systemic Toxicology Branch, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC.

Didoxadenosine (ddAdo) is a drug in clinical trial for the treatment of AIDS. Results of Tier I immunological studies showed that ddAdo administered to B6C3F1 female mice for 22 days over a 31 day period, suppressed the IgM antibody forming cell (AFC) response to sheep red blood cells. The effect was dose dependent between 85 and 350 mg/kg with the highest dose producing a 96% reduction. All other immunological functions examined were intact. The purpose of this study was to determine the immune cell targeted by ddAdo in the AFC response. The in vitro AFC assay was used to determine the immune targeted cell(s) by performing separations and reconstitution studies from vehicle and ddAdo treated mice. In the first series of studies, cross over experiments were used and showed that the adherent cells (antigen processing and presenting cell) were not altered by ddAdo treatment and these non-adherent cells were functionally deficient. The non-adherent cells were separated into T and B cells populations and crossover experiments performed. The T cells were unaffected by ddAdo treatment but the B cells were shown to be functionally deficient. This finding adds to the data base for projecting potential clinical toxicity. (Supported by NIEHS contract ES5094 and Training Grant ES07087.)
TOXIC EFFECTS OF PARTICULATE POLLUTANTS ADSORBED WITH BENZO(a)PYRENE (BaP) ON MAMMALIAN CELLS. J M Daisey, P N Atkins, and J T Zelekoff. New York University Medical Center, New York, NY.

Ambient particulate contaminants commonly exist in the environment as complex mixtures closely associated with other pollutants. Although toxic effects of individual pollutants have been studied, effects of combined pollutant exposures on human health and cellular functions have been poorly elucidated. In this in vitro study the cytotoxocities of particulate pollutants, namely wood charcoal and titanium dioxide (as a model particle), were evaluated. The relative particle toxicity, as measured by clonal survival of Chinese hamster cells, was wood charcoal > titanium dioxide. Results demonstrate that adsorption of BaP modifies the cytotoxicity of both particle types. In addition, it appears that the relative toxicity of the particles was enhanced with increasing particle storage time. HPLC analysis of solvent extracts from BaP adsorbed particles suggests that following adsorption, BaP bi-products were formed. Products formed as a result of these particle-substrate interactions may account in part for the observed changes in particle toxicity. The results of this study suggest that adsorption of BaP onto ambient particles can modify their toxic effects, and that this phenomena may occur via the oxidation products formed.

BILIARY EXCRETION OF BENZO(a)PYRENE IN BENTIC FISH FROM INDUSTRIALLY-POLLUTED AND REFERENCE SITES IN THE GREAT LAKES. G M Kirby, J R Smith, and M A Hayes. Department of Pathology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.

Hepatocellular carcinomas and biliary tract tumors occur more frequently in bottom-dwelling fish, especially white suckers (Catostomus commerson), from Hamilton Harbour in Lake Ontario. We compared the pharmacokinetics of orally administered [3H]-benzo(a)-pyrene (BaP), one of the most abundant polycyclic aromatic hydrocarbons (PAH) pollutants in Hamilton Harbour sediment, in white suckers from polluted (Lake Ontario) and reference (Georgian Bay) sites. [3H]-BaP was excreted almost entirely in the bile as water-soluble metabolites. At the time of capture, fish from the polluted site excreted [3H]-BaP twice as fast as fish from the reference site but both groups were similar after fish were kept for six weeks in laboratory tanks. The majority of excreted BaP in both groups remained water soluble after hydrolysis of bile with B-glucoamidase and aryl sulfatase. Fish recently captured from polluted sites produced 18 times more sulfated BaP than did reference fish (9.7 vs. 0.6 nmole/g bile). Liver cytosolic glutathione S-transferase (GST) activity (CDNB) was higher in reference fish but fish from Hamilton Harbour produced more nonhydrolysable BaP conjugates than reference fish. Analysis of bile metabolites by reverse phase HPLC revealed similar profiles of [3H]-labeled fluorescent peaks in fish from both sites. These findings suggest that white suckers have inducible hepatic GST excretion pathways for BaP. Hepatocellular adenomas and carcinomas, found only in Hamilton fish, were consistently deficient in immunoreactive GST when compared with surrounding liver. Altered hepatocellular foci were rarely found in fish with advanced liver neoplasms. This suggests that fish hepatocytes which have lost GST expression become more sensitive to PAHs during neoplastic progression. Supported by Ontario Ministry of the Environment and NSERC.

TOXICOKINETICS AND DISPOSITION OF BENZO(a)PYRENE (BaP) AND BaP 7,8 DIOL IN THE WINTER FLOUNDER. K M Kleinow* and A E McElroy+. *Dept. of Veterinary Pharmacology, Louisiana State University, Baton Rouge, LA and +Environmental Sciences, University of Massachusetts, Boston, MA. Sponsor: W Flour.

Benzo[a]pyrene (BaP) a polycyclic aromatic hydrocarbon can be biologically transformed into metabolites with mutagenic and carcinogenic potential. Intake, disposition, and elimination of BaP and the metabolite product BaP 7,8 diol were examined in flounder following intravascular and oral administration. Terminal elimination half-life of BaP and BaP 7,8 diol were 1.2 and 1.1 days respectively. BaP 7,8 diol demonstrated a higher apparent volume of distribution (1097 ml/kg) than BaP (1038 ml/kg). While biliary concentrations were higher for BaP 7,8 diol than for BaP, corresponding liver and intestinal levels were lower.

In contrast to the intravenous route oral administration resulted in intestinal concentrations equal (BaP 7,8 diol) or greater (BaP) than the liver. Extraction and 14C counting indicate that oral administration and BaP 7,8 diol administration results in a greater percentage of bound residues in the liver. This information will be useful in delineation of route and compound specific handling of carcinogens in aquatic species. (Supported by MDFBL Center for Membrane Toxicity Studies).

BIOAVAILABILITY AND BiotRANSFORMATION OF BENZO(a)PYRENE (BaP) AND BaP 7,8-DIHYDRODIOL (BaP 7,8-D) IN THE LOBSTER, HOMARUS AMERICANUS. M O James and D O Schoel. Dept. of Medicinal Chemistry and The Whitney Laboratory, University of Florida, Gainesville, FL.

The influence of biotransformation on the oral bioavailability of xenobiotics may be an important determinant of environmental toxicity. [14C]-BaP and a precarcinogenic metabolite, [14C]-BaP 7,8-D, were administered by intravascular (iv) or oral (po) routes to lobsters (450-520g). Serum hemolymph (HL) samples were analysed for 14C content and chemical composition. Animals were sacrificed at various times after the dose and tissues analysed for 14C content. Selected tissues were also analysed for chemical composition. Iv BaP was rapidly cleared from HL and taken up by HP where it was slowly metabolized and excreted in feces. At all times after po dosing, BaP concentrations in HL were very low, although concentrations in HP and whole animal were similar to those found after iv administration (68 ± 11% at 2 wks). After po administration of the more polar BaP 7,8-D, HL concentrations were maximal at 8-12 hr and thereafter HL and whole body 14C concentrations were similar after iv or po dosed. BaP 7,8-D was eliminated in feces and urine, such that <7% was left in the animal at 2 wks. For very lipophilic compounds such as BaP, HL concentrations are not a good indicator of systemic absorption in the lobster. Supported in part by CA44297, the US Army, and by a Lucille B. Markay Fellowship at Mt. Desert Isl. Biol. Lab.
BENZOLPYRENE (BeP) DNA-ADDUCTS IN SOUTHERN FLOUNDER (PARALICHTYS LETHOSTIGMA) FED CONTAMINATED SHELLFISH. J D Schell, E A Cromer, and M O James. The Whitney Lab and Dept. of Medicinal Chemistry, Univ. of Florida, St. Augustine, FL

The potential risks of eating contaminated shellfish were studied by measuring the extent of absorption and DNA binding of BeP in consumer target organs following exposure to pure BeP or meals of previously dosed spiny lobster (Panulirus argus). HPLC analysis of spiny lobster hepatopancreas (HP) indicated that 24 hr following an intrapericardial injection of [14C]BeP (6 μCi/kg), 95% of the dose in HP had been metabolized to polar metabolites including BeP-7,8 diol. In flounder fed HP from BeP-dosed lobster, 5.75 ± 1.15% of the dose was retained 24 hr after exposure. The liver contained 0.48 ± 0.08% of the dose. Exposure to HP spiked with pure [14C]BeP resulted in the absorption of 12.74 ± 0.64% of the dose at 24 hr, with 0.92 ± 0.16% of the [14C]BeP-equivalents retained in the liver. BeP DNA-adduct formation occurred in flounder liver following exposure to either HP containing BeP metabolites (0.59 ± 0.07 pmol [14C]BeP-equivalents/mg DNA) or BeP spiked HP (1.43 ± 0.15 pmol/mg DNA). These results indicate that the consumption of metabolized BeP present in contaminated shellfish can result in significant exposure and DNA damage to consumer organisms. Supported by CA44297.

EFFECT OF SOIL PROPERTIES ON BIOAVAILABILITY OF HEXACHLOROBIPHENYLS CONTAINED IN SOILS. C F Fries, ARS-USDA, Pesticide Degradation Laboratory, Beltsville, MD

Uniformly 14C-labeled 2,4,5,2',4',5'- and 2,3,5,2',3',5'-hexachlorobiphenyls (HCB) in solvent were added to three soils with high levels of sand, clay, or organic matter. The treated soils were added to standard rat diets at a 5% rate. HCBs added to normal diets without soil were used as positive controls. Effects of age of residues on bioavailabilities were determined by conducting studies at both 5 days and 6 months after the spiked soils were prepared. Total balance studies were carried out with 7-week-old male Sprague-Dawley rats. The experimental diets were fed for 3 days and the rats were sacrificed after 10 days. Quantities of 14C excreted in feces and concentrations of 14C in body fat were the major criteria for evaluating bioavailability. Retention of HCBs were in the range of 75 to 80% of the amount ingested. Differences in bioavailability between soils and control, among soil types, between compounds, and between degrees of aging were minor and were not statistically significant in any case.

METABOLISM OF DIETARY CARCINOGENS IN AQUATIC FOOD CHAINS. A E McElroy and J D Sisson, UMass./Boston, Boston MA. Sponsor: M O James.

Polycyclic aromatic hydrocarbons (PAH) are carcinogenic contaminants which accumulate in sediments in aquatic ecosystems. As such their availability to benthic organisms and potential transfer through aquatic food chains to man is a matter of environmental health concern. 14C-BeP, benzo(a)pyrene (BeP), and benzo(a)pyrene (BeP) was used to investigate in vivo production and retention of PAH metabolites and bound residues and their potential trophic transfer in and between the polychaete Nereis virens and the winter flounder Pseudopleuronectes americanus. Both the flounder and the polychaete metabolized single oral doses of BeP extensively to water soluble metabolites and bound residues which persisted for at least 4 to 20 days respectively. In fish, bile, intestine and liver contained the highest percentage of the dose while in all cases was extensively metabolized, although the pattern of products accumulated differed between tissue type. Mixtures of metabolites produced by worms and fed to fish appeared to be accumulated and resulted in bound residue formation in fish livers. These experiments characterized the rapid in vivo metabolism of BeP in two species of marine organisms and demonstrated the potential for food chain transfer and further modification of metabolic products and bound residues.

DEVELOPMENT OF ARYL HYDROCARBON HYDROXYLASE ACTIVITY IN EMBRYOS OF THE JAPANESE MEDAKA (Oryzias latipes). J D Wisk, T H Umbretti, M A Gallo and K R Cooper, Joint Graduate Program in Toxicology, Rutgers Univ.- UMDNJ, Piscataway, NJ.

Recent evidence suggests that fish possess measurable levels of cytochrome P-450 dependent monooxygenase activity during embryonic development and induction of this activity may be used as an indicator of environmental contamination. Studies were undertaken to examine the development of aryl hydrocarbon hydroxylase (AHH) activity in the Japanese medaka embryo and the effect of 2,3,7,8-tetrachlorodibenzop-dioxin (TCDD) on development of this activity. In non-treated embryos, AHH activity was detected as early as day 5 of development and remained relatively constant through day 10 of development in both the S9 fraction and 105,000 x g pellet of whole embryo homogenates. The liver rudiment appears on day 5 and hatch occurs on day 1. The mean activities in the S9 fraction and 105,000 x g pellet were 33 ± 14 and 103 ± 17 pmol/min/mg prt respectively. The AHH activity in embryos exposed to 12 parts per trillion TCDD beginning on day 0 of development was 2 to 3 times higher in both fractions by day 5 and remained elevated through day 10. The results demonstrate that a basal AHH activity is present in the Japanese medaka embryo during a substantial portion of its development and that this activity can be induced by TCDD. (NAES K-04107-1-89)
Differential Embryo Sensitivity to 2,3,7,8-Tetrachlorodibenzo-p-Dioxin (TCDD) in Fundulus Heteroclitus. R. Prince and K.R. Cooper. Joint Graduate Program in Toxicology, Rutgers University/UMDNJ, Piscataway, NJ.

A comparison of TCDD toxicity to embryos from both TCDD-impaired and nonimpacted feral killifish populations resulted in different sensitivity. The TCDD-contaminated site (200-400 ppb in suspended sediment) was located on Newark Bay, Newark, NJ; the TCDD-nonimpacted site was located on Long Island (LI). Eggs and sperm were stripped from the adult feral fish and exposed from fertilization to batch to concentrations (200 ppb-0.25 ppb) of [3H] TCDD in solution. At a nominal concentration of 200 ppb TCDD, the percentage of major lesions (tubed heart/collapsed yolk sphere) in the LI and Newark embryos was 55% and 5.5%, respectively; with embryo mortality at 20% and 12%, respectively. A laboratory-reared killifish, the Japanese medaka (Oryzias latipes), exhibited 100% embryo mortality and lesion occurrence at 200 ppb. Similar amounts of [3H] TCDD equivalents at 200 ppb were observed in medaka, Newark, and LI Fundulus embryos; 13.8, 14.5, and 13.2 pg, respectively. The differential sensitivity observed between the subpopulations of Fundulus and the Medaka cannot be attributed to an alteration in uptake. TCDD exposure appears to be acting as a selective pressure to confer resistance to Newark Bay Fundulus embryos. (NJAES K-01407-2-89)


The biosynthesis of steroid hormones takes place in several steroidogenic tissues and involves the participation of different forms of cytochrome P-450. Studies in fish have shown that polychlorinated biphenyl (PCB) mixtures adversely affect steroidogenesis in vivo and in vitro. The present study was conducted to determine whether individual PCB isomers such as HCB alter the cytochrome P-450-mediated production of steroid metabolites from [14C]-progesterone (PG) by head kidney (interrenal) of rainbow trout (Salmo gairdneri). At 1 mg/kg (I.P.), HCB significantly increased the conversion of PG to 17a-hydroxyprogesterone (17-OH PG) by kidney homogenates of both males and females (20°C, 90 min incubations). At 20 mg/kg, 17-OH PG was decreased in females but not in males. At both HCB doses, deoxycorticisol production was significantly increased only in males. Total PG metabolism was increased in both sexes by 1 mg/kg HCB, but was decreased in females at 20 mg/kg HCB. These findings suggest that HCB is altering steroidogenic P-450 activities in trout interrenal tissues in a dose- and sex-dependent manner. (Supported by NIH Grants ES-00230 and ES03850)


Field studies of fish living near sediments contaminated with PCBs, PAHs, PAEs (polyaromatic ketones) and quinones have indicated an increased incidence of hepatic tumors and epithelial lesions. Juvenile channel catfish (Ictalurus punctatus, 150 g) were exposed to either contaminated sediments from Black rock Harbor, RI (BRH fish) or reference sediments (RF fish) in 50-l flow-through aquaria. Four animals per group were sampled on days 2, 7, 14, and 28. Hepatic microsomes were assayed for ethoxyresorufin O-deethylase (EROD), ethoxyresorufin O-deethylase (EEDD), benzphetamine N-demethylase (BZND), and UDP glucuronyl transferase (UDPGT) activities. Cytosolic glutathione S-transferase (GST), total glutathione (GSH), oxidized glutathione (GSSG), total lipid peroxidation (LP), and DNA alkaline unwinding (ssDNA) were also assayed. Results indicate a 3-4 fold increase in EROD and a 50-100% increase in GSSG on all days, but no change in BZND in BRH fish, consistent with F-448 type induction reported for fish. On days 7, 14, and 28, GSH and GSSG were significantly increased (up to 70%) over controls while GST and UDPGT increased 15-20%. LP and ssDNA was significantly greater in BRH fish on all days. The greater increase in Phase I activities compared to Phase II activities suggests increased activation of sediment xenobiotics as a possible mechanism of increased ssDNA and LP in BRH fish.


A wide array of effects have been reported as a result of acute or subchronic exposure to O3, but few studies documenting chronic effects have been published. Although a number of chronic studies were possible, this study was designed to address three objectives: (1) to study the progressive effects of chronic exposure to O3 on the development of chronic lung disease as indicated by physiological, biochemical, immunological, and morphometric endpoints; (2) to determine the postexposure period; and (3) to determine extrapulmonary effects due to O3 exposure. A real world exposure regimen was used, consisting of a baseline exposure to 0.06 ppm O3, 13 hr/day, 7 days/wk, with a 9 hr spike Monday-Friday reaching a maximum concentration of 0.25 ppm O3. The square wave concentration was 0.19 ppm O3. Animals were evaluated after 1 wk, 3 wk, 3 mo, 12 mo, and 18 mo of exposure. After the 3, 12, and 18 mo exposures, separate groups of animals were held in clean air for 1.5, 6, and 4 mo, respectively, and evaluated at those time points. All exposure scenarios were conducted consecutively over a 3 yr period. The 12 and 18 mo exposures were run concurrently in two separate facilities. Stringent QA and QC procedures were followed to ensure quality animal and resultant data. (This abstract does not necessarily reflect EPA policy.)
The progression of lung structural changes caused by exposures to a simulated ambient pattern of \( \text{O}_3 \) was studied using microdissection and EM morphometric techniques. The proximal alveolar regions were isolated from lungs of rats exposed to \( \text{O}_3 \) for 1 and 3 wk, 3 and 18 mo. In addition, rats exposed to \( \text{O}_3 \) for 3 and 18 mo were returned to clean air for 1.5 and 4 mo respectively to study the reversibility of lung injuries. It was found that exposure to the simulated ambient pattern of \( \text{O}_3 \) caused a biphasic response in the proximal alveolar regions. After 1 wk of exposure, increased volumes of epithelium (+27%) and interstitium (+41%) and influx of alveolar macrophages (+166%) were observed. These responses subsided after 3 wk of exposure. Epithelial and interstitial injuries reappeared on prolonged exposure. Epithelial type I and II cells were hyperplastic. There were progressive increases of the volume of interstitial matrix after 3 mo (+10%), and 18 mo (+35%). Furthermore, connective tissue elements appeared to accumulate in the interstitium. When rats were allowed to recover in clean air for either 1.5 or 4 mo, the epithelial injuries were diminished. The volumes of the interstitial matrix remain elevated, but the increases were no longer statistically significant. Bundles of collagen fibers were still evident and it is not certain that these changes can be fully reversed.

Pulmonary enzymes were examined after exposure to \( \text{O}_3 \) in a simulated urban profile (see accompanying abstract; Grose et al.). Following 1 wk, 3 wk, and 3 mo of ozone exposure no changes were observed. However, after 12 months of \( \text{O}_3 \) exposure the following enzymes were significantly increased: selenium dependent GSH-peroxidase (23%), GSH-reductase (20%), GSH-transferase (13%) and NADPH cytochrome C reductase (68%). In addition nonselenium GSH-peroxidase (39%), and superoxide dismutase (12%) also showed nonsignificant increases in activity. After 18 mo of \( \text{O}_3 \) exposure these parameters returned to control levels except for superoxide dismutase which was still slightly elevated. The increased activity of the GSH-dependent enzymes after 12 mo of exposure have been observed following shorter \( \text{O}_3 \) exposures. Even though it has not been conclusively shown that these enzymes increase resistance towards \( \text{O}_3 \) exposure, it may indicate an adaptive response of the lung that aids in the overall resistance to oxidant injury. (This abstract doesn't necessarily reflect EPA policy.)

Various tissue, blood, and urine biochemical analyses were examined after 1, 3 wk, and 3, 12, 18 mo of exposure to \( \text{O}_3 \) in a simulated urban profile (see accompanying abstract; Grose et al.). There were no hematological changes due to \( \text{O}_3 \) exposure, although there were notable age related changes. All serum and urine chemistries were unaffected by exposure to \( \text{O}_3 \) through 12 mo except for a transient decrease in serum LDH, CPK, Creatinine, and BUN after 3 mo of exposure. However, after 18 mo of \( \text{O}_3 \) exposure serum CPK, BUN, and protein were elevated as were LDH and creatinine although non-significantly. Most biochemical changes in the tissues (liver, kidney, and heart) were unchanged through 18 mo of \( \text{O}_3 \) exposure. However, liver NADPH cytochrome C reductase and P450 were increased after 12 and 18 mo of exposure. These changes indicate a higher level of metabolism in the livers of \( \text{O}_3 \) exposed rats. This higher activity in \( \text{O}_3 \) exposed rats may indicate a greater susceptibility to certain bioactivated pollutants. (This abstract does not necessarily reflect EPA policy.)

Rats were exposed to a simulated urban profile of ozone (\( \text{O}_3 \)) or to filtered air (see abstract, Grose et al.). Spleens were removed after 1 and 3 wks and 3, 12, and 18 mo of exposure; cells were extracted, and assessed for natural killer cell activity (NK) and blastogenic response to T cell mitogens, phytohemagglutinin (PHA), and concanavalin A (ConA), and to the B cell mitogen Salmonella typhimurium glycopolypeptide (STG). Ozone had no effect on NK regardless of length of exposure. However, responses to all 3 mitogens were consistently lower in rats exposed to \( \text{O}_3 \); these differences were statistically significant following 3 mo of exposure. Maximum response to mitogens in air controls occurred in the 3 mo exposure group. In rats exposed for 12 and 18 mo mitogen responses were clearly depressed due to aging, which may account for our failure to see significant differences between \( \text{O}_3 \) and air at these time points. The results indicate an effect due to chronic \( \text{O}_3 \) exposure at a site distant from the lung. Ozone depression of PHA blastogenesis following acute exposure of humans to higher concentrations as well as following in vitro exposure of human cells have been reported. This data extends information on these effects to include chronic exposures of rats. (This abstract does not necessarily reflect EPA policy.)
The objective of this study was to detect biochemical changes in the respiratory tract of F344 rats resulting from chronic exposure to a simulated urban pattern of O₃ (see abstract, Grose et al.). Supernatants, cells, and whole lung tissue were assayed for protein, lipid phosphorous (LP), fatty acids (FA), alphatocopherol (AT), ascorbic acid (AH₂), glutathione and uric acid. Significant effects of O₃ exposure were as follows: BAL Supernatants: Protein was increased 40% at 1 and 3 weeks of exposure, and 20% at 12, 52, and 18 mo. This increase disappeared when the rats were allowed to recover 1.5 mo in clean air. Eight major FA's were decreased 20-40% at 12 weeks, but were unchanged at later time periods. Surfactant [AT] was decreased by 50% and 31% at 12 and 18 mo, respectively. AH₂ was increased 133% at 18 mo. BAL Cells: AH₂/protein ratio was increased 85% at 12 mo and 151% at 18 mo. [AT] was increased 34-55% in the BAL cells and in the whole lung at 18 mo. The surfactant AT decrement caused by chronic O₃ exposure indicates an impairment of a defensive mechanism. While other changes could represent unrecognized mechanisms of adaptation to the O₃ exposure. (This abstract does not necessarily reflect EPA policy).

Breathing patterns and mechanics were assessed in unanesthetized, restrained rats during challenges with 0.1% and 8% CO₂ after exposure to O₃ in a simulated urban profile (see abstract, Grose et al.) for 1, 3 wk and 3, 12, and 18 mo. The data indicate that O₃ caused a significant increase in expiratory resistance at each time point, but particularly at 18 mo. O₃ exposed rats showed a reduced ability to increase ventilation during CO₂ challenge as compared to control rats, although this difference was not statistically significant. The reduced CO₂-stimulated hyperventilation was the result of a significant decrease in frequency of breathing which was associated with a significant increase in the inspiratory time and not to changes in expiratory or apnea times. This data might suggest that chronic O₃ exposure alters autonomic responsiveness as evidenced by 1) increased resistance without associated histopathology and 2) diminished ventilatory response to CO₂ challenge. However, the effects were small and may only occur with increased ventilation.

There is evidence to suggest that chronic environmental O₃ exposure in man induces O₃ tolerance but this phenomenon has not been reported in animals. We chronically exposed rats to air and to a simulated urban profile of O₃ for 12 or 18 mo (see abstract, Grose et al.) and then assessed changes in ventilation during subsequent O₃-challenge. A similar postexposure O₃-challenge was done in cohort rats after recovery in clean air, 6 or 4 mo, respectively. O₃-challenge employed two techniques, a) O₃-challenge @ 1 ppm O₃ and b) O₂-challenge @ 0.5 ppm O₃ with CO₂ added to stimulate breathing. Results showed that rats chronically exposed to O₃ exhibited a delayed onset and attenuated O₃ response to the O₃-challenge, as seen in measures of frequency, tidal volume, expiratory time and peak expiratory flow. Quantification of the O₃-dose during O₃-challenge (cumulative O₃ removed by the rat) determined that chronic-air rats responded after 9-13 µg O₃ whereas the response dose was 3-21 µg O₃ for the chronic O₃ rats. Tolerance to O₃ was not detected in chronic-O₃ rats after recovery in air. These findings confirm experimentally that chronic exposure to O₃ can induce tolerance to its own irritancy and that this tolerance wanes with lack of continued exposure, hence implying reversibility of its impact on lung physiology. (This abstract does not necessarily reflect EPA policy.)

Pulmonary Immunology Profile in Fischer-344 Rats After Chronic Ozone Exposure. G R Burleson,1 LL Keyes,2 and JP Ehrlich,2 1 Inhalation Toxicology Division, Health Effects Research Laboratory, US EPA; 2NSI-ES, RTP, NC.

Ozone is an ubiquitous air pollutant that has been reported to exert deleterious health effects on pulmonary physiology, biochemistry, and immunology. The present study investigated the effects of chronic ozone exposure on pulmonary immune function. The chronic ozone exposure regimen is described in the accompanying abstract by Grose et al. Quantification of pulmonary immunocompetence was evaluated after 1 and 3 weeks, and after 3, 12, and 18 months exposure. Pulmonary immunological parameters evaluated included: (A) bronchoalveolar lavage cell differentials and phenotypic cell surface markers, (B) alveolar macrophages were quantified for (1) influenza virus-alveolar macrophage association, influenza virus-induced alveolar macrophage interferon production, and the kinetics of alveolar macrophage inactivation of influenza virus, and (2) alveolar macrophage tumor cytototoxicity, and (C) mediastinal lung lymph node cells were assayed for (1) NK activity, (2) mitogen stimulation, and phenotypic cell surface markers. None of the measures of pulmonary immunocompetence were affected by chronic ozone exposure. (This abstract does not necessarily reflect EPA policy.)
This study examines the effect of O₃ exposure (0.4 ppm, 2 hr) on protein (prot), uric acid (UA), ascorbic acid (AH₂), and alpha-tocopherol (AT) in humans, rats, normal and AH₂ deficient guinea pigs. Normal human volunteers were exposed twice, 6 weeks apart, to air or O₃ with intermittent periods of heavy exercise during exposure. Bronchoalveolar lavage fluid (BAL) was collected from humans 16 hrs post exposure and from animals 0 and 16 hrs post exposure. In human BAL supernatant (sup), O₃ double [prot], increased [UA] 124%, and did not change [AH₂] or [AT]. In rat BAL sup, O₃ decreased (15%, 0 hrs) [AH₂] then increased (8.3%, 16 hrs), and did not change [prot], [UA] or [AT]. In BAL sup from AH₂ deficient guinea pigs, O₃ increased [prot] and decreased [AT] but did not significantly affect [UA] or [AH₂]. There were no significant O₃ induced changes in prot, UA, AH₂, or AT in normally fed guinea pigs or in BAL cells of any species. In spite of reported high reaction rates of O₃ with AH₂ in vitro, O₃ had only a small effect on BAL [AH₂] in vivo. The O₃ induced increases in BAL [prot] in humans and guinea pigs, but not in rats, may be related to a 5-10 fold higher [AH₂] in the epithelial lining fluid of rats. (This abstract does not necessarily reflect EPA policy.)

Beta-aminopropionitrile (BAPN) is a lathyrogen which inhibits lysyl oxidase, an enzyme essential for the cross-linking of collagen and elastin. It was hypothesized that interference with connective tissue repair following oxidant injury would lead to lung dysfunction and structural remodeling consistent with emphysema genesis. Six groups of ten, 90-day-old, F-344 male rats were fed either standard chow or chow with 0.5% BAPN for 1 wk prior to a 5 day (6 hr/d) exposure to 0.0, 0.5, or 1.5 ppm O₃. Diets were maintained during exposure and for 3 wk post. Lung function was then evaluated in each rat including: lung volumes, airflow dynamics, lung compliance (Crs), and CD diffusing capacity (D_{LCO}). Left lung lobes were then prepared for morphologic/morphometric appraisal. As anticipated, BAPN itself significantly slowed body weight gain (-12%; BW); however, lung/BW ratios were elevated in these groups. Also, Crs and D_{LCO} were (-15%) depressed as result of BAPN. No significant interactions between BAPN and O₃ exposure were observed in either physiologic or morphometric parameters. O₃ alone, however, did seem to suppress lung Crs, regardless of diet. Hence, repressed connective tissue repair does not seem to alter the outcome of lung injury induced by subacute exposure to O₃. This abstract does not necessarily reflect EPA policy.)

CONCENTRATION-TIME MODELS FOR THE EFFECTS OF INHALED OZONE ON BRONCHOALVEOLAR LAVAGE PROTEIN. J W Highfill, G E Hatch, and D L Costa. HERL, USEPA, Research Triangle Park, NC. Sponsor: J S Tepper

Data from recent human studies suggest that the current 1 hr National Ambient Air Quality Standard for ozone (O₃) may be inappropriate for exposures of several hours. USEPA and the NIVA of The Netherlands collaborated to provide relevant animal data. We report on the matrix of concentrations (C) and exposure times (T) for rats and the modeling of C and T on protein concentration of bronchoalveolar lavage fluid (BALF) obtained from rats. BALF is known to be related to protein leakage into the airspace. Replicate experiments were done for five O₃ concentrations (0.0, 0.1, 0.2, 0.4, and 0.8 ppm) at each of three durations (2, 4, and 8 hr). One proposed least squares response surface model for C and T is log(C) = 5.13 - 0.9C - 0.1T + 0.9C² + 1.1CT. Our results indicate that a constant response of BALF for equal CXT products underestimate the toxicity observed after exposure to O₃. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)

EFFECTS OF ACUTE OXIDANT STRESS ON ADULT AND AGED RAT LUNG PYRIDINE NUCLEOTIDES. N R Montgomery, P Raska-Zemry, L Zychlinski, and J U Balis. Colleges of Public Health and Medicine, Univ. of South Florida and VA Hospital, Tampa, FL.

Adult (4 mos) and aged (24 mos) Fischer 344 male rats were exposed to 0-3.0 ppm ozone for 8 h and sacrificed immediately. Exposure to 0.5 ppm O₃ did not affect the whole lung conc of either NADPH of NADP⁺, their sum, or ratio in adults or aged. However, at 1.5-3.0 ppm, the aged lung had markedly greater decrements in all parameters. Investigation of the hexose monophosphate shunt confirmed that O₃ decreased triphosphonucleotide synthesis and that aged rats had lower metabolic rates than adults. For NAD/H, exposure to 0.5 ppm O₃ was without effect on adults or aged. Again, 1.5-3.0 ppm dramatically reduced nucleotide conc in adults, but only slightly altered [NAD+] in the aged. Mitochondrial ox-phos at 3.0 ppm O₃ was decreased equally in adult and aged rats, suggesting that the difference in NAD/H metabolism did not affect the mitochondrial oxidation. Pyridine nucleotide balance is affected by acute oxidant stress and aged rats respond differently than adults. The functional consequence of this difference is under investigation. Supported by NIH Grants AG07801 and HL34793 and VA Research Funds.

The presence of 3-(cystein-S-y1)-acetaminophen (3-cys-A) protein adducts correlate with acetaminophen hepatotoxicity. Previously we reported an ELISA specific for acetaminophen adducts which is not dependent on radiolabeled samples (JPET 241: 527). The particle concentration fluorescence immunoassay (PCFIA) was developed to replace the ELISA and uses a fluorometer and specially designed assay plates (Baxter Healthcare Corp.). Solid phase antigen for PCFIA was prepared by coupling metallothionein-acetaminophen to amino-substituted polystyrene beads using N-succinimidyl 3-(2-pyridyaldithio)propionate as a coupling reagent. The ELISA and PCFIA had similar limits of detection (20 pmole/mg) and recognized the same epitope as demonstrated by similar relative inhibitory potencies for N-acetylcycteine-A, A-bound glutathione-S-transferase and A. Serum samples and liver fractions containing 3-cys-A protein adducts assayed by ELISA and PCFIA produced similar results (r= 0.89). PCFIA was used to evaluate the role of 3-cys-A protein adducts in acetaminophen toxicity and has advantages over ELISA including: a covalently coupled solid phase assay time, availability of internal standards, and absence of drift and edge effects permitting the assay of 40% more samples per 96-well plate.


The mechanism of the nephrotoxicity of acetaminophen (APAP) is not understood. It has been shown that this toxicity is apparently not mediated by N-acetyl-p-benzozquinone imine (NAPQI), the metabolite that produces the hepatotoxicity.

Since cysteine conjugates of related compounds have been shown to be nephrotoxic, we determined the relative nephrotoxicity of the cysteine conjugate of APAP, 3-(cystein-S-y1)-acetaminophen (3-cys-A). This metabolite is produced by conjugation of NAPQI with glutathione followed by further metabolism. 3-Cys-A was synthesized by reaction of NAPQI with cysteine. Its structure was confirmed by MS and proton NMR. APAP (750 mg/kg and 1000 mg/kg) produced a necrosis of the renal proximal tubules in male Fischer 344 rats as evidenced by increases in blood urea nitrogen and serum creatinine, as well as histopathological lesions. 3-Cys-A was not nephrotoxic at doses up to 810 mg/kg, iv. However, the known nephrotoxin p-aminophenol (PAP) was toxic at doses as low as 75 mg/kg. These data indicate that the nephrotoxicity of acetaminophen is apparently not mediated by further metabolism of 3-cys-A via cysteine conjugate beta lyase; however, APAP nephrotoxicity may be mediated via PAP.


3-(Cystein-S-y1)-acetaminophen (3-cys-A) protein adducts in liver correlate with acetaminophen hepatotoxicity. In this work, we utilized an immunochemical assay to quantitate 3-cys-A protein adducts in kidney following nephrotoxic doses of acetaminophen (APAP) and its deacetylated form p-aminophenol (PAP) to male F344 rats. At 6 hrs following APAP (500, 750 and 1000 mg/kg) or PAP (200 mg/kg) there was histological evidence of proximal tubular necrosis with PAP and a dose dependent necrosis with APAP. Also, necrosis was observed in the livers of some animals at 750 and 1000 mg/kg APAP. Following PAP, 3-cys-A protein adducts were not found in kidney, serum or liver. Following APAP, 3-cys-A protein adducts were found in liver, kidney, and serum but not in heart. In the renal cortex the same level of 3-cys-A protein adducts (0.2 nmol/mg) was observed at each APAP dose. 3-Cys-A protein adducts in liver and serum correlated with liver damage. These data indicate that the nephrotoxicity of PAP is not mediated by 3-cys-A protein adducts. Moreover, the positive dose response for APAP-induced nephrotoxicity coupled with the absence of a dose response for formation of 3-cys-A protein adducts indicate that these adducts do not correlate with kidney toxicity.


The hepatotoxicity of acetaminophen (A) correlates with 3-(cystein-S-y1)A (3-cys-A) protein adducts. We localized these adducts in microwave-fixed liver of B6C3Fl mice given 400 mg/kg A. We correlated these immunohistochemically findings with serum transaminases and with immunohistochemical detection of the adducts in serum and hepatic S-9. At 30 minutes, 3-cys-A adducts were detected in the centrilobular area of the liver immunohistochemically and were quantified in S-9. The livers were histologically normal and neither adducts nor elevated transaminases were present in the serum. However, hepatic glutathione was 90% depleted. Thereafter, the staining intensity and the width of the affected centrilobular areas increased until 6 hrs, along with increase in adducts in S-9. At 8 hrs the histochemical staining intensity was markedly decreased, correlating with parallel elevations in serum 3-cys-A protein adducts and serum transaminases. Histologic changes in affected hepatocytes were first noted at 1 hr and varied from cloudy swelling and vacuolization to necrosis. Collectively, these data indicate that binding of the reactive metabolite precedes evidence of toxicity, and that binding progresses from central to peripheral in the hepatic lobule.

The hepatotoxicity of acetaminophen correlates with the formation of 3-(cystein-s-y1)acetaminophen (3-cys-A) adducts on protein. Using a sensitive and specific immunochemical assay, we quantitated the formation of these adducts in liver fractions and serum after administration of a hepatotoxic dose of acetaminophen (400 mg/kg) to B6C3F1 mice. Adducts in the cytosolic fraction increased to 2.9 nmoles/mg at 2 hrs then decreased to 0.8 nmoles/mg by 8 hrs. Concomitant with the decrease in adducts in the cytosol, 3-cys-A adducts appeared in serum and their levels paralleled increases in serum alanine aminotransferase. Mitochondrial adducts peaked at 1 hr (1.2 mmol/mg) and subsequently decreased to 0.4 mmol/mg at 8 hrs. The 4,000g pellet (nuclei and plasma membrane) had the highest level of adducts (6 mmol/mg) which remained constant from 1 to 8 hrs. Evaluation of fractions purified from a 1,000g pellet indicated that the majority of 3-cys-A adducts were localized in plasma membrane and mitochondrial peak levels were 5.6 and 1.6 mmol/mg respectively. 3-Cys-A adducts were not detected in nuclei. The localization of high levels of 3-cys-A protein adducts in plasma membranes may play a critical role in acetaminophen toxicity.


Electron microscopic examination of pancreatic tissue from B6C3F1 male mice treated with acetaminophen (500 mg/kg, ip) revealed alterations of beta cell ultrastructure at 8 hrs (distended intercellular spaces, cytoplasmic vacuolization, disrupted plasma and organelar membranes). Concomitant with these alterations were increases in serum insulin as measured by a commercial radioimmunoassay. These increases correlated with an increase in hepatotoxicity as assessed by serum ALT levels. At acetaminophen doses of 100 mg/kg and 200 mg/kg, serum ALT and insulin levels were not significantly different from control levels; however, at doses of 300 mg/kg and greater, both serum ALT and insulin levels were significantly increased. Acetaminophen-protein adducts were not detected in pancreatic homogenates using a sensitive immunochemical assay. Also, an immunohistological assay revealed acetaminophen-protein adducts in hepatic centrilobular areas; however, these adducts were not detected in beta cells. These data suggest that acetaminophen-induced beta cell alterations are apparently not a direct result of acetaminophen-protein binding.

191 COMPARISON OF THE DISTRIBUTION OF SUBCELLULAR COVALENT BINDING AND THE EFFECTS ON CALCIUM HOMEOSTASIS PRODUCED BY ACETAMINOPHEN AND 3-HYDROXYACETANILIDE IN MOUSE LIVER. M A Timmerstein and S D Nelson. Department of Medicinal Chemistry, University of Washington, Seattle, WA.

Overdoses of acetaminophen (APAP) have been shown to produce hepatic necrosis in man and in experimental animals. This toxicity has not been observed with the regiosomer 3-hydroxyacetanilide (AMAP). Radiolabeled APAP (250 mg/kg) and AMAP (600 mg/kg) were administered ip. to phenobarbital induced mice which were fasted 16 hours prior to injections. One hour after administration, these doses yielded similar levels of covalent binding in liver homogenates. However, subcellular fractionation indicated that the pattern of subcellular binding was unique for each compound. Greater levels of mitochondrial covalent binding were seen following APAP administration while higher cytosolic and microsomal levels were observed following AMAP treatment. APAP also depleted mitochondrial glutathione (GSH) levels to a greater extent than AMAP. One hour after administration, mitochondrial GSH levels were lowered to 8% of control levels in APAP treated animals while AMAP only depleted GSH to 63% of control levels. Moreover, APAP impaired the ability of mitochondria to sequester calcium, whereas AMAP did not. Similarly, APAP but not AMAP inhibited plasma membrane Ca++-ATPase activity. The data suggest that reactive metabolites of APAP and AMAP can bind to cellular proteins to a similar extent, but that the distribution of subcellular binding is different as well as their effects on calcium homeostasis.


Acetaminophen (APAP) administration (600 mg/kg, p.o.) to fasted male CD-1 mice resulted in cellular damage to liver, lung, and kidney. An affinity purified antibody against covalently bound APAP was used to identify APAP-protein adducts in microsomal and cytosolic extracts from these target organs. The proteins were resolved on SDS-PAGE, transferred to nitrocellulose membranes and analyzed immunologically. Covalent binding of APAP to intracellular proteins was only observed in those organs which exhibited cellular damage; no APAP adducts were detected in tissues which did not undergo necrosis. In all target tissues the arylation of proteins was not random but highly selective with two adducts of 44 and 38 kD accounting for the majority of the total APAP-bound proteins which were detected immunologically. In addition, a third major APAP-protein adduct of 33 kD was also observed in kidney cytosol. The severity of tissue damage and the amount of adducts present in these tissues could be significantly reduced when mice were pretreated with the mixed function oxidase inhibitor, piperonyl butoxide, prior to APAP dosing. Immunohistochemical analysis of plasma from APAP treated animals indicated the presence of several protein-adducts by 4 hr following drug administration. These adducts did not appear to be of plasma origin. Incubation of cytosolic proteins from liver, lung, kidney, spleen, brain and heart with an APAP metabolite generating liver microsomal system demonstrated that the cytosolic 38 kD protein target was native to all tissues tested. By contrast, the 38 kD protein target did not appear to be endogenous to plasma since it was not detected when plasma was incubated in vitro with the liver microsomal system. These studies indicate that, although the 38 kD proteins appear to be endogenous to both target and non-target tissues, the APAP-protein adducts are detectable only in tissues which become damaged by APAP. (NIH GM 31469)
To evaluate the mechanistic importance of covalent binding in acetaminophen (APAP)-induced hepatotoxicity, we have compared the effects of 2,6-dimethyl acetaminophen (2,6-DMA) to those of APAP in primary cultures of mouse hepatocytes. Immunohistochemical analysis has shown that the majority of covalent binding after a cytotoxic dose of APAP occurs on two major bands of molecular weight 44 and 58 KD (Biochem. Pharmacol. 36, 1193, 1987). At equimolar concentrations, 2,6-DMA bound proteins 30% as much as APAP, but was not cytotoxic in the hepatocytes from non-induced mice. However, when the hepatocytes were obtained from phenobarbital-induced mice, the administration of APAP or 2,6-DMA resulted not only in increased binding but a more rapid onset of cytotoxicity for APAP and a change from an apparent cytotoxic effects to overt cytotoxicity for 2,6-DMA. Since the affinity purified anti-APAP antibody exhibited no anti-2,6-DMA activity, a new antibody specific for 2,6-DMA was required. An immunogen was constructed by covalently linking 2,6-DMA via a p-aminobenzoic acid linker to keyhole limpet hemocyanin and following immunization, the antibodies specific for 2,6-DMA were purified using a 2,6-DMA affinity Sepharose 6B matrix. This new antibody was used for Western blotting to detect electrophoretically resolved proteins. Results indicate that in both phenobarbital and non-induced mice, the binding of 2,6-DMA is highly selective with the most prominent target being a 58 KD cytosolic protein. No binding to the 44 KD protein was observed with 2,6-DMA. The phenobarbital treated animals exhibited an increase in the extent of arylation to the 58 KD protein as well as the addition of new protein adducts. These data suggest that both the specificity of covalent binding as well as the extent of binding to the major targets may play an important role in the ensuing toxicity. (NIH GM 31460)

THE EFFECT OF 8-METHoxyFORSALLEN (8-MOP) POST-TREATMENT ON THE COVALENT BINDING OF ACETAMINOPHEN (APAP) TO ELECTROPHORETICALLY SEPARATED HEPATIC PROTEINS. J. Brady, J. Bartalone, R. Birge, S. Hart, D. S. Wyand, E. Khaireallah and S. D. Cohen. Univ. of Conn., Toxicology Program, Storrs, CT 06268.

Fasted (18hr), 3 month old male, CD-1 mice were given APAP (400mg/kg, po) or water. Two hrs later, 8-MOP (54mg/kg), or corn oil was given ip. Covalent binding to liver macromolecules was measured radiometrically and immunochemically at 0.2, 4 and 6hrs after APAP. Plasma sorbitol dehydrogenase (SDH) activity, mortality and liver histopathology were assessed 12hr after dosing. Radiometric covalent binding was maximal 2hrs after dosing. 8-MOP given 2 hrs after APAP reduced mortality and SDH levels by 74 and 50%, respectively, and decreased liver necrosis, suggesting that 8-MOP diminished liver damage by reducing APAP bioactivation. However, when measured immunohistochemically, the covalent binding of APAP to electrophoretically separated proteins was not changed by a 2 hr post-treatment with 8-MOP. Taken together, these data suggest that the post-treatment protection with the cytochrome P450 inhibitor, 8-MOP may block APAP adduct formation in addition to the reported inhibition of APAP activation. (Supported by the CDTX, NIH grants GM31460 and ES07163 and an ICI Americas Fellowship in Toxicology to JTB.)

A NEW APPROACH TO DERIVING EXPOSURE GUIDELINES. S. C. Lewis, J. R. Lynch, A. I. Nikiforov, and K. A. Scala. Exxon Biomedical Sciences, East Millstone, NJ.

Since the introduction of "safety factors" to derive the "acceptable daily intake", significant advancements have been made in toxicology and regulatory decision-making. To capitalize on those advances, a procedure for exposure guideline derivation is offered. The derived guidelines are based on no-observed-adverse-effect level (from human or animal studies), which is downwardly adjusted to provide adequate safety. The distinctive features of this improved approach are: (1) each adjustment factor may take a range of values (typically from 1 to 10); (2) uncertainty is addressed separately and is reflected in the final value; (3) all adjustments reflect scientific consensus. Flexibility in the selection of data adjustments exploits the most contemporary scientific understanding. Separating best estimates for intra- and inter-species differences from adjustments for uncertainty improves communication between risk assessors and risk managers. Scientific consensus assures broad acceptance of the guidance value. The overall framework, with illustrative examples, will be presented.
ABAMECTIN: A RISK ASSESSMENT FOR DEVELOPMENTAL TOXICITY. K. Pfeifer, R. Krieger, California Department of Food and Agriculture, Sacramento CA.

The California Department of Food and Agriculture (CDFA) has responsibility for pesticide evaluation and regulation in the State of California. A toxicology review, as mandated under various state statutes, includes the evaluation of adverse effects through a risk assessment process. The lead branches for risk assessment are Medical Toxicology and Worker Health and Safety; however, other branches within the Division of Pest Management and the California Department of Health Services participate in the risk assessment process. A risk assessment for abamectin, a naturally occurring microbial insecticide/miticide developed by Merck, Sharp and Dohme, is presented to illustrate this process for a compound with adverse developmental effects which were reported in animal studies at relatively low dosages. The basis for the risk assessment was a NOEL of 0.05 mg/kg/day for maternal toxicity in mice. Highest exposure estimates using surrogate data were for handgun applicators in greenhouses or shadehouses and ranged from 0.0018 to 0.042 ug/kg/day. Corresponding margins of safety after implementing all protective requirements on the product label were 2,777 and 1,190. The risk assessment for abamectin demonstrates the importance of exposure and other potentially mitigating factors in determining the risk to humans working with this compound. Some of these other factors include: relative application rates, dermal absorption, pharmacokinetics and environmental fate.


An interim 2,3,7,8-TCDD toxicity equivalency approach for estimating the health risks associated with exposure to PCDD/PCDF mixtures has been developed. Interim 2,3,7,8-TCDD toxicity equivalency factors (TEF) were assigned based on a weight-of-evidence evaluation of the available toxicological data. In general, more weight was given to data derived from chronic or subchronic studies than to acute toxicity or in vitro data. Acute reproductive/developmental effects were given slightly more weight than other acute toxicological data (decreased body weight gain, thymic atrophy, immunotoxicity, guinea pig LD50 and in vitro test data (Ah receptor binding, AHH induction). To simplify the approach and to acknowledge the approximate nature of the TEFs, all TEFs were rounded to the nearest whole (e.g., 0.1) or half (e.g., 0.05) order of magnitude. This general approach for estimating TEFs is similar to that taken by other regulatory agencies.

RETRIEVING ASSIGNMENT CONTEXT OF OBSERVATION FOR F-344 RATS CHRONICALLY EXPOSED TO FORMALDEHYDE. W. H. Lowry, T. M. Monticello, T. B. Starr, K. T. Morgan. Chemical Institute of Toxicology, Research Triangle Park, NC.

One approach to estimating age-specific tumor incidence rates without using scheduled sacrifice information involves determining the context of observation for each tumor-bearing animal. Context of observation is defined as the determination of whether the tumor of interest contributed directly or indirectly to the cause of death, or alternatively, if the tumor was an incidental finding in an animal dying from an unrelated cause. The present study was undertaken to assign retrospectively the context of observation for nasal squamous cell carcinoma in F-344 rats exposed to 14.3 ppm of formaldehyde for up to 24 months. Information utilized included clinical observations, necropsy data, histopathology data, and light microscopic tissue examination. Animals were assigned to one of four context categories based on five different criteria: tumor size, invasiveness, multi-level involvement, necrosis, and inflammation. Results indicate that greater than 90% of the tumors were classifiable as definitely incidental (2%) or definitely fatal (88%) with a high degree of certainty. This study demonstrates that the context of observation can be assigned even long after a bioassay has been completed, provided the data has been properly archived.


Under Section 304(a)(1) of the Clean Water Act of 1977, as amended in 1987, the U.S. Environmental Protection Agency is required to publish criteria for water quality. In accordance with this act, the U.S. EPA in 1980 developed ambient water quality criteria for 129 priority pollutants. Separate criteria were developed for the protection of aquatic life and for the protection of human health from adverse toxic effects associated with the consumption of contaminated water, fish and shellfish.

In an effort to provide criteria for water quality which accurately reflect the most current scientific knowledge, the Agency is currently re-evaluating the criteria for human health protection. This re-evaluation includes a comprehensive review of pertinent data on toxicologic and carcinogenic effects, bioconcentration in seafood, and daily seafood consumption. This presentation details the approaches used in the evaluation of scientific information and the application of risk assessment guidelines for the development of updated ambient water quality criteria.
Sponsor: Harlan Choudhury

With the passage of the Clean Water Act in 1977 and subsequent amendments in 1987, the U.S. EPA is required to develop and publish criteria for contaminants that may occur in ambient water which reflect the latest scientific knowledge on the kind and extent of all identifiable effects on health and welfare. Contaminants classified as known, probable or possible human carcinogens are considered to have no threshold effect levels. For other chemicals, exposure levels are identified below which no effects are expected to occur. Criteria are calculated to provide guidance for estimating allowable concentrations of chemicals in ambient water. These criteria are expected to protect the human population over a lifetime of exposure to contaminated water, fish and shellfish. Application of risk assessment methods is demonstrated through examples. Approaches employed in estimating scientifically defensible criteria using either complete or deficient data sets are shown. A review of the scientific information and its associated uncertainties is presented for these examples.

203 APPROACHES TO USING FIELD DATA TO ASSESS HUMAN HEALTH EFFECTS FROM MUNICIPAL WASTE COMBUSTORS. F. McGinnis, M. Fragge, D. Basu and L. Fradkin, Syracuse Research Corporation, Cincinnati, OH and *Syracuse, NY, and **ECAO, U.S. Environmental Protection Agency, Cincinnati, OH.

Municipal waste combustion (MWC) is being used in the United States as a means of disposal. Concomitant with the use of this disposal process has been the development of methodologies to assess human health and environmental effects of emitted pollutants. The U.S. Environmental Protection Agency has developed a risk assessment methodology for direct and indirect exposure pathways from MWC. Additionally, field studies are being implemented to determine levels of emitted pollutants in the environment. The use of field data in various models of multiple pathway exposure can serve to strengthen the acceptability and further develop models but poses a challenge to the risk assessor. Outlined are problems associated with environmental sampling, application of the models to field data, and interpretation of resultant human risk. Air and environmental media data are used in a risk assessment to illustrate various solutions for these issues.

202 ESTIMATING DERMAL EXPOSURES FOR SUPERFUND RISK ASSESSMENT. J. Herrinton, J. Vandewen, and D. Shelton. CH2M HILL, Reston, VA.

At Superfund sites, the primary exposure routes are inhalation, ingestion, and dermal exposure. Typically, only inhalation and ingestion are quantified. The methodologies available for assessing dermal exposure are poorly developed, in large part because of the uncertainties associated with necessary exposure parameters, particularly absorption and permeability. However, at many Superfund sites, dermal exposure with contaminated soil or water may present exposures that are of similar magnitude to ingestion or inhalation exposures. This paper presents an overview of dermal exposure—the theoretical basis for exposure calculations for air and water, the sources and magnitude of uncertainties, and methods to calculate dermal exposure. Quantitative information contrasting dermal exposures with ingestion and inhalation exposures will be presented, along with illustrations of the effects of uncertainties in exposure parameters on dermal exposure estimates.

204 TOXICOKINETICS OF INTERSPECIES SCALING: IMPLICATIONS FOR RISK ASSESSMENT. J. B. Todhunter, Todhunter, Vandave Associates, Wash., DC.

In assessing risk to humans based on animal data, interspecies scaling of dosage and potency is required. This is commonly done on the basis of either dose per body weight or per the 2/3rd power of body weight (i.e., volume of distribution or metabolic size). To date, there has not been a sound conceptual basis for predicting which basis is appropriate for a given toxicant. In particular, interspecies scaling of carcinogens has been done on the basis of the metabolic size by some since it is considered that in many cases metabolic activation to the active carcinogen is required. We present a toxicokinetic analysis of differences in dose relationships between acute/short term exposures and sub-chronic/chronic exposures. This indicates that tissue steady state levels of toxicant species are the dominant factor in interspecies scaling and allows for construction of a sound model for predicting the appropriate basis for interspecies scaling. In general it appears that once steady state levels are reached, that volume of distribution scaling is the most appropriate way to scale dosage and potency in chronic exposure situations. In acute/short term exposures, it is necessary to consider specific properties of the substance. The compound 1,1,1-TCE is used as a case example and it is shown that this substance scales as dose per body weight.

Toxicological evaluation of chemical contaminants in drinking water is conducted to protect public health in response to actual or potential contamination situations such as those resulting from leakage of underground storage tanks (trichloroethylene), leaching through soil (selenium, nitrate), agricultural runoff (thiocarbamate, molinate, bentazon), or from the proposed use for weed control in reservoirs (diquat). Mandated activities to protect groundwater contamination have included the assessment of atrazine, simazine, bromacil, diuron, and prometon. Ongoing work includes the development of water quality standards (maximum contaminant levels, or MCLs) for over forty chemicals, selected because they have been detected in California groundwater. About half of these chemicals do not currently have water standards established at the federal level. The case samples presented describe the considerations involved in health risk assessments and water quality standards development.

OZONE-INDUCED EFFECTS ON LUNG ARACHIDONIC ACID METABOLISM AND MACROPHAGE FUNCTION. J T Zelikoff, A Gunnison and R B Schlesinger, NYU Medical Center, NY, NY.

Ozone (O₃) poses a significant threat to human health. Arachidonic acid metabolites (eicosanoids) produced in the lung by cells including macrophage (MΦ), are potent biological mediators, thought to contribute to the development of lung disease. To begin to determine the role of eicosanoids in mediating O₃ toxicity we have examined the effects of acute in vivo O₃ exposure on MΦ phagocytosis and on eicosanoid production. Rabbits exposed to 1 ppm O₃ for 2 hrs were sacrificed and lungs lavaged immediately, 5 and 24 hr after exposure, to determine temporal metabolite patterns in acellular lavage fluid and in media conditioned (for 5 hrs) by cultured MΦ (MCM) recovered from exposed animals. Results determined by radioimmunoassay show that relative to controls, PGE₂, PGG₂, 6-keto PGF₁α, and possibly TXB₂ "levels" were elevated in the lavage fluid and MCM collected immediately after exposure. PGE₂ levels also appeared elevated in MCM from MΦ recovered 5 and possibly 24 hrs after exposure. No change in LTB₄ values were observed. Following O₃ exposure, the phagocytic activity of MΦ collected immediately and 24 hrs after exposure was depressed. Specific eicosanoids acting via intracellular CAMP may be responsible for this observed change. These results provide evidence for a possible mechanism of O₃ involvement in the pathogenesis of lung disease.

EFFECTS OF SIDESTREAM CIGARETTE SMOKE ON MACROPHAGE PHAGOCYTOSIS AND LUNG ARACHIDONIC ACID METABOLISM. A Gunissone, I Finkelstein, W Y Su and R Schlesinger, NYU Medical Center, NY, NY.

There is currently concern over the issue of passive smoke toxicity. We are reporting on two aspects of the potential questions of passive smoke, namely its effects on lung arachidonic acid (AA) metabolism and macrophage (MΦ) phagocytosis. Rabbits were exposed to sidestream smoke (a major constituent of passive smoke) from 1R4F research cigarettes for 90 minutes/day, up to 9 consecutive days. The mean exposure concentration of particles was 23 mg/m³. Rabbits were sacrificed after 1, 4 or 9 days of exposure, their lungs lavaged and recovered cells cultured. Macrophage conditioned media (MCM), as well as acellular lavage fluid, were analyzed for selected eicosanoids by radioimmunoassay. Concentrations of 6-keto-PGF₁α and LTB₄ in lavage fluid did not change significantly during the exposure period, but levels of PGE₂, PGG₂ and TXB₂ increased after 4 days of exposure, returning toward baseline after 5 additional days of exposure. The phagocytic index of MΦ (fraction of MΦ ingesting inert latex particles in vitro) paralleled the changes in eicosanoid concentration in lavage fluid. There was, however, no change in PGE₂, elaboration by cultured MΦ from exposed animals compared to controls. These results are consistent with the suggested inhibition by cigarette smoke of prostaglandin dehydrogenase.

MODULATION OF FULMORARY EICOSANOID BIOSYNTHESIS BY SULFURIC ACID AEROSOLS. R B Schlesinger and J T Zelikoff, NYU Medical Center, NY, NY.

Eicosanoids (arachidonic acid metabolites) are potent biological mediators. Air pollutants may modulate eicosanoid synthesis, a possible factor in the pathogenesis of environmentally-related lung disease. H₂SO₄ aerosols are components of ambient air and may cause exposure may result in immune hypersecretion and airway hyperresponsivity. To begin determining possible underlying mechanisms, lung eicosanoids were assessed in rabbits exposed to H₂SO₄ (0.3 μm) for 5 or 10 days at: 50 μg/m³, 4 h/day; 100 μg/m³, 2 hr/day; or 200 μg/m³, 1 hr/day. Animals were then sacrificed and the lungs lavaged. No changes (from control) in the numbers of macrophages (MΦ) or neutrophils were observed. Eicosanoid analyses were performed by radioimmunoassay of acellular lavage fluid (assesses production by the whole lung), and media which had been conditioned (for 5 hr) by lavaged MΦ (assesses production by a specific cell type). Lavage fluid showed no changes (from control) in PGE₂, and 6-keto-PGF₁α and increases in PGF₁α and TxB₂ after only some exposures. However, LTB₄ was increased by 10 days at 50 μg/m³ and with all exposures at higher acid concentrations. No changes were found in MΦ-conditioned media. This study, the first to examine eicosanoids after in vivo H₂SO₄ exposure, indicates that low acid concentrations stimulate metabolism. LTB₄ induces mucous secretion and airway hyperresponsivity, suggesting a role in these responses to H₂SO₄.
Neutrophil (PMN) infiltration is the hallmark of an inflammatory response. Increases in alveolar space neutrophil numbers have previously been demonstrated in rabbits exposed to \( O_3 \), a potent pulmonary irritant. Although eicosanoid (arachidonic acid metabolite) production by PMNs is most likely an important regulator of the course of an inflammatory reaction, there have been no prior studies of eicosanoids released by PMNs exposed to \( O_3 \). For this pilot study, human and rabbit PMNs were isolated from donor whole blood using discontinuous plasma-Tercoll gradients. Following attachment to culture dishes, the PMNs were exposed in vitro for 1 or 2 hr to clean air, or \( O_3 \) at 0.2, 0.3, or 1.0 ppm. Levels of selected eicosanoids in the PMN-conditioned media harvested 2 hr post-exposure were measured by radioimmunoassay. TXB\(_2\) was increased in a similar dose-dependent manner in the media conditioned by with the human or rabbit PMNs. No changes were detected in levels of PGE\(_2\), or \( \delta \)-keto-PGF\(_{1\alpha}\). TXB\(_2\) is a vasoconstrictor; it is also the stable derivative of TXA\(_2\), which is involved in PMN adhesion, an important step in the early inflammatory response. The results of this study suggest the rabbit model could be a useful tool for evaluation of eicosanoid production by PMNs responding to ozone exposure.

ALTERATIONS OF EICOSANOID BIOSYNTHESIS BY ALVEOLAR MACROPHAGES EXPOSED TO NITROGEN DIOXIDE. T W Robinson and R J Forman. Dept. of Pediatrics and Inst. for Toxicology, Children's Hospital of L.A., USC School of Medicine, Los Angeles, CA.

Alveolar macrophages were exposed to nitrogen dioxide (NO\(_2\)), a ubiquitous air pollutant, to examine potential alterations of eicosanoid biosynthesis. Cells (3 x 10\(^6\)/plate) were labeled with \( ^3H \)-arachidonic acid at a level of 20 \( \muCi/plate \) for 4 hr. Cells were then exposed to NO\(_2\) in air for 1 hr. NO\(_2\) at 0.08 or 0.8 ppm had no effect on eicosanoid biosynthesis. NO\(_2\), at 5 ppm, stimulated the production of an aldehyde to a level of 43\( \times \)14 picomoles/mg protein. NO\(_2\), at 20 ppm, stimulated the production of the aldehyde, d\( _1 \)HETE\(_3\), and monoHETEs to levels of 6\( \times \)10, 2\( \times \)8, and 1\( \times \)14 picomoles/mg protein, respectively. Control levels for the aldehyde, d\( _1 \)HETE\(_3\), and monoHETEs were 2\( \times \)14, 9\( \times \), and 8\( \times \)1 picomoles/mg protein, respectively. NO\(_2\) at 5 or 20 ppm stimulated macrophage production of eicosanoids with potent biological actions which could play roles in the development of lung injury. (Supported by NTH Grant HL37556)
LUCIGENIN CHEMILUMINESCENCE (CL) BY ALVEOLAR MACROPHAGES (AMs) AND ITS RELATIONSHIP TO MITOCHONDRIAL RESPIRATION. R L Esterline and R L White. Division of Toxicological Sciences, Johns Hopkins Univ., Baltimore, MD.

Lucigentin (LUC) is a chemilumigenic probe which has been used to assess O2- generation by inflammatory cells. CL from LUC is observed in unstimulated AMs but is not seen with unstimulated polymorphonuclear leukocytes (PMNs). The LUC CL in AMs was found to be both SOD (10 µg/ml) and Cu/Zn SOD (1 µM) inhibitable, indicating the involvement of O2- in the process. One major difference between AMs and PMNs is the higher level of mitochondrial respiration in the AMs. Rotenone (1 µM) and antimycin (3 µM), two inhibitors of mitochondrial respiration, were found to inhibit AM LUC CL. The addition of NADH to rat liver submitochondrial particles (SMP) generated LUC CL which was both SOD- and Cu/Zn SOD-inhibitable. Rotenone and antimycin were found to alter LUC CL in SMP however not to the degree observed in AMs. SMP CL was pH sensitive with the CL diminished in acidic medium. This suggests that intracellular pH changes brought about by mitochondrial inhibitors may account for the diminished CL response as opposed to a direct effect on mitochondrial O2- generation. Stimulation of AMs with LPS or zymosan caused an additional increase in LUC CL response from these cells. In contrast, the addition of TPA to AMs resulted in a substantial loss of the unstimulated CL response although O2- production in these cells, as indicated by cytochrome c reduction, was ongoing. This may be indicative of TPA effects on mitochondrial respiration which do not occur following stimulation with OZ. In conclusion, LUC CL from AMs containing a component not accounted for by O2- generation by the NADPH oxidase which may be explained by the generation of O2- by AM mitochondrial succinate-supported by ES 0760, CAAT and Amer. Cancer Soc. S16-3.

BLEOMYCIN-INDUCED ALTERATIONS IN THE LUNG'S SUBPOPULATIONS OF LEUKOCYTES. Y E Valdez, L A Dethloff, and B E Lehner. Los Alamos National Laboratory, Los Alamos, NM.

Fischer 344 rats were intratracheally instilled with Bleomycin (Bleo) and sacrificed on days 6, 14, and 28 in order to: (1) assess changes in the lung's free cell population, and (2) enumerate the sizes of the lungs' interstitial macrophage (IM), eosinophil (EOS), polymorphonuclear leukocyte (PMN), lymphocyte (LC), and mast cell (MC) subpopulations. Cell suspensions on days 6 and 14, alveolar macrophage (AM) numbers remained within control limits, although IM-and-blood monocyte (BM)-like cells represented -36% and -21% of the AM at these times. By day 28, the AM increased nearly two-fold but only ~6% of them were IM-, BM-like cells. Lavaged PMN, EOS, and LC were all normally increased on day 6 and subsided thereafter. Interstitial LC subsets were identified with the monoclonal antibodies W3/25, OKB, OX12, and W3/13. The LC subpopulations were increased on days 6 and 14, but decreased below control values as of day 28. The T-helper/T-suppressor cell ratio was decreased on day 6, markedly elevated on day 14, and returned to control limits by day 28. The sizes of the IM and interstitial PMN, EOS, and MC populations were elevated on days 6 and 14. These results demonstrate the complexity of lung cell changes induced by Bleo and suggest numerous roles for lung leukocytes in fibrogenesis.

CHARACTERIZATION OF A NEUTROPHIL CHEMOTACTIC FACTOR IN THE LUNG GENERATED BY EXPOSURE OF RATS TO CADMIUM CHLORIDE. S H Gavett, G Oberdoerster, and J N Finkelstein. University of Rochester Environmental Health Sciences Center, Rochester, NY.

Acute exposure of the rat lung in vivo to cadmium chloride (CdCl2) induces an inflammatory response characterized by the early loss of alveolar macrophage viability and the subsequent influx of neutrophils (N) and protein. The goal of this research was to characterize the chemotactic factor generated by this exposure which is responsible for the migration of N into the lung. Fisher 344 rats were intratracheally instilled with 10 µg of cadmium as CdCl2 in saline and killed 24 hr later. The lungs were lavaged with 5 ml saline, and the lavage fluid was centrifuged first to remove cells and then to remove aggregate lipid. Concentrated lavage supernatant was applied to a Sephadex G-100 column, and fractions were concentrated to determine chemotactic activity using 2-1-Cr labelled neutrophil peaks of chemotactic activity were found in fractions corresponding to molecular weights of approximately 80,000 and 51,000. These peaks coincide with the reported reaction products of alpha-1-antitrypsin inhibitor (aPI) with leukocyte or macrophage elastase which form two chemotactically active complexes. Reaction of the concentrated lavage supernatant with an antibody to aPi significantly reduced its chemotactic activity. The results suggest that exposure of the rat lung in vivo to CdCl2 induces production of chemotactic factors for N which could be chemically modified forms of aPI.


Little is known of changes in the pleural cell population that may occur following inhalation of mineral fibres. In our investigations pleural cells were observed containing partly phagocytosed fibres 3 days after a 5 day exposure of PVC rats to MMF. Thus the time required for fibres to reach the pleural cavity may be shorter than usually suggested. High number (15-25%) of mast cells and eosinophils were recovered from the pleural cavity of both air- and fibre-exposed rats. The majority of cells classified as mononuclear phagocytes had eosinophilic, uniform nuclei and were smaller than alveolar macrophages. Pleural cells were negative or in contrast to both alveolar and peritoneal macrophages. Both alveolar and pleural cells exhibited heterogeneity with respect to two anti-rat peritoneal macrophage antibodies, MRC OX-41 and MRC OX-42. OX-41 recognises a surface antigen associated with complement receptor function. Alveolar macrophages were 99% positive with OX-41 but 95% of pleural mononuclear phagocytes were positive with OX-42. Further studies on pleural cells with respect to both surface phenotype and function are in progress with emphasis on changes that may occur following fibre inhalation.

(Supported by the UK Health and Safety Executive)
Different long-term pulmonary responses are induced by GaAs and silica. This study investigated possible cytological differences in lung of male Fischer-344 rats following instillation of 100 mg/kg GaAs or silica (DQ-12). After 1 day, bronchoalveolar lavage fluid (BALF) was analyzed for key biomolecules and cytology. Cultures of pulmonary alveolar macrophages (PAM) from control or instilled animals were treated with known stimulants of activation [phorbol ester (PMA) and opsonized Zymosan A] or test particles, for comparison of superoxide (O$_2^-$) production. The toxicity of the test compounds to PAM were assessed by release of lactate dehydrogenase into the media. The BALF cytology had an increase in total cell numbers in GaAs and DQ-12 treated animals due to an influx of polymorphonuclear cells. PAM recruitment was also evidenced since BALF acid phosphatase activities of particle treated animals were twice the control levels. Both GaAs and DQ-12 stimulated C5 production above control levels when tested in vitro or in vivo. GaAs and DQ-12 also exhibited similar toxicities to the PAM. The initial cellular responses produced by GaAs and silica cannot account for the differences seen in the later progression of pulmonary pathologies.

Manganese shares the unique mechanism of mitochondrial calcium influx, accumulates in mitochondria, and is cleared only very slowly from brain. Mn toxicity may thus be related to accumulation in brain mitochondria or to interference with mitochondrial Ca$^{2+}$ transport. Using a metallochroic indicator at wavelengths where it is sensitive to Mn (but not Ca), together with dual-label isotope techniques, we investigated both Mn$^{2+}$ and Ca$^{2+}$ mitochondrial efflux kinetics. We report that 1) there is no Na-dependent Mn$^{2+}$ efflux from either liver or brain mitochondria; 2) Mn strongly inhibits both Na-dependent and Na-independent Ca$^{2+}$ efflux in both brain and liver, in a mode that appears to be primarily competitive; and 3) Ca competitively inhibits Mn$^{2+}$ efflux in liver. These findings suggest the possibility of significant mitochondrial accumulation of both Mn and Ca in Mn-intoxicated brain and liver.

The high contamination of soil with chromium at a number of residential sites in New Jersey, resulting from slag from local chrome manufacturing industries, has necessitated studies of the bioavailability of chromium. For this purpose, samples of chromium contaminated soil were collected at several sites by the New Jersey Department of Environmental Protection. Analyses indicated 1.6-3.6% chromium, along with high levels of calcium but low sodium content. Samples of the chromium-contaminated soils and the calcium salt were given per os to male adult rats at doses of 150 umol and 240 umol Cr/kg in acute (2 day) and subchronic (14 day) studies. Recovery of chromium from tissues (adrenals, brain, kidneys, liver, lung, spleen, blood and muscle) at 24 hr after 2 day dosing or 72 hr following 14 day dosing was not above 5% in any of the animals. Chromium levels were highest in blood with kidney and liver next highest. The amount of chromium excreted by rats in the 2 day experiment was greater in rats fed the chromium containing soils (21.5%) than by those fed the calcium salt (3%). These data will be used for risk assessment determinations.

Co$^{3+}$, a compound with low water solubility, has been used as a drying agent in paints. The in vivo disposition and in vitro dissolution profile of this cobalt-containing compound was investigated. Disposition studies were conducted after the oral administration to male Fischer 344 rats of 2.8 mg/kg or 280 mg/kg Co$^{3+}$ dissolved in emulphor/ethanol (1:2); ca. 0.01, and 0.1 x LD$_{50}$. Cobalt levels were determined in urine, faces, and tissues by flameless atomic absorption spectroscopy. By 48 hr, the low dose animals excreted more of the dose in the urine, while the high dose animals eliminated more in the feces. Significant tissue accumulations were found in the heart, kidneys, and liver. Thus, cobalt can be released from Co$^{3+}$ and absorbed after oral dosing. In vitro dissolution of Co$^{3+}$, soluble (cobalt chloride, CoCl$_2$), and insoluble (cobalt oxide, CoO) cobalt compounds were examined. Dissolution of Co$^{3+}$ was strongly media and pH dependent with increasing dissolution in the presence of protein and at low pH. Aside from protein binding of CoCl$_2$, CoCl$_2$ and CoO dissolution were unaffected by solution conditions. These results suggest that the order of in vivo bioavailability of various cobalt compounds may be indicated from their in vitro dissolution behavior. (Supported by N01-ES-3-5501)
A PHYSIOLOGICALLY-BASED TOXICOKINETIC MODEL FOR INCORPORATION OF LEAD INTO THE GROWING RAT SKELETON. E.J. O’Flaherty. Department of Environmental Health, University of Cincinnati, Cincinnati, OH.

A model of the growing rat skeleton has been developed (O’Flaherty, E.J., Tox. Lett., in press (1988)). In this model, four processes are considered to be responsible for bone growth and exchange of mineral between bone and blood: rapid exchange within a small surface compartment, slow exchange within the entire bone volume, deposition of new bone mineral, and resorption of existing bone. This model has been expanded to describe incorporation of lead into the growing bone. Rapid exchange of lead between plasma and bone occurs at all bone surfaces. Deposition and resorption of lead are assigned to the haversian region of bone, while slow exchange takes place in deeper bone regions by diffusion outward from canaliculi. The model is relatively insensitive to the size of the rapidly-exchanging pool. Deposition of lead with forming bone and slow diffusion are the critical processes determining the shape of the blood lead curve after cessation of lead exposure. Lead blood concentrations predicted by the model are compared with actual blood lead measurements in rats for several different lead exposure patterns. (Supported by USPHS Grant ES04125).

THE KINETICS OF CADMIUM UPTAKE BY HEPATIC SINUSOIDAL PLASMA MEMBRANE VESICLES. H B Eastman and J M Frazier. The Johns Hopkins University, Baltimore, MD.

The kinetics of 109Cd uptake across hepatic sinusoidal plasma membranes were investigated using sinusoidal plasma membrane vesicles isolated from male Wistar rats (175 - 225g). This investigation was undertaken to determine the nature of the uptake mechanisms for Cd. Uptake of 109Cd was measured over a range of Cd concentrations from 0.14 μM to 20 μM and v1 measurements were made at 5 min. Plots of v1 vs [Cd] indicated that both a mediated transport mechanism and diffusion are involved in the uptake of 109Cd by sinusoidal plasma membrane vesicles. The data are consistent with the following equation, which combines both mediated transport and diffusion, to describe the uptake of 109Cd by sinusoidal plasma membrane vesicles:

\[ v = \left( V_{max}[S]/K_m[S] \right) + P[S]. \]

Where P is the permeability of the sinusoidal membrane vesicles for Cd and K_m and V_max are constants with their usual meaning. Upon kinetic analysis of the data, an apparent K_m of 1.32 μM, V_max of 2.16 x 10^{-5} mmole/μg x min and P of 5.69 x 10^{-9} l/min x μg were calculated. These data suggest that a carrier mediated mechanism is involved, in part, in the uptake of Cd across sinusoidal hepatic plasma membranes.

COMPARISON OF THE PLACENTAL TRANSFER OF ORGANIC MERCURY (0-Hg), INORGANIC MERCURY (I-Hg), CADMIUM (Cd) AND LEAD (Pb) IN HEALTHY PREGNANT WOMEN. A Nakano and T Kurosu, National Institute for Minamata Disease, Minamata, Japan. Sponsor: K Imura.

To characterize the mode of placental transfer of O-Hg, I-Hg, Cd and Pb to the fetus and the accumulation of these elements in the placenta, the maternal and umbilical cord blood and placenta were collected from 21 healthy pregnant women and concentrations of the 4 elements in these specimens were determined. In the placenta another 11 elements were also analyzed. Levels of O-Hg, I-Hg, Cd and Pb in the maternal blood were 8.3 ± 3.2, 1.2 ± 0.3, 1.8 ± 0.7 and 76.1 ± 23.5 ppb (Mean ± SD), respectively, whereas the levels in umbilical cord blood were 12.7 ± 8.7, 1.5 ± 0.8, 1.1 ± 0.4 and 67.7 ± 40.7 ppb, respectively. The ratio of the concentration of each metal in the umbilical cord blood to that in the maternal blood showed that O-Hg, I-Hg, Pb and Cd decrease in this order, which may reflect that O-Hg can be easily transferred from the placenta to the fetus. In the placenta Pb concentration was also the highest and levels of Cd, O-Hg and I-Hg became lower in this order. The ratio of the concentration of each metal in the placenta to that in the maternal blood showed that Cd, I-Hg, O-Hg and Pb decrease in this order, suggesting that Cd is highly retained in the placenta. Pb level in the placenta had strongly positive correlations with levels of elements relevant to bone metabolism, suggesting that placental Pb may be associated with the calcification of this tissue.

ROLE OF METALLOTHIONEIN (MT) IN BILARY METAL EXCRETION. S Jav and E H Jeffery. University of Illinois, Urbana, IL.

The effect of acute induction of MT on biliary excretion (BExn) of a bolus of Cd or Zn was determined. Female Sprague-Dawley rats (200-220 g) were treated daily for 3 days with ZnCl_2 (6.5 mg/kg ip, in saline) or saline alone. Zn caused a 17-fold induction of hepatic MT. A bolus of Zn or Cd, 1 mg/kg iv, was administered as the chloride, and bile and plasma samples analyzed for metal content by flame AA. Rats were killed at 3 h, livers excised, and MT and MT-bound metals estimated. This acute induction of MT inhibited BExn of Cd but was without effect on BExn of Zn. This finding is in contrast to our earlier finding (J Nutr 1988, in press) that chronic MT induction by high dietary Zn inhibited BExn of both metals. This difference is due to the extent of Zn-saturation of MT before administration of the bolus metal. MT induced by dietary Zn was not Zn-saturated. The MT induced by Zn injection was saturated with respect to Zn, so that the bolus of Zn remained unchanged and free for BExn. However, Cd displaced Zn from MT and so was unavailable for BExn. In conclusion, the degree of metal saturation of MT and the in vivo affinities of metals for MT are important determinants of the effect of MT induction on BExn. Supported by Environmental Toxicology Program, University of Illinois.
HEPATIC CADMIUM ACCUMULATION IS ELEVATED BY 
ESTRADIOL. M E Black and Z A Shaikh, University 
of Rhode Island, Kingston, RI.

We have previously demonstrated that female rats accumulate more cadmium in their livers than males (J. Appl. Toxicol. 8:217, 1988). The purpose of the present study was to investigate the role of estradiol on hepatic cadmium and metallothionein levels. Adult male rats were castrated and injected with estradiol (0.5 mg/kg; 2X/day) for 6 days prior to a single sc injection of 75Cd labelled CdCl2 (10 μmol/kg). A time course study was also performed in which rats of both sexes were sacrificed between 1 and 72 hr after receiving cadmium. Control females and castrated animals given estradiol had significantly higher hepatic cadmium and metallothionein levels than the control males 24 hr after the injection. Blood levels of cadmium in the control female and castrated estradiol-treated rats were significantly lower than the control males. Castration or the injection of estradiol did not cause any significant change in hepatic metallothionein. The sex difference in hepatic cadmium uptake was apparent as early as 1 hr after the injection. These results demonstrate that estradiol causes an increased uptake of cadmium in the liver (Supported by PHS grant No. ES03187).

THE EFFECT OF TESTOSTERONE ON THE DISTRIBUTION 
OF CADMIUM TO THE RAT PROSTATE. S W Rhodes, Z Z 
Wahba, R M Bare, D E Devor, and M P Waalkes. 
NCI-FCRF, Frederick, MD.

Cadmium (Cd) will induce prostatic tumors (PT) in the rat only when given in regimes which avoid testicular tissue destruction, suggesting Cd-induced PT formation is dependent on normal testicular function. The structural maintenance of the prostate is dependent on testosterone (T), the primary hormonal product of the testes. Thus, this study assessed the effect of T on the distribution of Cd to the prostate. WF/NCr rats were castrated and given sc silastic tubing T implants approximating 1-3x normal circulating levels. Two weeks later rats were given Cd (40 μmol/Kg, sc). At 1 and 2 weeks after Cd, prostates were removed, weighed and subjected to metal analysis. Gravimetric analysis indicated that the T implants overcame the negative effect of Cd on prostate weight. The Cd burden of the prostate (n = 4 to 6) increased up to 2-fold, from 0.996 (control) to 1.855 (3XT) and 0.977 (control) to 1.937 (3XT) μg Cd/prostate at 1 and 2 weeks respectively, with increasing T support. At both time points the prostatic Cd increased in a T dose-related fashion as compared to intact controls. Results indicate the distribution of Cd to the rat prostate is dependent on circulating T levels and support the assertion that appropriate testicular function is required for Cd-induced PT formation.

RENAI Cd UPTAKE FROM BLOOD IN RABBITS. E C 
Foukes and S Blank, Dept. Env. Health Physiol.-Biophys., Univ. of Cincinnati Coll. of Med., Cincinnati, OH.

During chronic exposure renal Cd uptake probably reflects largely the reabsorption of filtered CdMT (Cd metallothionein); non-filtered CdMT is not taken up (TAP 45:505, 1978). If Cd is injected with mercaptoethanol (ME) to keep it diffusible in plasma, uptake is observed from both blood and filtrate (JCP 227: 1356, 1974). We report here preliminary tests of the hypothesis that extraction of Cd from blood resembles the process of Cd uptake from the lumen of the rat jejunum (Toxicol, in press).

In that case Cd transfer from blood into cells should be diffusion-limited, and prolonging the insulin A-V transit time from a normal 15 to 56 sec should lead to increased fractional extraction of Cd. Cd was accordingly trapped in the kidney for 40 sec by aortic occlusion. Such short anoxia did not depress secretion of PAA or reabsorption of cyclooxygenase during their constant IV infusion. After an IA bolus of Cd with excess ME or BAL, extraction of non-filtered Cd rose from <1% (free flow) to >40% (occluded). This result points to a diffusion-limited uptake of Cd.

EARLY TIME-DEPENDENT CHANGES IN THE TISSUE DIS- 
TRIBUTION OF CADMIUM AFTER ORAL BUT NOT INTRA- 
VENOUS EXPOSURE. MM Jonah and M Bhattacharyya, 
Argonne National Laboratory, Argonne, IL.

To investigate causes for reported differences in liver-kidney (L:K) cadmium ratios after oral vs. intravenous (IV) exposure, we studied 109Cd distribution in mice after gavage (100 μg Cd/kg) or IV (1 μg Cd/kg) 109Cd administration. Unexpectedly, within 1 h, we found L:K 109Cd ratios of ~10 for both modes of administration. In mice receiving oral Cd, however, the high L:K 109Cd ratio at early times decreased approximately fourfold by 72 h, whereas in mice receiving IV Cd, the ratio remained high and relatively constant with time. The time-dependent decrease in the L:K 109Cd ratio after oral exposure was caused by a four- to fivefold increase in renal Cd content, which occurred between 30 min and 72 h after oral but not IV exposure. Results suggest that, soon after exposure, Cd bound to albumin may enter the blood from the GI tract and deposit mainly in the liver, as has been observed after IV exposure. With time, however, Cd leaving the intestinal cells may bind to metallothionein and deposit almost exclusively in the kidney. The different pathways of deposition after oral vs. IV exposure may in part explain why acute parenteral Cd exposure causes liver toxicity while chronic oral exposure causes renal toxicity. Work supported by the U.S. Department of Energy under Contract No. W-31-109-ENG-38.
A CC14-CHC13 INTERACTION STUDY IN ISOLATED HEPATOCYTES - THE ROLE OF P-450 METABOLISM. JF Borzelleca, TM O'Hara, C Connings, and L Condie. Medical College of Virginia, Richmond, VA and U.S.E.P.A., Cincinnati, OH.

The toxicity of CC14 and CHC13 alone and in combination was evaluated in isolated hepatocytes. Parameters evaluated were % initial K+ (cell injury) and % LDH leakage (cell death). Response Surface Methodology was used to describe and analyze the interactions. Greater than additive interaction was demonstrated. To explain this interaction, the role of P-450 metabolism was evaluated in hepatocytes from rats pretreated with phenobarbital (P-450 induction) or metyrapone (P-450 inhibition). P-450 was involved in the toxicity of CC14 and CHC13 alone and in combination. Supported by U.S.E.P.A. Cooperative Agreement CR-812558.

IN VITRO CYTOXICITY OF COMPLEX WASTE MIXTURES IN CULTURED HEPATOCYTES. D L McKeen, D M DeMarini*, and R A Merrick. USEPA, HERL, Cincinnati, OH and Research Triangle Park, NC.

Millions of tons of hazardous wastes are produced annually which need rapid, comparative toxicity characterization. Primary cultures of rat hepatocytes were used as a model system for exposure to 10 complex waste mixtures obtained from different industrial sites (A-D) to rank their in vitro cytotoxicity. Results were then compared with their in vivo toxicity rank order. Also analyzed in the in vitro system were positive controls representing inorganics, cadmium, and volatile organics, carbon tetrachloride for a relative toxicity comparison. Hepatocytes were seeded onto 96-well microtiter plates and dosed within 4 hours. The concentrations tested ranged between 0.01 to 100 ng sample per ml tissue culture medium. Cytotoxicity was determined 18 hours later using the NBT (methylthiazolyl diphenyltetrazolium) vital dye assay. The in vitro LC50's from most toxic to least toxic are: J>


This study was undertaken to critically evaluate the purported causative role of lipid peroxidation in the potentiation of carbon tetrachloride hepatotoxicity by trichloroethylene. Hepatocytes in suspension were exposed to carbon tetrachloride together with various concentrations of trichloroethylene over a 40 fold range. Samples were taken for estimation of toxicity by leakage of cytoplasmic enzymes and potassium ion. Some incubations included the antioxidant N,N'-diphenyl-p-phenylenediamine (DPDD) while others contained dithiothreitol (DTT), a chelating reducing compound. Assays to estimate total and nonprotein bound sulphhydryl groups were also performed. Results clearly showed potentiation by trichloroethylene of both lipid peroxidation and toxicity due to carbon tetrachloride. DPDD inhibited lipid peroxidation while DTT did not. Neither, however, was able to inhibit the potentiation of toxicity. Therefore, our data show that lipid peroxidation is not responsible for the trichloroethylene-induced potentiation of toxicity in hepatocytes due to carbon tetrachloride. Furthermore, there is no evidence to indicate a role for sulphhydryl groups in this response.

ENHANCEMENT OF CARBON TETRACHLORIDE (CC14) HEPATOTOXICITY BY PRIOR EXPOSURE TO A MIXTURE OF 25 GROUNDWATER CONTAMINANTS. J E Simmons1, R S H Yang2, J C Seely2, D Syvendsgaard1 and A McDonald1, 1HERL/US EPA, CEN/NSP/SPATHCO, 4NSI, Research Triangle Park, NC.

Groundwater is an important source of drinking water. Therefore, we examined CC14 hepatotoxicity as affected by exposure to a mixture of 25 chemicals frequently found in groundwater. Male F344 rats were exposed to one of two dose levels of the groundwater mixture, at environmentally relevant levels, via drinking water for 14 days then gavaged with 0 or 0.275 ml CC14/kg. Indices of hepatotoxicity were measured 24 hours later. As was the case for denitized water controls, exposure to the groundwater mixture alone did not result in histopathological changes. Denitized water/CC14 treatment resulted in centrilobular vacuolar degeneration. Combined exposure (chemical mixture + CC14) appeared to increase vacuolar degeneration as well as produce centrilobular necrosis, accompanied by increased serum AST and ALT activity. Exposure to the chemical mixture resulted in decreased consumption of water and food. The influence of decreased water-and-feed consumption on the observed enhancement of CC14 toxicity is being examined. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)
233 TOXIC INTERACTIONS BETWEEN CARBON TETRACHLORIDE (CCl₄) AND CHLORDOPHORM (CHCl₃). H G Lamb, C Gennings, J F Borzelleca and L W Condie. Depts. of Pharmacology/Toxicology and Biostatistics, MCV, Richmond, VA, and USEPA, Cincinnati, OH.

CHCl₃ increases the hepatotoxicity of CCl₄ in the intact animal. However, there is limited information about CHCl₃ and CCl₄ interactions in cultured hepatocytes. These studies were undertaken to determine the toxicity of various concentrations of CCl₄ and/or CHCl₃ on primary cultures of adult rat hepatocytes. Cultured hepatocytes were incubated 24h with CCl₄ (0-1 mM) and/or CHCl₃ (0-2.5 mM). Agent-dependent increases in hepatocyte injury were associated with a rise in medium AST (plasma membrane integrity) and a reduction in the cell's capacity to reduce NADP (mitochondrial function) and incorporate U-[H]-Choline into phosphorylcholine (endoplasmic reticulum function). Cultured hepatocytes incubated with CCl₄ and/or CHCl₃ displayed dose-dependent alterations in all three markers of cell injury. Response surface methodologies were used to analyze the dose-response data. Results suggest that CHCl₃ increases the toxicity of CCl₄ since all combinations of CCl₄ and CHCl₃ are more toxic than CCl₄ and CHCl₃ alone. Therefore, cultured hepatocytes may be an appropriate model in vitro to identify and study hepatotoxic interacants. (Supported by EPA Cooperative Agreement 812558 and NIH Grant DK-31115). (Abstract does not necessarily reflect EPA policy).

234 POTENTIATION OF CARBON TETRACHLORIDE HEPATOTOXICITY AND LETHALITY BY VARIOUS ALCOHOLS. S D Ray and H M Mehendale. Dept of Pharmacology and Toxicology, Univ of Mississippi Medical Center, Jackson, MS

Several aliphatic alcohols are capable of potentiating CCl₄ hepatotoxicity. Whether this potentiating effect is a true synergism or simply an inhibition of the clearance of CCl₄ is not known. The present investigation was designed to test whether (i) a nontoxic low dose of alcohol can potentiate CCl₄ hepatotoxicity, and (ii) this potentiation leads to greater animal lethality. We have employed methanol, ethanol, isopropanol, t-butanol, pent, hexa, octa, deca and eicosanol at equimolar doses (10 mmol/kg) in the present investigation. These alcohol were given orally to male SD rats (175-250g) 18h prior to CCl₄ administration (p.o.). Liver injury was assessed by serum transaminases. None of these alcohol alone increased serum ALT or AST, whereas CCl₄ administered to alcohol-treated animals resulted in significant elevation of serum transaminases and decreased ID50 values of CCl₄. The order of potentiation was hexa>penta>butanol>octa>isoprop>deca>metha>etha>eicosanol. The present study establishes a positive correlation between hepatotoxicity and lethality. (Supported by EPA and Medical Research Command, Wright Patterson AFB, CR-814053; Abstract does not necessarily reflect EPA policy).

235 A CCL₄/CHCL₃ INTERACTION STUDY IN ISOLATED HEPATOCYTES - SELECTION OF A VEHICLE. T M O'Hara, J F Borzelleca, and L Condie. Medical College of Virginia, Richmond, VA and U.S.E.P.A., Cincinnati, OH.

Emulphor, ethanol, and DMSO were evaluated as vehicles in studying the toxicity of CCl₄ and CHCl₃ in isolated hepatocytes. Appropriateness of the vehicle was determined by evaluating the following parameters: solubility of CCl₄ and CHCl₃ in the vehicle, cell injury (% Initial K+), cell death (LDH leakage) and interaction (protection or enhanced toxicity) with CCl₄ and CHCl₃. The maximum no effect levels (v/v) were: emulphor (0.125 %), ethanol (1.0 %) and DMSO (5.0 %). Emulphor at toxic levels was inadequate to dissolve enough CCl₄ to evaluate in this system. Ethanol (5.0, 2.5, 1.0, 0.5 %) was more toxic than DMSO and interacted with both CCl₄ and CHCl₃ to enhance toxicity. DMSO (15.0, 5.0, 2.5 %) did not significantly alter the toxicity of CCl₄ and CHCl₃ (no test interaction). These data suggest that DMSO should be the vehicle for evaluating the toxicity of CCl₄ and CHCl₃ and their mechanisms of action in the isolated hepatocyte. Supported by U.S.E.P.A. Cooperative Agreement CR-812558.

236 POTENTIATION OF CCl₄ HEPATOTOXICITY AND LETHALITY BY KETONES. M P S Portia, P R S Kodavanti, and H M Mehendale. Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS

Potentiation of haloalkane hepatotoxicity by ketones is well established. The present studies were designed to investigate the potential of ketones as enhancers of CCl₄ toxicity in isolated hepatocytes. The ketones potentiated CCl₄ hepatotoxicity by a varying extent as indicated by serum enzyme elevations. Branched chain ketones appear to potentiate CCl₄ liver injury and toxicity to a greater extent than straight chain ketones. (Supported by EPA and Medical Research Command, Wright Patterson AFB, R-814053, Abstract does not necessarily reflect EPA policy.)
237 PHENOTOLAMINE ANTAGONISM OF IODOBENZENE HEPATOTOXICITY IN B6C3F1 MICE.
M A Smith, J Gandy, S M Roberts, R C James, and R D Harbison. Division of Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR and Center for Environmental Toxicology, University of Florida, Gainesville, FL.

Previous studies from our laboratory have shown that high-dose exposure to the halocarbon bromobenzene (BBz) results in hepatotoxicity and lethality that can be substantially diminished by co-treatment with the alpha-adrenergic antagonist, phenotolamine. The purpose of this study was to determine if administration of the related halocarbon iodobenzene (IBz) results in hepatotoxicity and lethality that can be substantially diminished by co-treatment with the alpha-adrenergic antagonist, phenotolamine. The purpose of this study was to determine if administration of the related halocarbon iodobenzene (IBz) results in hepatotoxicity and lethality that can be substantially diminished by co-treatment with the alpha-adrenergic antagonist, phenotolamine.
Compounds most likely to be metabolized in skin are frequently insoluble in water. They will not partition readily into the receptor fluid used in an in vitro diffusion cell study and, therefore, an accurate absorption rate cannot easily be determined. Addition of a surfactant (PEG 20 oleyl ether) to the receptor fluid can destroy enzyme activity in skin. We have found that various procedures can be used to enhance appearance of absorbed material in the receptor fluid: (i) addition of serum protein; (ii) preparation of thin sections of skin with a dermatome or by enzyme separation of skin to isolate the epidermis; and (iii) increased mixing of the contents of the flow-cell. The permeation of the water insoluble compound acetyl ethyl tetramethyl tetralin (AETT) into the receptor fluid was enhanced 50-fold compared to its absorption through full-thickness hairless guinea pig skin when these procedures were utilized. After 24 hr, 23.8% of the applied dose appeared in the receptor fluid and 16.9% of this material had been metabolized. Approximately 40% of the applied dose (all unmetabolized) was found in the skin at the end of the 24 hr study. Calculation of total percutaneous absorption must include the absorbed compound and metabolites in the receptor fluid as well as those found in the skin at the end of an experiment.
PRELIMINARY EVALUATION OF IN VITRO PERMEABILITY OF MONKEY BUCCAL MUCOSA AND SKIN TO TRITIATED WATER (THO) AND PbTx-3 (RED TIDE TOXIN). M. Mehta, B. W. Kemppainen and R. G. Stafford, School of Pharmacy, Auburn, AL.

Discs of excised monkey buccal mucosa and skin were mounted on static diffusion cells. Some of excised discs were placed in between large pore nylon filter membranes to determine if it is necessary to provide support for the delicate buccal mucosa. Hanks balanced salt solution (HBSS) was used as buffer which bathed the epithelial (outer) and dermal (inner) surface of tissue discs. Sequential samples were removed from receptor fluid (buffer bathing the dermal side) during the length of experiment (24 hr) and radioactivity analyzed. Permeability coefficient (KP) of monkey buccal mucosa (BM), BM with nylon (BMN), monkey skin (MS) and MS with nylon (MSN) to THO, was 187, 2050, 116 and 1.6 respectively. KP of BM, BMN, MS and MSN to PbTx-3 was 5.4, 577, 1.6 and 62 respectively. Nylon membranes changed the penetration of THO and PbTx-3 through tissue by factors of 0.01 and 106.8. It was concluded that nylon support membranes alter the tissue barrier characteristics and it is better to support tissue by preparing thicker discs of mucosa. It was observed that at the end of the experiment only 2% of THO was within mucosa as compared to 36% of a lipophilic compound, PbTx-3. This suggests PbTx-3 readily penetrates into the mucosa and then slowly diffuses into receptor fluid.

THE BIOCHEMICAL AND MORPHOLOGICAL EFFECTS OF VEHICLES ON THE ISOLATED PERFUSED PORCINE SKIN FLAP. R. R. KING and N.A MONTEIRO-RIVIERE. COL. OF VET. MED. AND TOXICOLOGY PROGRAM, N.C. STATE UNIV., RALEIGH, N. C.

The isolated perfused porcine skin flap (IPPSF) is a new in vitro model which has possible applications in cutaneous toxicology. Since many xenobiotics are applied to the skin in vehicles that enhance their absorption, this study was designed to evaluate the response of this model to the application of cutaneous vehicles. The vehicles chosen were: acetone(A), 100% ethanol(E), 90% dimethyl sulfoxide(D), toluene(T), cyclohexane(C) and untreated controls. Each IPPSF was treated with 200 ul of the vehicle, (n=4 per group). For cumulative glucose utilization (CGU), E showed significantly lowered average glucose utilization per hr of perfusion than control. No other treatments differed significantly from the control for CGU or for the rate of change of the cumulative glucose utilization: venous lactate (CGU/L) ratio. Peripheral vascular resistance in the IPPSF over time was characterized by three patterns: E most closely mimicked control; D, T, and C were similar; and acetone was unique. The significance of these flap resistance profiles is unknown. Morphological changes indicated an apparent increase in intracellular edema with D. These vehicle effects should be considered when designing absorption and cutaneous toxicity studies for the IPPSF.
SPECIES DIFFERENCES IN PEROXISOME PROLIFERATION. C R Elcombe and J A Styles. ICI, Central Tox Lab Macclesfield, UK. Sponsor: P M D Foster

The present studies have compared the in vivo and in vitro responses of rat and guinea pig hepatocytes to methylclofenapate (MCP) - a potent peroxisome proliferator (PP). The effect of MCP on cultured human hepatocytes has also been examined. DNA synthesis (S-phase) was determined by flow cytometry following BrdUrd labelling (in vivo) or by 3H-Tdr labelling and autoradiography (in vitro). Cytochrome P-450 was measured as lauric acid hydroxylation (Lah) and PP was determined using 3H-palmitoyl CoA oxidation (PCO). Oral administration of MCP (5-25 mg/kg/day) to rats for up to 7 days resulted in increased liver weights and dose-dependent stimulation of S-phase, Lah and PCO. In contrast, MCP had no effect upon guinea pig liver. In cultured rat hepatocytes MCP (5-150µM) stimulated S-phase, Lah and PCO; however MCP had no effect upon these parameters in cultured guinea pig or human hepatocytes. These experiments highlight the marked species differences in sensitivity to PP and associated hepatic phenomena. Considering the well established relationship between PP and liver cancer it is probable that similar species differences exist in susceptibility to tumour development. These data reinforce the hypothesis that the non-mutagenic PPs present little or no carcinogenic hazard to man.

STUDIES IN VIVO AND IN CULTURE ON THE MECHANISM OF LIVER HYPERPLASIA IN RATS INDUCED BY METHYL-CLOFENAPATE. J A Styles and C R Elcombe. ICI Cl, Macclesfield, UK. Sponsor: P Foster

The peroxisome proliferator methylclofenapate (MCP) induces a transient acute hyperplasia in the livers of rats. Our studies have shown that when MCP was administered daily to rats by gavage the occurrence of S-phase was mainly in bi-nucleated (2 x 2N) cells. At the end of the acute hyperplasia approximately 12% of the 2 x 2N cells had undergone S-phase and become 4N cells following cytokinesis, while about 10% remained unresponsive. This observation indicates that the transient hyperplastic response to MCP is due to the depletion of sensitive 2 x 2N cells. Similar results have also been obtained from cultured hepatocytes. Interrupted dosing studies have revealed that cessation of MCP dosing following acute hyperplasia resulted in the regeneration of 2 x 2N hepatocytes within 10 weeks to the original proportions, and these regenerated cells were responsive to stimulation by MCP. The above observations suggests a mechanism in which MCP acts as a growth factor. This hypothesis has been examined in experiments with cultured rat hepatocytes. Rats were dosed with MCP to induce hyperplasia, and the cells then cultured in the presence of EGF. The results showed that hepatocytes from pretreated animals were un-responsive to EGF, whereas cells from untreated animals exhibited a stimulation of S-phase activity.

DEVELOPMENT OF A CULTURED HEPATOCYTE SYSTEM TO STUDY NONGENOTOXIC MECHANISMS OF HEPATOCARCINOGENESIS. A P Li and J C Merrill, Monsanto Company Environmental Health Laboratory, St. Louis, MO.

To complement short-term genotoxicity assays in the screening of carcinogenic chemicals, assays for endpoints relevant to nongenotoxic mechanisms of hepatocarcinogenesis were established using cultured hepatocytes from Sprague-Dawley rats and CD-1 mice. The endpoints were: 1. cytotoxicity: assayed by quantifying the release of cytoplasmic enzymes lactate dehydrogenase and glutamic-oxaloacetic transaminase; 2. mitogenicity: measured by quantifying cells in S phase by autoradiography and/or total 3H-thymidine incorporation; 3. induction of gene expression: via measuring induction of microsomal P450 content and P450 enzymes such as cellular ethoxy- coumarin O-deethylase activities as well as the quantification of c-myc oncogene expression; 4. induction of peroxisomes: via measuring palmitoyl coenzyme A oxidation and carnitine acetyl transferase activities. This mechanistic approach using cultured hepatocytes is now being validated with known rodent hepatocarcinogens and structurally similar noncarcinogens in our laboratory.

A NOVEL HYPOTHESIS TO EXPLAIN THE MECHANISM OF ACTION OF STRUCTURALLY DISSIMILAR PEROXISOMAL PROLIFERATING AGENTS. B Keller and R G Thurman. Dept. Pharmacol, U N Carolina at Chapel Hill, NC.

Ethylexanol, a known non-genotoxic carcinogen and a peroxisomal proliferator, inhibited state 3 respiration in isolated mitochondria and selectively damaged O&lt;sub&gt;2&lt;/sub&gt;-rich peripheral regions of the liver lobule. The purpose of this study was to test the hypothesis that these events occurred with a variety of structurally dissimilar peroxisomal proliferators. Ciprofibrate(CIP), valproate(VL), pentadecafluorooctanoate(PFO), diolactone(CLO), ethylexanol(EH), and diethylhexylphthalate-late(DEHP) were compared for their effects on isolated mitochondria and toxicity in the perfused rat liver. Mitochrondia were isolated from livers of fasted rats by standard procedures and were incubated at room temperature in 2 mL of buffer containing 100 mM KCl, 50 mM sucrose, 20 mM Tris-HCl, 5 mM Tris-phosphate, and 10 µM rotenone, pH 7.2. Oxygen uptake was measured polarographically with a Clark-type O₂ electrode employing succinate as substrate. All peroxisomal proliferators studied inhibited state 3 but not state 4 rates of oxygen uptake (CIP＞PFO＞EH＞CLO＞VL＞DEHP; half-maximal inhibition occurred at concentrations ranging from 0.3 to 7 mM depending on the specific compound). In the perfused liver, oxygen uptake was about 3 times greater in O₂-rich upstream peripheral than pericentral regions of the liver lobule due to more active mitochondria. Perfusion with EH, PFO, and CLO led to LDH release and damaged predominantly peripheral regions of the lobule as reflected by trypan blue uptake. Therefore, we propose that peroxisomal proliferators in general inhibit actively respiring mitochondria in perinatal regions of the liver lobule leading to local toxicity. This in turn initiates cell replication leading to the well known tumor-promoting effects of this broad class of agents (ES-04325).
Chronic toxicity of CI-924, a lipid-regulating agent chemically described as 5,5'-[11,1'-biphenyl]-2,2'-diylbis(oxy)]bis[2,2'-dimethylpentanoic acid], was investigated in rats and dogs. Rats tolerated daily doses up to 150 mg/kg for 52 weeks with dose-related body weight gain suppression. Laboratory findings included slight nonprogressive decreased red blood cell indices and serum triglycerides and mild to marked increased serum AST, ALT, alkaline phosphatase, and bilirubin. Hematology at all doses was more pronounced in males than females and correlated with hypertrophic hepatocytes with granular eosinophilic cytoplasm, fatty change, and minimal to moderate bile retention. Laboratory and pathologic changes were reversible. Dogs tolerated doses up to 300 mg/kg for 52 weeks. There were decreased serum triglycerides and activated partial thromboplastin times in 300 mg/kg females. No pathologic changes were observed. Changes observed in the liver of the rat and reduced triglycerides in both species were expected findings consistent with lipid-regulating drugs.

The objective of this study was to examine the relationship between hepatic lipid content and the activity of peroxisomal β-oxidation (β-ox) after administration of the peroxisomal proliferator. Rats were administered to rats in the diet at concentrations of 0.05, 0.1, and 0.3%. After 24 hours, there was a dose-related increase in hepatic cellular lipid droplets detected histologically and triglycerides measured biochemically in mid- and high-dose rats. After three days, both parameters in high-dose rats returned to control levels and remained there through the three months of treatment. The regression of the lipid corresponded with marked induction of β-ox in high-dose rats. In contrast, the hepatic lipid content of mid-dose rats became progressively greater throughout the study such that after three months triglycerides were increased 2.5-fold and there were numerous large lipid vacuoles observed histologically. There was only minimal induction of β-ox in mid-dose rats. In low-dose rats, β-ox was not increased at any time during the study and there were only minor increases in lipid content. The reversal of the lipid accumulation correlated with the induction of β-ox suggesting a role for peroxisomal proliferation in correcting disturbances in hepatic lipid metabolism.
BIOCHEMICAL AND PEROXISOMAL PROLIFERATING EFFECTS OF BLEACHED KRAFT PULP AND PAPER MILL EFFLUENT IN CHANNEL CATFISH. E. Platter-Michals and R. Di Giulio. Ecotoxicology Laboratory, Duke University, Durham, NC. Sponsor: M.B. Abou-Doina

Bleached kraft pulp and paper mill effluent (BKME) induced components of both the mixed function oxidase system and the free radical/oxidant defense system and caused peroxisome proliferation in channel catfish exposed to subacute concentrations. Whole BKME caused significant (p < 0.05) dose-dependent inductions of hepatic catalase and ethoxyresorufin O-deethylase activities and a significant decrease in hepatic reduced glutathione concentrations. Hepatic palmitoyl-CoA oxidase and laurayl-CoA oxidase activities, measures of peroxisomal proliferation, were induced in exposed fish 7-10 fold over controls. A chlorinated phenolic mixture but not a resin acid mixture (the two major classes of compounds in BKME) caused an increase in both catalase and palmitoyl-CoA oxidase activities in catfish liver. Pure compound studies with pentachlorophenol and 2,4-dichlorophenol will be presented.

RELATIONSHIP BETWEEN TESTICULAR CELL CULTURE AGE AND SUSCEPTIBILITY TO 1,3-DINITROBENZENE INDUCED TOXICITY. C D Brown and M G Miller. Department of Environmental Toxicology, University of California, Davis. Sponsor: L. Shull.

It is well known that the drug metabolizing capacity of cells in vitro rapidly declines after isolation. The present studies have used 1,3-DNB as a model testicular toxicant which is proposed to undergo metabolic activation in the testis. The effect of testicular culture age on the toxicity of 1,3-DNB was examined. Cells were isolated from 26 day old SD rats and used either immediately, after 24 hr, or after 96 hr in culture. Cell viability was assessed using Neutral Red incorporation (NR), MTT reduction (MTT), lactate secretion, and intracellular ATP. Metabolism and covalent binding (CB) of (14C)-1,3-DNB were also studied. 1,3-DNB (5-50 mU) caused a dose dependent depletion of ATP in fresh cells while 24 and 96 hr were refractile to ATP depletion. NR and MTT were depressed above 100 mU 1,3-DNB in 24 and 96 hr cells. No significant change in lactate secretion was noted in fresh cells while lactate increased in a dose dependent manner in 24 and 96 hr cells. (12C)-1,3-DNB was metabolized (<5%) in all three systems, with fresh cells metabolizing most rapidly and nitroaniline being a major metabolite. (14C)-1,3-DNB was covalently bound in fresh cultures, but not significantly in 24 or 96 hr cultures. Moreover, the rate of CB in fresh cells decreased with incubation time. The relationship between metabolism and the mechanism of 1,3-DNB induced testicular toxicity is being further explored.

REDOX CYCLING OF m-NITROSONITROBENZENE (NNB): A POSSIBLE MECHANISM FOR THE SERTOLI CELL TOXICITY OF m-DINITROBENZENE (DNB). P M D Foster, M K Ellis and D A Cave. ICI, CTE, Macclesfield, UK.

DNB is a testicular, Sertoli cell toxicant in rats. It is nitroreduced by Sertoli cells via NNB (NNB producing greater toxicity in vitro than DNB). Further, pyruvate (P) and lactate (L) secretions by Sertoli cells are increased (P>L) by DNB, which may be related to a change in cellular redox state. DNB or NNB (5-100 mU) were added to Sertoli cell cultures together with [14C] 1- or 6-glucose or alanine as substrates to determine the role of the glycolytic (GLY), pentose phosphate (PPP) and gluconeogenic amino acid (GAA) pathways in contributing to the increases in P and L. Further chemical studies were conducted to ascertain the reactivity of NNB with NAD(P)H and GSH. DNB and NNB produced significant increases in P (max 2000%, 250%) and L (700%, 350%) derived from [14C] glucose, but with no change in the relative contributions of GLY or PPP. Both compounds also increased the conversion of [14C] alanine to P and L. NNB was chemically reduced in seconds to m-nitrophenylene-diolamine (NPH) by the co-factors. Since NNB is a metabolite of DNB, an oxidation of NPH to NNB is likely to have occurred, establishing a redox cycle to exhaust reduced co-factors. The NNB- or DNB-induced stimulation of Sertoli cell GLY, PPP and GAA pathways to P, which yields a net increase in NAD(P)H, is a compensatory measure.

IS STIMULATION OF LACTATE SECRETION BY CULTURED RAT SERTOLI CELLS A USEFUL INDEX OF TESTICULAR TOXICITY? N A Worrell, D M Cressy, G A Thompson and T J B Gray. MIRA, Garston, Harrow, UK.

A number of testicular toxicants (e.g. certain phthalate esters, AP 1312/TS) stimulate lactate secretion in primary rat Sertoli cell-enriched cultures. Initial studies were conducted to investigate whether increased lactate secretion occurs in vivo. Tubule fragments prepared from rats pre-treated with an oral dose of either the toxic phthalate ester di-2-ethylhexyl phthalate (200 mg/kg) or AP1312/TS (400 mg/kg) secreted significantly more lactate than tubule fragments isolated from control animals. A subsequent study investigated the relationship between increased lactate secretion and toxicity. Previous studies had shown that stimulation of lactate secretion by the toxic phthalate ester mono-2-ethylhexyl phthalate (MEHP) can be blocked by incubation in glucose-free medium. MEHP (200 mg/l) was equally toxic to Sertoli-germ cell co-cultures in the presence or absence of glucose, as judged by increases in germ cell detachment. These results suggest that stimulation of lactate secretion may be an early response to testicular toxicants both in vivo and in vitro, but that its relationship to toxicity is causal and not causal. In view of this and the failure of some known toxicants to affect lactate secretion in vitro, this measurement may have limited utility as an in vitro index of testicular toxicity. (Supported by the UK Ministry of Agriculture, Fisheries and Food)
261 RESPONSE OF RAT TESTICULAR CELL CULTURES TO REPRODUCTIVE TOXINS IS INFLUENCED BY OXYGEN CONCENTRATION. J Wytowicz, T Fabel and M Brabec. Eastern Michigan University, Ypsilanti, MI.

Cell culture methods are increasingly employed in the study of the effect of toxic chemicals. Most often, the cells are cultured in air (20% O2) although tissue concentrations are lower. In this study, the response of cocultures of rat Sertoli-germ cells to the known male reproductive toxins, DBCP (dibromochloropropane), MA (methoxyacetic acid), and C6 was different when O2 was lowered from 20% to 5%. Detachment of germ cells doubled when cultures were exposed to 100 mM MA, while no change was observed at 20% O2. Conversely, germ cell detachment at 2 mM C6 decreased by 50%, relative to that at 20% O2. 0.5 mM DBCP failed to elicit germ cell detachment, while 0.1 mM MA caused a release of germ cells at 20% O2. 100 mM Pb did not cause germ cell release at either O2 concentration. Lactate accumulation in the media was increased at 5% O2. Lactate accumulation at 5% was elevated by 100 mM MA. However, Pb and C6 elicited biphase patterns of lactate accumulation that did not correlate with germ cell detachment. Since P02 is less than 40 mmHg in the seminiferous tubule, it appears important to measure toxicity responses under physiological concentrations. Supported by Michigan Research and Excellence Development Fund.


Using ethane dimethanesulphonate (EDS), a potent Leydig cell (LC) toxicant, we developed a protocol to assess testicular and epididymal effects, following both in vivo and in vitro exposures. When IC50s were incubated in vitro for 3 hr with 200 μM EDS, HCG-stimulated testosterone (T) production was inhibited 30% in 36% using either crude (15%) or purified (9%) LC preparations. This response was dose dependent: T production by purified LCs was 500, 480, 350, and 225 ng/10^8 LCs at 0, 100, 200 and 500 μM EDS. Exposure of animals to 3 hr EDS in vivo and subsequent determination of HCG-stimulated T production by LCs in vitro also revealed an inhibition of LC function: IC50s in crude preparations from animals injected with 0, 5, 25, 50 and 100 mg/kg EDS produced 320, 210, 190, 150 and 70 ng T/10^8 LCs. To assess an epididymal effect, rats were killed 4 days after injection of 100 mg/kg EDS. Only slight decreases in testis weight and spermatid numbers existed at this time; however, sperm granulomas were found in the caudal epididymis of all treated rats and the proximal cauda sperm showed significant decreases in both motility and velocity, suggesting that EDS has direct epididymal effects. Thus, this protocol determined the in vivo/in vitro dose-responsiveness of LCs to EDS and identified non-testicular epididymal effects.

262 TRI-O-CRESYL PHOSPHATE (TCP) TOXICITY TO SERTOLI CELLS IN VITRO REQUIRES LEYDIG CELLS. R. Chapin and J. Phelps. DAMT, NTP, NIEHS. Research Triangle Park, NC.

Previous studies on the testicular toxicity of TCP have found that the Sertoli cell is the first cell to show pathology in vivo and that the testis contains considerable quantities of the parent compound (TCP) and the active metabolite, saligenin cyclic-c-teryl phosphate (SCOP). Because Leydig cells contain steroidogenic P450 isozymes, and the activation of TCP is P450-mediated, we hypothesized that the activation of TCP occurs in Leydig cells, and adversely affected Leydig testosterone (T) synthesis and Sertoli cell function. TCP and SCOP inhibited T production by rat Leydig cells in primary culture after 3 and 18 hrs by up to 8% in a dose-dependent fashion (0.3 - 100 μM). Addition of sterol intermediates with TCP showed that mitochondrial and SER P450-mediated steps in T synthesis were affected. A target enzyme, nonspecific esterase (NSE) was inhibited up to 50% in Leydig cells after TCP, SCOP, but not the parent compound TCP, inhibited NSE by 20%-80% in Sertoli cells in vitro. Sertoli cells were then grown on a semipermeable (Costar Transwell) membrane separating two communicable chambers in vitro. The addition of TCP to the outer chamber lowered Sertoli NSE only when Leydig cells were also in the outer chamber. These data are consistent with a necessary interaction between the two cell types in producing the testicular toxicity of this organophosphate.

264 THE TOXICITY OF METHOXYACETIC ACID (MAA) TO RAT PACHYCTENE SPERMATOGETES. W. M. Cook, N. R. Worrell and T. J. B. Gray. BIBRA, Carshalton, Surrey, UK.

Administration of MAA to rodents results in a depletion of pachytene spermatocytes (PS) from the testis. To determine whether this represents a direct action on PS or an effect secondary to Leydig cell injury, we have compared the toxicity of MAA in primary rat Sertoli-germ cell cocultures and isolated PS. Necrosis of PS in Sertoli-germ cell cocultures incubated with MAA (100 μM) was first evident 9 hr after treatment, with depletion of PS from the cultures evident after a 12 hr exposure. However, pulse treatment experiments revealed that a 5 hr exposure was sufficient to produce degeneration and necrosis 24 hr after the start of treatment. A similar time course for the development of signs of toxicity was evident when isolated PS were treated with MAA with a decrease in viability and a reduction in cellular ATP levels apparent after a 12 hr exposure and a pulse exposure of 6 hr sufficient to cause a reduction in viability 24 hr after dosing. The viability of round spermatids exposed to MAA for up to 24 hr was, however, unaffected. Exposure of Sertoli cell enriched cultures to MAA for 24 hr prior to the attachment of untreated isolated PS had no effect on the degree of attachment observed. These results suggest that MAA acts directly on PS and that irreversible cell damage occurs during the first 6 hr of exposure. (Supported by the UK Ministry of Agriculture, Fisheries and Food.)
265 ATTENIUATION OF 2-METHOXAMETHANOL-INDUCED TESTICULAR TOXICITY IN THE RAT BY SIMPLE PHYSIOLOGICAL COMPOUNDS. C A Nebus, F Weish, and P K Working. GITR, Research Triangle Park, NC.

We have shown that the developmental toxicity of 2-methoxyethanol (2-ME) in mice is attenuated by physiological compounds such as serine, acetate, sarcosine, glycine, and D-glucose. In the present study we examined the ability of the same agents to protect against 2-ME-induced testicular toxicity. The degeneration of late-stage pachytene spermatocytes that was observed histologically 24 hr after 2-ME treatment (6.6 mmol/kg) did not occur after concurrent administration of serine (16.5 mmol/kg). The depletion of mature, elongated spermatids prevalent 23 days after 2-ME exposure was also prevented by concomitant dosing with serine. Daily sperm production (DSP) was determined 23 days after 2-ME treatment using either serine, acetate (43 mmol/kg), sarcosine (16.5 mmol/kg), glycine (43 mmol/kg), or D-glucose (43 mmol/kg) as the attenuating agent. 2-ME treatment alone resulted in DSP that ranged from 57-59% of control. Serine completely blocked the decrease in DSP whereas D-glucose was without effect. Acetate, sarcosine and glycine were of similar efficacy resulting in DSP values ranging from 75-80% of control. The effectiveness of these compounds may result from their ability to donate one-carbon units and other essential building blocks for purine bases which are needed for RNA synthesis by pachytene spermatocytes.

267 Uptake and Distribution of Microcystin in Cultured Hepatocytes. W L Thompson, N A Robinson, and J G Pace. US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, MD. Sponsor: E W Wannemacher.

Microcystin-LR (MC), a hepatotoxin isolated from the blue-green algae Microcystis aeruginosa, causes liver necrosis in several animal species and affects attachment in cultured hepatocyte monolayers. However, the mechanism of action of MC is unknown. Radio-labeled MC (sp. act. 197 mCi/mmol) was used to determine if there was a direct association of toxin with cultured hepatocytes. Uptake of labeled MC (1.0 μg/ml) by hepatocytes reached a maximum at about 30 min. Higher concentrations did not increase the maximum amount of label taken up by the cells. Competition for uptake by unlabeled MC was demonstrated, but displacement did not occur after the first 10 min of exposure to the labeled toxin. The maximum concentration of MC associated with the cells was about 10 ng per 10^6 cells, of which 80% or more was in the TCA-precipitable fraction. Subfractionation of treated hepatocytes, resulted in the following distribution of [3H]-MC: plasma membrane, 2.8%; nuclear fraction, 9.6%; 100K x g supernatant, 68.6%; 100K x g pellet, 8.9%. These results indicate that uptake of MC by hepatocytes is a saturable process during which the toxin becomes firmly associated with the TCA-precipitable, cytosolic fraction.


High doses of retinoids induce testicular toxicity in experimental animals which is morphologically similar to the toxicity produced by hypovitaminosis A. Thus testicular function is dependent on retinol status. The hypothesis tested is that high doses of retinoids induce testicular toxicity through alterations in tissue and plasma retinol. Male rats Crl:CD(SD)BR 8 weeks old were dosed PO with 90 mg/kg/day of the retinoid Ro 23-2895 (all-E)-[2-(m-nitroxy) phenyl]-3,7-dimethyl-2,4,6,8 nonatetraenoic acid, dissolved in Tween 80. Controls received an equal volume of vehicle (2.5 ml/kg). Rats (n = 6) were sacrificed after 1, 3, 7, 15, and 21 days of treatment. Retinol in plasma and testes was determined by HPLC following extraction in acetonitrile: butanol (1:1). Plasma retinol was significantly decreased (77%) from control after 24 hrs and remained decreased throughout dosing. After 21 days, plasma retinol in treated rats was 13% of controls. Retinol in the testes was significantly reduced by day 3 of treatment and by day 15 was reduced 50-60% from control. These data and the morphologic similarities between Ro 23-2895-induced testicular toxicity and vitamin A deficiency, indicate that reductions of endogenous retinol in plasma and testes might be a mechanism for the testicular toxicity induced by high doses of retinoids.


tBuBHQ is a potent inhibitor (K1=1 μM) of both ATP-dependent Ca2+ sequestration by microsomes and microsomal Ca2+-stimulated ATPase activity. In contrast, the hydroquinone does not alter mitochondrial Ca2+ homeostasis and the plasma membrane Ca2+-ATPase activity (Moore et al., FEDS Lett. 224, 331, 1987). We now report that in isolated rat hepatocytes, tBuBHQ increased the cytosolic Ca2+ concentration to a new steady state level within seconds (ED1=1-2 μM), as measured by the fluorescent indicator, Fluo 2. This effect did not involve an increased influx of Ca2+ into the cell and was independent of the presence of extracellular Ca2+, but resulted from a rapid release of Ca2+ from the same store that is mobilized by the hormone, vasopressin. However, unlike vasopressin, which mobilizes Intracellular Ca2+ by stimulating the breakdown of phosphatidylinositol 4,5-bisphosphate to release inositol 1,4,5-trisphosphate (IP3), tBuBHQ did not cause a significant accumulation of inositol phosphates. Pretreatment of the cells with tBuBHQ abolished the vasopressin-mediated increase in cytosolic Ca2+ without altering inositol polyphosphate formation. Thus, tBuBHQ may be a useful tool to study the role of endoplasmic reticular Ca2+ in cells.
SUPPRESSION OF CELL DIVISION IN REUBER HEPATOMA CELLS PRE-EXPOSED TO CHLORDECONE BY CCl₄. H M Mehendale and S D Ray. Dept of Pharmacology and Toxicology, Univ of Mississippi Medical Center, Jackson, MS

Chlordecone (CD) pretreatment is known to markedly potentiate CCl₄ hepatotoxicity. Our earlier work has indicated suppressed hepatocellular regeneration to be responsible for the accelerated progression of CCl₄ hepatotoxicity. This study examines if this combination treatment arrests cell division in a rapidly dividing rat Hepatoma cell line in vitro. Reuber Hepatoma cells were cultured in vitro using serum-supplemented Swine-77 medium. Upon confluence, monolayers were subcultured and grown either in the presence or absence of 10 ppm CD for 11 or 16 days. Then the cells were exposed to various concentrations of CCl₄ (5, 10, 20, 40 mmol/ml). CD (10 ppm) was consistently stimulatory, whereas CCl₄ had either no effect or an inhibitory effect on cell division depending upon the concentration. When CD-treated cells were exposed to a subtoxic dose of CCl₄, there was a dose-dependent inhibition of cell division. Cell doubling time was significantly increased. Cell death, after CCl₄ treatment to normal or CD-treated cells was constant over 96 h period indicating that cell division rather than cell death is involved in the phenomenon. This observation supports our hypothesis that the CD+CCl₄ treatment suppresses cell division. (Supported by AFSR-88-0009).

DISSOCIATION OF DNA DAMAGE FROM THE KILLING OF CULTURED HEPATOCYTES BY AN OXIDATIVE STRESS. IB Coleman, D Gillor, JL Farber. Dept. of Pathology, Thomas Jefferson University, Philadelphia, PA.

The role of DNA damage in oxidative cell injury was explored by exposing cultured rat hepatocytes to 3 agents known to cause an oxidative stress, tert-butylihydroperoxide (TBHP), H₂O₂ (from glucose oxidase), and menadione. Each agent produced DNA single strand breaks prior to the loss of cellular viability. DNA damage and cell killing were dependent upon a cellular source of iron. Pretreatment with the iron chelator deferoxamine prevented both, and addition of iron to the medium after deferoxamine treatment restored the DNA damage and cell death. The radical scavenger KTB showed a similar effect. DNA damage was restored by TBHP and glucose oxidase. Acidification of the medium also prevented the cell killing without any effect on the extent of DNA damage. These data indicate that DNA damage and cell killing produced by an oxidative stress depend upon the iron-catalyzed formation of a potent oxidizing species. However, such DNA damage can be dissociated from the mechanisms that lethally injure the cells over the time course studied.


Incubation of 3-methylcholanthrene-induced hepatocytes with 1-20 mM acetaminophen (APAP) produced a substantial decrease in GSH and GSSG in the medium without a loss of viability. With 20 mM APAP, there was a 21% decline in protein thiols. Approximately 20% of this depletion was due to the formation of GSH mixed disulfides, whereas atrylation by APAP metabolites accounted for the remainder. The presence of the GSH reductase inhibitor BCNU enhanced the accumulation of GSSG and the depletion of protein thiols. With 1 mM APAP and BCNU, 73% of the cells died within 4 hours and protein thiols were depleted by 22%. GSH mixed disulfides and atrylation now accounted for only 20% of the protein thiol loss. The major proportion was a consequence of lipid peroxidation and was prevented by the antioxidant DPD. With 20 mM APAP and BCNU, there was no loss of viability at 4 hours, a result that reflects the antioxidant action of the toxin. Nevertheless, GSSG increased 4-fold over that of control, and 40% of the protein thiols were lost. GSH mixed disulfide formation contributed nearly 50% to this loss. These data document that APAP metabolism generates an oxidative stress. Also, as much as 40% of total protein thiols can be depleted due to the reaction with GSSG and the metabolites of APAP without any loss of viability.

COMPARATIVE TOXICITY OF CYCLIC POLYPEPTIDES AND DEPSIPEPTIDES ON CULTURED RAT HEPATOCYTES. K A Mereish, R Solow, Y Singh* and R Bhatnagar. Pathophysiology Division, USAMRDC, Fort Detrick, Frederick, MD and Laboratory of Chemical Pharmacology, National Institutes of Health, Bethesda, MD. Sponsor: R W Wannemacher

Primary cultures of adult rat hepatocytes were used to investigate the comparative toxicity of three cyclic polypeptides (cyclosporine, gramicidin-s, microcystin-LR) and three cyclic depsipeptides (enmitin-b and valinomycin). Cell injury was assessed by the release of cellular adenine nucleotides and lactate dehydrogenase (LDH) into the media. At 1 µM, the cyclic polypeptides (cyclosporine and gramicidin-s) and depsipeptides (enmitin-b and valinomycin) did not induce a significant release of adenine nucleotides or LDH from cultured rat hepatocytes as compared to controls. However, gramicidin-s, valinomycin and cyclosporine induced significant cytotoxicity at 50 µM. Microcystin-LR dose-response studies indicated that maximum cytotoxicity was found at 1 µM. Comparatively, gramicidin-s, valinomycin and cyclosporine were at least 50 times less cytotoxic than microcystin-LR to rat hepatocytes. The release of adenine nucleotides from hepatocytes treated with microcystin-LR was distinctly different from that observed in hepatocytes treated with the other peptides by the presence of a lag phase.
MECHANISMS OF METHYLATING AGENT-INDUCED CYTOXICITY IN ISOLATED HEPATOCYTES. H G Shertzer, M Sainsbury* and ML Berger. University of Cincinnati College of Medicine, Cincinnati, OH; *University of Bath, Bath, England.

Although N-methyl,N-nitro,N-nitrosoguanidine (MNNG) and methylmethanesulfonate (MMS) cause injury and malondialdehyde formation in rat hepatocytes, only MMS toxicity can be diminished by pretreatment with the antioxidant promethazine (Biochem Pharm 37:3183, 1988). Hence, we compared 14 antioxidants that protected against MNNG and MMS toxicity. Chemoprotection was quantified as the concentration that delayed 50% cell killing by 1 h. While chemoprotection against MNNG and antioxidant efficacy were directly related (R=0.88), chemoprotection against MMS and antioxidant efficacy were unrelated (R=0.32). Since the compounds protected against MMS, we hypothesized that they stabilized membranes; therefore, their capacity to prevent erythrocyte osmotic lysis was assessed. Chemoprotection against both MNNG and MMS correlated with reduced RBC fragility (R=0.94 and 0.72, respectively). We propose that methylating agents destabilize cellular membranes resulting in hepatocellular injury. For MNNG, radical-mediated events may result in membrane destabilization; for MMS, membranes are destabilized without concurrent radical events. The current studies provide a basis for future work to determine structure-activity relationships of chemoprotective agents.

SPECIES COMPARISON OF COCAINE HEPATOTOXICITY IN CULTURED RAT LIVER SLICES. S Connors, A J Gandolfi, C L Krumdieck, and K Brendel. Departments of Pharmacology, U of Arizona, Tucson, AZ and Department of Nutrition Science, U of Alabama, Birmingham, AL.

The hepatotoxicity of cocaine depends upon a number of factors including species, sex, strain and pretreatment. There is controversy in the literature, i.e. no study was found in the rat, guinea pig or rabbit even after pretreatment with phenobarbital (PB) but others were not in agreement. The purpose of the present study was to find a sensitive in vitro model in which to investigate cocaine hepatotoxicity. Precision-cut liver slices from various species were incubated with cocaine (0.5 mM) for up to 6 hr. Slice K+ retention and Lactate uptake were used as viability indicators. Toxicity was seen at 0.05 mM (25% control level) in PB-treated rats whereas only minimal effects were seen at 1 mM (90% control level) with human liver slices. The differential species sensitivity (males) was: PB-treated rat > CD-1 mouse > DBA/2J mouse > pig > human > rat > rabbit. While PB pretreatment enhanced cocaine toxicity, slices of 18 hr fasted PB-treated rats demonstrated even greater intoxication (additional 15% decrease; 0.25 mM). This in vitro system thus allows to study species, strain and metabolic influences on hepatotoxicity. (NIH GM 38290).

Oligomycin (OM), an inhibitor of the mitochondrial ATPase, blocks oxidative ADP phosphorylation causing marked ATP depletion and rapid cell death in hepatocytes. Glycolytic ATP formation is not affected by OM. Fructose (FR) is metabolised by hepatocytes to FR-1-phosphate by fructokinase. FR-1-phosphate is further broken down to glyceraldehyde-3-phosphate (G-3-P) which enters glycolysis and is ultimately metabolized to pyruvate thereby increasing the rate of ATP turnover. The purpose of these experiments was to examine the role of ATP depletion in OM-induced cell injury. Hepatocytes exposed to 200 nM OM had a >85% decrease in [ATP], a marked increase in [AMP], and membrane blebbing. Cells exposed to FR (50 nM) had decreased levels of ATP (40%) and P. By the kinase action of fructokinase, FR did not produce cell death despite marked ATP depletion. Cells exposed to both OM (200 nM) and FR (50 nM) had a slight but significant increase in ATP content when compared to OM alone. FR also protected hepatocytes from OM-induced cell death. Iodoacetate (25 mM) an inhibitor of pyruvate kinase (PK) at the 25-P level, caused a decrease in ATP to levels observed with OM alone and reversed the FR protection on OM-induced lethality. These data indicate that FR metabolism to G-3-P and ultimately to pyruvate increases glycolytic ATP phosphorylation and ATP availability which protects cells against OM-induced lethal cell injury. Thus, cell death following disruption of mitochondrial function with OM may be critically linked to the availability of ATP.

MITOCNDRIAL POISONS BLOCK HERNAL RESPONSE AND RAISE CYTOSOLIC CALCIUM IN SINGLE HEPATOCYTES. M T Smith, L Blank, T Kawashiki, and R Y Tsien, School of Public Health 1 and Dept. of Physiology-Anatomy 2, University of California, Berkeley, CA.

A procedure has been developed for loading freshly isolated hepatocytes with significant concentrations (~250 nM) of Ca²⁺ in a de-esterified Ca²⁺ responsive form. Following loading, fluorescence ratio imaging of individual hepatocytes revealed that various hormones, including phentolamine and vasopressin, induce a series of oscillations in cytosolic Ca²⁺ in a large proportion of cells. These oscillations are similar to those previously observed in hepatocytes microinjected with Ca²⁺-sensitive indicator and resemble a train of periodic spikes (Nature 319: 600, 1986). The frequency but not the magnitude of these spikes is highly dependent upon the extracellular Ca²⁺ concentration. Ca²⁺ oscillations were stopped soon after the addition of a number of mitochondrial poisons including FCCP, oligomycin, nigericin and valinomycin. The cytosolic Ca²⁺ concentration then rose gradually prior to cell death and leakage of fur-2 from the hepatocytes. Exposure of hepatocytes to mitochondrial poisons prior to addition of hormone also resulted in a rise in cytosolic Ca²⁺ and prevented oscillations. Compounds which damage mitochondria may therefore inhibit the hormonal responsiveness of hepatocytes and raise cytosolic Ca²⁺. The latter may play a role in the cytotoxic effects of such compounds.

ISOLATION OF IgE ANTIBODIES FROM THE GUINEA PIG. M E Bennedsen, P L Thorne, and H Karol. Department of Industrial Environmental Health Sciences, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA.

This laboratory has developed an animal model for pulmonary sensitivity to industrial chemicals which utilizes the guinea pig. In efforts to prove the mechanisms underlying both immediate- and late-onset pulmonary responses, it became necessary to develop procedures for the separation, isolation and identification of specific antibodies from this species belonging to the IgE class. Male, Hartley guinea pigs were immunized with an antigen consisting of Ascaris suum extract and the haptenic sensitizing chemical, tolucene dithiocynate (TDI). Blood was drawn from the animals and the IgG globulin fraction prepared using 50% ammonium sulfate. The globulins were fractionated using an FPLC system employing a Mono Q ion exchange column. Antibody to TDI was determined using both ELISA and passive cutaneous anaphylaxis procedures. Using a linear phosphate buffer gradient (0.01-0.3 M), the IgG was eluted at a single fraction which was distinct from that which contained the other guinea pig cytotoxic antibody (IgG). These studies demonstrate the ability to separate and characterize IgE from the guinea pig and enable the investigation of the role of IgE antibodies in immediate- and late-onset pulmonary sensitization reactions to TDI. Supported by NIH grant #10532.

A MULTISPECIES COMPARISON OF TRIMELLITIC ANHYDRIDE (TMA)-INDUCED PULMONARY SENSITIZATION. N S Hategan, C L Leach, C R Zeiss, M R Andresen, L K Verrykou, and P J Garvin. IIT Research Institute, Veterans Administration, and Amoco Corporation, Chicago, IL.

We developed a rat model which simulates the lung lesions and high titers of TMA-specific serum antibody seen in TMA-exposed workers. In order to examine whether the rat model can be duplicated in other species, inhalation studies were conducted using rats, mice, guinea pigs and rabbits of both sexes. All animals, except the filtered air controls, were exposed to a target concentration of 500 μg/m³ of TMA, 6 hrs/day for 10 days. Half of the animals/species were sacrificed following the 10-day exposure, while the other half was allowed a 2-week rest period followed by a 6-hr TMA challenge and sacrifice. External hemorrhagic lung foci were counted, and lung weight and volume determined. TMA-specific IgG antibody was also determined for all animals. Rats exhibited the typical respiratory sensitization response which was more pronounced following the 10-day exposure than after rest and challenge. The response in rabbits, guinea pigs and mice was more variable and less prominent than that in rats. Antibody levels were significantly elevated in rabbits both at the termination of exposure and following challenge with TMA; the number of lung foci was higher at the end of the 10-day exposure than after rest and challenge, but was not significantly different from controls. Guinea pig response was minimal with antibody levels being significantly higher than controls only following TMA challenge. No significant responses were seen in the TMA-exposed mice. Due to its greater immunological responsiveness and because it more closely reflects the human response, the rat seems to be the species of choice to study the pulmonary sensitization induced by TMA and possibly other anhydrides.
EVALUATION OF THE MOUSE EAR SWELLING TEST (MEST) AS A REPLACEMENT FOR GUINEA PIG SENSITIZATION TESTING. S Hignet, J D Dorko, H E Kennah and C S Barrow, PPG Industries, Inc., Environmental Sciences Center, Pittsburgh, PA.

The MEST has been proposed to replace traditional guinea pig (GP) tests because it provides quantitative data instead of subjective data used in GP tests. The objectives of this study were (1) to evaluate the MEST using model compounds to determine the dose at which 50% (SD50) or 100% (SD100) of the animals were sensitized and (2) to test the compounds on GP at a dose to compare to the MEST results. For the MEST, 5-10 mice/group were exposed to 3 daily doses of 10-333 mg/ml of hydroxylamine hydrochloride (HAHCl) or 0.01-10 mg/ml of 1-chloro-2,4-dinitrobenzene (DNB). Challenge occurred 6-7 days later with the maximum nonirritating dose of each compound. SD50/SD100 values could not be obtained with HAHCl because only 10% of the mice responded at the highest concentration. DNBC had SD50/SD100 values of 0.01/10.0 mg/ml, respectively. Groups of 10 GP were exposed to 333 mg/ml HAHCl or 1.0 mg/ml DNBC for 6 hr/day, 3 days a week over a 2-week period. Challenge occurred 13 days later with the maximum nonirritating dose. HAHCl and DNBC were both very strong sensitizers with 90% and 100% of the GP showing positive responses, respectively. These results indicate the MEST test is unreliable in detection of sensitization even using very strong sensitizers since it did not detect HAHCl as such, while GP testing showed it to be a very strong sensitizer.

DEVELOPMENT OF DIAGNOSTIC ANTIGENS FOR DETECTION OF ANTIBODIES TO TOLUENE DIISOCYANATE. R Jin and M H Karol, Department of Industrial Environmental Health Sciences, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA.

Isocyanates have been recognized as agents capable of causing pulmonary hypersensitivity reactions in appropriately exposed individuals. This laboratory has been engaged in development of diagnostic reagents to detect antibodies to chemical allergens as an indication of chemical exposure and possible sensitization. Using a guinea pig animal model, studies were undertaken to characterize TDI-antigens which were found to be effective in antibody detection. Sets of animals were exposed by inhalation to 5 ppm TDI for 3 hr per day on 5 consecutive days. Others received TDI by intradermal injection. Serum was obtained 2-3 weeks later and examined, by ELISA, for TDI-specific antibodies. The assays employed a series of synthetic TDI-serum albumin conjugates which varied in TDI content from 11-80 groups per mole protein. Antibodies were produced in all animals. Detection depended on the composition of the test antigen. Highest titers were obtained using hapten conjugates which contained high TDI density. These results emphasize the importance of antigen composition in detecting antibodies to hapten and indicate that standardized procedures must be used for preparation and characterization of antigens to be used for diagnostic purposes. Supported by NIHES 01532.


Epidermal cell injury is commonly seen during contact sensitivity reactions, but the mechanism causing this injury is unknown. We have developed a quantifiable histological test for contact sensitivity based on epidermal cell injury: the epidermal vacuolization test. Following active sensitization, the contact sensitizers, DNBC or oxazolone, were topicaly applied to full-thickness 1 cm² explants of guinea pig skin. Compared to the response of skin from a control guinea pig, apparently normal skin from a specifically-sensitized guinea pig showed a dose-related increase in the number of epidermal vacuoles. Skin from a actively-sensitized guinea pig was not nonspecifically hyperreactive as two primary irritants and an unrelated contact sensitizer produced similar numbers of vacuoles in skin of both sensitized and control animals. IgG and immune sera from oxazolone-sensitized guinea pigs could passively-sensitize control skin explants in-vitro to oxazolone. In these studies, application of oxazolone not only specifically increased the number of epidermal vacuoles, but also decreased the number of detectable mast cells in the explants. These findings suggest that contact sensitizers could induce IgG antibodies that specifically enhance epidermal cell injury by causing mast cell degranulation.

IMMUNOGENICITY OF SYNTHETIC FOOD COLOURS. S Nicklin, W P Hutchinson and K Miller, NIBRA, Carshalton, Surrey, UK, Sponsor S D Gangoli.

In order to gain more information on the biological activity of synthetic food colours we have examined the immunogenicity of FD & C yellow 2, FD & C Red 2 and brown FK in Brown Norway strain rats. Rats received graded doses of either free colour, an associated ovalbumin-colour complex or ovalbumin bound colour reactive metabolite(s) i.e. d 0 and d 28 using carrageenan as adjuvant. Serum was obtained weekly. IgE antibody production was determined by passive cutaneous anaphylaxis, total antibody activity was determined by ELISA. Yellow-2 was negative throughout. Red 2 and the ovalbumin-red 2 conjugate also proved negative, protein-red 2 metabolite(s) however, were positive by ELISA. Brown FK failed to initiate antibody production but both ovalbumin-brown FK and the brown FK protein-metabolite(s) elicited dose dependent antibody production detectable by both PCA and ELISA. These results provide some insight into the biological activity of the selected colours and clearly have relevance in the future development of predictive screening assays for food chemicals.

(Supported by the UK Ministry of Agriculture, Fisheries and Food.)
AN OPTIMIZED IN VITRO LYMPHOCYTE BLASTOGENESIS ASSAY FOR CONTACT SENSITIVITY TO NICKEL SULFATE IN MICE. M K Robinson, E R Fletcher, and D L Sneller, Procter & Gamble, Cincinnati, OH. Sponsor: M J Murray.

Nickel sulfate (NS) is among the most common contact allergens. However, it is difficult to assess the allergenicity of NS using in vivo animal tests. Guinea pig skin testing and the mouse ear swelling assay have yielded variable sensitization data. In vitro lymphocyte blastogenesis has been useful in clinical diagnosis but has rarely been used to evaluate NS-induced sensitization in animals. We used an optimized lymphocyte blastogenesis assay for contact sensitizers (J. Invest. Dermatol., in press) to determine if mice treated with NS would show NS-specific lymphocyte proliferation and whether this response would correlate with ear swelling. BALB/c mice were given 10 open applications (2X/day for 5 days) of 20% NS and ear challenged 3-5 days later at the same dose. After recording 24 and 48 hour ear thickness measurements, lymphocytes from the draining nodes were cultured with Langerhans cell-enriched epidermal cells (EC), EC + NS (soluble NS), or EC/NS (EC modified by preincubation with NS). The EC/NS co-culture stimulated significant NS-specific proliferation above the autologous response to EC alone. EC + NS was mitogenic. We saw no ear swelling response to NS, suggesting that the in vitro response to EC/NS was more sensitive in detecting sensitization to NS after open application in mice.

LATE-ONSET AIRWAY AND FEBRILE RESPONSES IN AN ANIMAL MODEL OF PULMONARY HYPERSENSITIVITY TO AIRBORNE ALLERGENS. P S Thorne and M H Karol, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA.

We have described a guinea pig model for pulmonary hypersensitivity to airborne allergens. A prominent feature of the model is 24 hr continuous monitoring of respiratory rate, breathing volume and body temperature which allows detection of immediate-onset (IR), late-onset (LR) and febrile responses. Experiments were undertaken to probe the mechanisms underlying LR by determination of febrile responses, pulmonary function and histopathology at the time of peak response. Animals were sensitized by ip injection with 1 mg ovalbumin on Day 0 and inhalation boost on Day B. Hypersensitivity reactions were elicited by inhalation challenges on Days 15 and 29. All animals demonstrated severe IR which occurred during the 15-20 min challenge and were not accompanied by fever. LR were produced about 50% of the time and were accompanied by fever which reached 1.6°C above prechallenge values. Pulmonary function determination and histopathological examination performed at the peak of the LR demonstrated airflow disturbance and eosinophilic infiltration into airway mucosa. These results indicate LR is an airway response which is accompanied by fever and demonstrate the use of the animal model in elucidation of the mechanisms underlying pulmonary hypersensitivity reactions. Supported by NIH 01532.

INHALATION SENSITIZATION OF GUINEA PIGS TO TOLUENE DIISOCYANATE (TDI): GENERATION OF ANTIBODIES THAT RECOGNIZE TDI AND GUINEA PIG SERUM ALBUMIN (GPIA). R Carlo and E Clark, Procter & Gamble, Cincinnati, OH. Sponsor: M J Murray.

Guinea pigs were sensitized to TDI by inhalation of 0.12 ppm or 4 ppm TDI vapor for 3 hr/day for 5 consecutive days. Inhalation challenge with 50 ug/m3 TDI-GPSA aerosol resulted in immediate respiratory reactions (increase in rate and retracted breathing) in 0/8 animals in the 0.12 ppm group, 5/7 animals in the 2 ppm group and 4/8 animals in the 4 ppm group. No reactions were elicited when challenged with aerosolized GPA, TDI-bovine albumin (BSA) or TDI vapor. Antibodies (Ab) to TDI were detected by active and passive cutaneous anaphylaxis and ELISA using TDI-GPSA, TDI-BSA and TDI-ovalbumin antigens. Anti-TDI Ab was detected in sera and lung lavage fluid from the 2 and 4 ppm exposure groups but not in sera and lung lavage fluid from the 0.12 ppm exposure group. Ab to self protein, GPSA, were detected in sera but not lung lavage from the same animals. Ab to GPSA was present in lower titers than Ab to TDI. Neither anti-TDI or anti-GPA Ab were found in baseline sera. Ab to self protein was found in sera after the TDI sensitization regimen but before any challenge regimen. This observation suggests that exposure to haptenating low molecular weight chemicals can induce an immune response to self carrier protein.

PHENYLETHYLISULFONYL FLUORIDE (PMSF) PREVENTS MIPAFOX-INDUCED CHANGES IN HEM PERIPHERAL NERVE PHOSPHOINOSITIDE METABOLISM. C N Pope and S Padilla, US Environmental Protection Agency, Research Triangle Park, NC.

Some organophosphorus (OP) compounds can induce a delayed, primarily distal, neuropathy (OPIDN). The serine protease inhibitor PMSF can protect against OPIDN if given prior to OP exposure. We compared the effects of either pre- or post-treatment PMSF administration on the development of OPIDN and on peripheral nerve phospholipid metabolism in hens treated with the neuropathic OP compound mipafox. Both mipafox (50 mg/kg/im) and PMSF (67 mg/kg/sc) caused >90% inhibition of brain neurtotoxic esterase activity, but clinical signs of OPIDN, beginning 7 to 8 days after treatment, developed only in hens receiving mipafox first (n=10/group). Five days after treatment, marked reductions (21% to 30%) in the in vitro phosphorylation of distal sciatic nerve phosphoinositides were evident in the neuropathic group (i.e., those receiving mipafox first) but no significant differences were seen in the protected group (i.e., those receiving PMSF first) (n=6/group). Considering that the distal nerve is most sensitive to the neuropathic effects of OP inhibitors, changes in phosphoinositide metabolism in the distal nerve may participate in the pathogenesis of OPIDN. (*Supported by a NRC Research Fellowship.)
BIOCHEMICAL, PHYSIOLOGICAL AND PATHOLOGICAL CHANGES IN HENS BETWEEN INHIBITION OF NEUROTOXIC ESTERASE (NTE) AND ONSET OF CLINICAL SIGNS DURING ORGANOPHOSPHATE-INDUCED DELAYED NEUROPATHY (OPIDN). M Ehrich, H El-Fawal, L Gay and BS Jortner, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

Indices of OPIDN in the hen model have traditionally been restricted to early inhibition of NTE, which returns to control levels within days, and ataxia with associated pathological changes in rear limb peripheral nerve or spinal cord more than 10 days later. Other manifestations of OPIDN were evaluated in adult hens that had been given phenyl saligenin phosphate (PSP) 2.5 mg/kg im and sacrificed 1, 4, 15 and 21 da later. NTE activity was <20% of control in PSP-treated hens at 24 hr. Clinical signs were evident by 10 da and progressed in severity to paralysis by 21 da. Hen biventer cervicis nerve-muscle preparations showed increased sensitivity to acetylcholine (5x) and elevated rheobase (2x) as early as 4 da after PSP. These effects continued to progress to 21 da. Activity of calcium-activated neutral protease in partially purified gastrocnemius muscle was significantly over control at time periods tested after 7 da (152%, 172%, 148% and 132% at 7, 10, 15 and 21 day, respectively). Histopathological examination of the biventer cervicis nerve was consistent with a pattern of progressive nerve degeneration beginning on day 7. Results indicate OPIDN can be detected in the interval between NTE inhibition and onset of clinical signs. (Supported by NIHES031384)

MODIFICATION OF ORGANOPHOSPHATE-INDUCED DELAYED NEUROPATHY (OPIDN) IN HENS WITH NIFEDIPINE. HN EL-Fawal, BS Jortner and M Ehrich, Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA

The involvement of Ca++ in the pathogenesis of OPIDN was tested using the Ca++ channel blocker nifedipine (NIF). Twenty hens were given NIF 1.0 mg/kg/day in 0.5 mg/kg doses for 5 da. Phenyl saligenin phosphate (25 mg/kg/day) was administered on the second day to 15 of these hens and to 15 hens not given NIF. Untreated hens served as controls. Ataxia appeared earlier in hens treated with PSP alone than in those treated with NIF+PSP (scoring 0-5, unaffected to those with both leg and wing paralysis). Strength-duration curves of sciatic & tibial nerves, gastrocnemius muscle and biventer cervicis nerve-muscle preparations at 4-5, 7-8 and 15-16 da post-PSP exposure showed significant increases in excitability threshold for hens given PSP only. Rheobase values for these preparations were 3-4x those of control hens on all days tested. Rheobase in NIF+PSP-treated hens increased only in the biventer cervicis and only at 15 da. The gastrocnemius muscle of hens treated with PSP alone was 50-100x more sensitive than controls to close-arterial injection of acetylcholine (ACH); the biventer muscle was 100-200x more sensitive. Hypersensitivity to ACH in muscles from NIF+PSP-treated hens only appeared 15 da post-PSP and only in the biventer muscle. This indicates that NIF block of Ca++ channels can attenuate clinical and pathological changes that occur during OPIDN. (Supported by NIHES031384)


Avin neurotoxicity studies may be undertaken on organophosphorus compounds intended for use as industrial chemicals or pesticides. Adult female hens are dosed with either test compound, TOCP as the positive control at 500 mg/kg or vehicle only. The compounds are administered by oral gavage and the positive results are noted. If a positive result is noted a second dose is given 21 days later. Results are assessed by daily observation for clinical signs of ataxia and neuropathological examination at termination. Following perfusion, sections of brain, spinal cord and peripheral nerve are taken and relevant stains used. Interpretation of the results depends upon the degree of agreement between the clinical signs seen and the pathological examination with background data being used to support the conclusions drawn. Results are presented on 149 birds dosed with TOCP over a 3-year period and compared with a similar number dosed with vehicle only. Of those birds dosed with TOCP 114 showed clinical signs of ataxia, the majority at grade 3 or above. Significant neuropathological lesions were seen in the four areas of spinal cord and three sections of peripheral nerve examined. The incidence of lesions in the two brain sections was low. No signs of ataxia or significant lesion incidence were seen in the negative controls.

WEUROTOXIC ESTERASE ASSAY: CORRECTED WAVELENGTH AND EXTINCTION COEFFICIENT. US Kayyali, TB Moore, JC Randall, and RJ Richardson, Toxicology Program, The University of Michigan, Ann Arbor, MI.

A assay for neurotoxic esterase (NTE) was developed by Johnson (1969; 1973) to assess the delayed neurotoxic potential of organophosphorus compounds. Activity is calculated from the rate of phenyl valerate hydrolysis resistant to parathion and sensitive to mipafox inhibition. The amount of phenol produced is measured colorimetrically by coupling with aminotyramine to yield a product with \( \lambda_m = 510 \text{ nm and } c = 13,900 \text{ M}^{-1} \text{ cm}^{-1} \). The assay was improved and simplified later by Johnson (1977) without any change in the \( \lambda_m \) or \( c \), even though the chromophore solvent was altered by adding sodium dodecyl sulfate (SDS). In the present work, we find that the NTE assay is performed according to the improved procedure the \( \lambda_m \) of the chromophore in the assay mixture is shifted from 510 nm to 490 nm. The same shift in \( \lambda_m \) is observed with phenol standards coupled with aminotyramine in an SDS solution. The sensitivity of the NTE assay increases when measurements are made at the corrected \( \lambda_m \) (corrected to \( c = 15,600 \text{ M}^{-1} \text{ cm}^{-1} \)). We suspect that the inclusion of SDS in the improved procedure is responsible for the shift in the absorbance spectrum of the chromophore. (This research was supported, in part, by a gift from the Dow Chemical Company.)
293 SELECTIVE DEGENERATION IN THE CHICKEN CENTRAL NERVOUS SYSTEM AFTER EXPOSURE TO BIS (1-METHYL-ETHYL) PHOSPHOROFLUORIDATE (DFP). D Tanaka, and S J Burnia. Dept of Anatomy and Animal Science, Michigan State University, East Lansing, MI.

This study examined the extent of axonal and terminal degeneration in the CNS after a single exposure to DFP. Birds were injected with 1mg DFP/kg body weight and killed 3hr, 24hr, and 3wk after dosing. Three hour brains were processed for AChE histochemistry, 24hr brains for determination of NTE activity, and 3wk brains for the presence of axonal degeneration using the Fink-Heimer method. At 24hr, the birds had cholinergic signs and the brainstem and spinal cord showed complete loss of AChE+ fibers. At 24hr, whole brain NTE was inhibited in excess of 70%. At 3wk degeneration was noted in the lumbar ventral horn, medial pontine-spinal tract, dorsal spino-cerebellar tract, and fasciculus gracilis of the spinal cord. In the medulla, degeneration was present in the dorsal and ventral spinocerebellar tracts and in the spinal lemniscus. Terminal degeneration was present in the gracile, lateral cervical, external cuneate, and solitary nuclei as well as in the reticular formation. Mossey fiber degeneration was also present in the cerebellar anterior lobe. These results indicate that in addition to its acute effects on AChE and NTE, DFP can also cause axonal and terminal degeneration in specific brainstem nuclei and spinal pathways.

294 EFFECT OF GANGLIOSIDES ON NEUROPATHY TARGET ESTERASE. A Moretto and M Lotti. Istituto di Medicina del Lavoro dell’Università di Padova, Padua, ITALY.

Ganglioside (GS) treatment was reported to reduce the severity of organophosphate-induced delayed polyneuropathy (OPIDP) in hens dosed with tri-o-cresyl phosphate (Berry et al. Toxicologist. 1986, 6, 882). OPIDP is initiated by inhibition/aging of axonal neuropathy target esterase (NTE) within hours after dosing and expressed 2 weeks later. Incubation of hen brain with GS GM1 (100-100 uM, 0-120 min, pH 8, 37°) had no effect on NTE (87-102% of control). DFP 150s (20 min, pH 8, 37°) were 0.7 uM in control and 0.6 uM in GM1-preincubated NTE. Hens (n=4) were daily dosed with GS GM1 (10 mg/kg i.m.) or saline for 7 days before DFP (1 mg/kg s.c.) or solvent. After 24 h NTE in nervous system of GM1+solvent group was 86-109% of controls. NTE inhibition in GM1+DFP and saline+DFP groups was similar. These data show that GS has no effect on the inhibition of NTE activity but suggest that GM1 might ameliorate the recovery from OPIDP induced by DFP (0.9 mg/kg s.c.) as clinically assessed by comparing GS or saline treated hens (n=5). Partly supported by FIDIA spa, Abano Terme, Italy.

295 AGE-RELATED SENSITIVITY TO ORGANO-PHOSPHATE-INDUCED DELAYED POLYNEUROPATHY. M Lotti, A Moretto, and P Borlina. Istituto di Medicina del Lavoro dell’Università di Padova, Padua, ITALY.

Neuropathy target esterase (NTE) is defined in vitro among nervous system phenyl-Valerate (PV) esterases as resistant to paraoxon (PX) (40 uM) but sensitive to mipafox (50 uM). Inhibition/aging of hen axonal NTE (>70%) initiates organophosphate-induced delayed polyneuropathy (OPIDP). Chicks are resistant to OPIDP. Total PV-esterases in brain (B), spinal cord (SC) and peripheral nerve (PN) of 10-80 day old chicks were not age-dependent nor different from those of hen. NTE was measured using 256 uM PX for complete inhibition of PX-sensitive PV-esterases; it decreased from 39.2±0.5 in B, 17.1±0.6 in SC and 2.1±0.4 in PN of 10 day old chicks to 21.6±1.4, 5.0±0.8 and 1.0±0.1, respectively in hens (nmol/min/mg protein, mean±SD, n=3). PV-esterases dissected with PX (40 uM) changed with age either in their proportion or sensitivity in chick PN but not in B and SC. Groups of chicks aged 20, 40 and 60 days, treated with DFP (1 mg/kg s.c.) had high NTE inhibition correlating with OPIDP in the 80-day group, only.

296 TRIPHENYL PHOSPHITE INHIBITION OF CATECHOLAMINE SECRETION FROM BOVINE ADRENOMEDULLARY CHROMAFFIN CELLS. M B Abou-Doint and J K Knoth. Univ Med Ctr., Durham, NC.

Triphenyl phosphite (TPP) induces a neuropathy similar to organophosphorus-induced delayed neurotoxicity (OPIDN) except that in addition to axonal degeneration, direct cell damage occurs. To understand the mechanism of TPP-induced neuropathy we are biochemically investigating its effects in vitro using primary cultures of bovine adrenomedullary chromaffin cells as a neural model system. Since TPP affects the cell body in vivo, we assessed what effects it may have on presynaptic events by determining its action on exocytosis (i.e. secretion of catecholamines). TPP inhibited catecholamine secretion in both a time- and dose-dependent manner. By 4 hrs TPP inhibited nicotine-induced secretion by 75-85%. About 35% inhibition was seen as early as 15 min. Twenty-four hour incubations with concentrations as high as 100 uM TPP did not cause cell death. Inhibition also occurred in a dose-dependent manner with maximal inhibition at 25 uM TPP. In contrast to TPP's effects, dimethyl phosphite, which is an OPIDN producer in vivo, had only minimal effects on catecholamine secretion at concentrations as high as 100 uM. These in vitro results correlate well with in vivo findings which demonstrate direct cell body effects with TPP and not OPIDN-producing compounds such as DFP.
Some phosphoramidates (e.g. isofenphos and tabun) produce symptoms of organophosphate induced delayed neuropathy (OPIDN) at high doses. The neuropathic potential of acephate, another phosphoramide, was assessed by determining inhibition of hen brain acetylcholinesterase (AChE) and neuropathy target esterase (NTE) in vivo. Laying hens were treated with acephate technical (5 to 1000 mg/kg, oral) or disopropylfluorophosphate (DFP, 50 to 200 ug/kg, im) 24 hours before sampling. The approximate LD50 of acephate was 800 mg/kg. DFP, a known neurotoxicant, was as good an inhibitor of NTE as it was of AChE. ED50 (50% inhibition) values were 133 ug/kg (NTE) and 145 ug/kg (AChE). In contrast, acephate was much weaker an inhibitor of NTE than it was of AChE. ED50 values were 1500 mg/kg (NTE) and 9.5 mg/kg (AChE). The high ED50 NTE:AChE ratio suggests a single non-lethal dose of acephate would not cause OPIDN even with atropine protection and assuming that aging of the inhibited NTE occurs.

The potential for the organophosphorus insecticide chlorpyrifos to produce delayed neuropathy was investigated using the cat. Animals (five per group) were dosed once, IM, with either corn oil (vehicle control), DFP, 5.0 mg/kg (positive control), or chlorpyrifos, 300 mg/kg. Clinical signs of delayed neuropathy and lymphocyte neuropathy target esterase (LNTI) activity were monitored during a 60 day observation period. The mean interval between dosing and the onset of hindlimb ataxia was 19 days. Ataxia was characterized by hypermetria, waddling gait, and conscious proprioceptive deficits. With regard to paresis, the DFP treated cats were less severely affected than those given chlorpyrifos. Unlike the chlorpyrifos treated cats, those treated with DFP improved over time. The mean maximal depression of LNTI in the chlorpyrifos treated animals was 46% of the pretreatment activity on day 0. The mean maximal depression of LNTI in DFP treated cat's was 96% and occurred at one day after dosing. Lesions were confined to the white matter of the central nervous system and to the peripheral nerves, however, there was a moderate variation in severity between the areas examined. The onset and character of ataxia coupled with lesions restricted primarily to the white matter of the central nervous system were consistent with delayed neuropathy. However, the maximal depression of LNTI in the chlorpyrifos-treated animals was significantly delayed and less severe than the positive control animals.

Male and female rats were conditioned in nose-only exposure tubes 25 min/day for two days, then exposed to cigarette smoke for eight days. Mainstream cigarette smoke was generated by a modified Walton smoking machine from 193 research cigarettes. Deposition studies were then conducted by placing the rats in plethysmograph tubes to measure minute respiratory volume during exposure, then exposing them to 4C-drotilaconate labeled cigarette smoke at mass concentrations of 200 or 624 mg/m³ for 25 min. Particle size distribution, real-time concentration, and specific activity were determined using a multi-channel Mercor impactor, a real-time aerosol monitor, and filter samples, respectively. Immediately after exposure, the rats were sacrificed to determine the distribution of the 4C.

REGIONAL DEPOSITION PATTERNS FOR INHALED PARTICLES AND FIBERS ARE DEPENDENT UPON AIRWAY BRANCHING PATTERNS. M A Harkavy, C G Plopper, and D W Macherl. Du Pont-Haskell Lab., Newark, DE and Univ. of Cal. Davis, CA.

Recently it was demonstrated that regional differences in asbestos fiber burdens were inversely related to the pathlength of intrapulmonary airways, i.e., increased fibers were retained in cranial regions containing the shortest pathlengths from the main bronchus. The present study investigated whether this was due to enhanced deposition or reduced clearance of inhaled particles.

Rats were exposed to aerosols of carbonyl iron (CI) particles for 1 or 6 hrs at concs. of 100 mg/m³ and were either vascularly-fixed immediately after exposure for deposition studies or airway-fixed 24 or 48 hrs postexposure to assess macrophage clearance. The left lobe was then microdissected to the level of the bronchial-alveolar junctions. Particle deposition and macrophage recruitment were quantified by scanning electron microscopy. Increased numbers of particles deposited in cranial (p<0.05) compared to caudal regions, and this correlated with an enhanced pulmonary macrophage chemotactic response. Our results suggest that airway pathlengths influence regional deposition patterns for both particulate and fibrous materials.

DEPOSITION OF ULTRAFINE PARTICLES IN F-344 RAT NASAL CASTS. Y S Cheng, G X Hansen, Y F Su, H C Yeh and K T Morgan*. Levelate Inhalation Toxicology Research Institute, Albuquerque, NM. *CIDT, RFP, NC. Sponsor: C H Hobbs.

Information is available on deposition of larger particles (30.02 μm) in the nasal passages of laboratory animals, with the deposition fraction increasing with increasing particle size. Little information is available for ultrafine particles less than 0.2 μm. Molds (models) were prepared from replica casts of mouse passages of F-344 rats, using clear casting plastic. Total deposition of ultrafine aerosols in these casts was then determined using a unidirectional flow system. Measured pressure drops in the casts were a function of flow rate to the power of 1.4-1.6, indicating that the flow through the nasal passage was not laminar. Deposition data was obtained from these casts, using monodisperse sodium chloride aerosols with particle size ranging from 0.2 to 0.005 μm. Inspiatory and expiratory flow rates of 200 to 500 cc/min, Similarly tight deposition data were obtained for the three cast studied. The deposition efficiency was greatest for the smallest particles and decreased with increasing particles size and flow rate indicating that diffusion was the dominant mechanism. At an inspiratory flow rate of 400 cc/min, which is comparable to a respiratory minute volume of 200 cc/min for mature male F-344 rats, deposition efficiency was 79% for 0.01 and 0.005 μm particles, respectively. Turbulent diffusion was considered to be the dominant mechanism for deposition of ultrafine particles in the nasal passage. This information is important for understanding the toxicity and carcinogenicity of particles, including diesel soot, radon progeny, and vapors. (Research supported by US DOE/ORE under Contract No. DE-AC04-76EV01013 and the 01F.)
REVERSIBILITY OF BIOCHEMICAL ALTERATIONS IN BRONCHO-ALVEOLAR LAVAGE UPON CESSATION OF DUST EXPOSURE.
O. Creuzenberger, H. Muhs, B. Bellmann, R. Klipper, R. Mermeister and P. Morrow
1. Fraunhofer Institute for Toxicology, Hannover 3000, FRG 2. Corporate Environmental Health & Safety, Xerox Corp. Rochester NY 3. University of Rochester, Rochester, NY. SPF-VAF, F-344 rats were exposed to a chronic inhalation study for up to 24-months to a special test toner at 0, 1, 4, and 16 mg/m³. BAL parameters were measured at the end of the exposure period and 6 weeks subsequently. Biochemical and cytological parameters indicated inflammatory process at the high exposure level without evidence of recovery. In a follow-up investigation, the persistence of such responses upon cessation of dust exposure was investigated. Female F-344 rats were exposed to 0, 10, and 40 mg/m³ of the test toner for three months, resulting in lung burdens of 0.4 and 3.0 mg respectively. Lung overloading was evident at the 40 mg/m³ exposure level. BAL parameters were measured at 3 months intervals over a one year recovery period. At the low exposure level, only a few of the parameters were slightly elevated, and generally returned to normal after a 6 months recovery period. At the high dust exposure level, inflammatory reactions were evident in all parameters and only limited recovery was observed after a 6 months post exposure period.

KINETICS OF APPEARANCE OF POLYMORPHONUCLEAR LEUKOCYTES AND THEIR PARTICLE BURDENS DURING THE ALVEOLAR CLEARANCE OF A HIGH LUNG BURDEN OF PARTICLES. B. E. Lehner, A. Cline, J. E. London. Los Alamos National Laboratory, Los Alamos, NM.

We quantitated polymorphonuclear leukocytes (PMN) in lung free cell populations during the alveolar clearance of three lung burdens of particles, and quantitated particle burdens in the PMN. Fischer-344 rats were intratracheally instilled with 1.6x10^10 (L), 2.0x10^10 (M), or 6.8x10^10 (H) polystyrene microspheres (2.0 μm dia), or with carrier vehicle alone, and the lung retention kinetics of the particles were determined over 176 days. 50% of the particles cleared the L and M lungs by day 35 while half of the H burden remained up to day 80. On days 7, 14, -55, -85, and -176, the lungs of the animals were lavaged and the numbers of PMN were enumerated. The numbers of particles in the PMN were also quantitated. PMN numbers from the PBS, L, and M lungs were similar during the study but became progressively elevated in the H lungs. Particles were not found in the PMN from the L and M lungs. However, they were observed in PMN from the H lungs and their abundance in the PMN gradually increased 10-fold over the course of the study. These results support the possibility that: 1) previously phagocytized particles may be continuously released over the course of alveolar clearance, and 2) PMN may play a role in causing pathologic changes in particle "overload" conditions.

REGIONAL UPTAKE OF VAPORS IN THE RESPIRATORY TRACTS OF RATS AND BEAGLE DOGS. A. R. Dahl, M. B. Snipes, J. L. Mauderly, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

Methods for measuring vapor uptake in the nose and lungs were developed to provide data for testing mathematical models that include terms for uptake in blood, nasal metabolism, mucociliary clearance, "back-pressure" desorption, and chemical reactivity. Rats were anesthetized with urethane and were fitted with a cuffed endotracheal tube -5 mm caudal to the larynx. The free end of the tube was attached to a small animal respirator that simulated natural breathing through the nose. The rat's nose was placed in a nose-only exposure cone attached to a pneumotach and a non-rebreathing valve. Catheters were employed to obtain air, tracheal air and exhaled air. Vapor concentrations were determined automatically using a gas chromatograph. Use of both living and freshly-killed rats allowed for separation of the contribution of blood uptake from uptake due to nasal metabolism. Dogs were sedated and fitted with 2 tracheal catheters for sampling inhaled and exhaled air as well as air exhaled at the nose. The concentration of vapor during an entire breath or at the end of a breath could be determined by electronically triggering air sampling with signals from the pneumotach. Early results using ethyl acetate indicate that nasal uptake is the major route of uptake and that nasal metabolism contribute substantially to the metabolism of the absorbed vapors. (Research supported by NIH/NIOSH Grant ES04482 and U.S. DOE/DOE under Contract No. DE-AC04-76EV01013.)
SULFUR DIOXIDE DEPOSITION IN THE HUMAN RESPIRATORY TRACT. D B Menzel, R L Wolpert and J R Boger, III. Depts. Pharmacology and Medicine, Comprehensive Cancer Center and Inst. for Statistics and Decision Sciences, Duke University, Durham, NC.

Sulfur dioxide (SO₂) occurs widely in the urban atmosphere from the combustion of fossil fuels and is a known pulmonary irritant. Using the Miller-Overton mathematical model of ozone regional deposition in human and animal lungs as a basis, a mathematical model of the regional deposition of SO₂ was developed. To adjust for upper airways deposition, the elimination of SO₂ reaction products from the plgma (plasma S-sulfonates) was also modeled using a one compartment pharmacokinetic model. Combining the two simulations provided a close fit to measured plasma S-sulfonate levels in humans inhaling SO₂. About 86% of the inhaled SO₂ is removed by the human nasopharyngeal region. Deposition within the lung is simulated to occur mostly in the upper conducting airways (2.3 x 10⁻⁷ μmol/min/ppm) with less than 1% reaching the respiratory region. The sensitivity of the model to differences in the chemical reaction rate of SO₂ with lung macromolecules suggests that the physical properties of SO₂, rather than chemical reactivity, dictate the pattern of regional deposition in the lung. Supported by grants from the Electric Power Research Institute and the ILSI Risk Sciences Institute.

EXERCISE IS PREDICTED TO ENHANCE THE DEPOSITION OF SULFUR DIOXIDE IN THE LUNG. R L Wolpert, D B Menzel and J R Boger, III. Depts. of Pharmacology and Medicine, Comprehensive Cancer Center and Inst. of Statistics and Decision Sciences, Duke University, Durham, NC.

Exercise exacerbates the pulmonary toxicity of sulfur dioxide (SO₂) especially in asthmatic subjects. The mechanism(s) of exacerbation is unknown. Using a mathematical model, the deposition of SO₂ in each of 24 generations of bifurcations of the human lung were calculated under mild exercise and heavy exercise. Resting respiration is predicted to result in 2.3 x 10⁻⁸ μmol/min/ppm in the tracheal region of the lung. Mild exercise and heavy exercise are predicted to result in 6.3 and 8.3 x 10⁻⁸ μmol/min/ppm, respectively. Despite the increased deposition of SO₂ in the tracheal region due to exercise, little SO₂ reaches the respiratory regions of the lung. While the thickness of the mucus layer in the tracheal region affects the amount of SO₂ reaching the underlying tissues, an enhanced mucus layer in asthmatics is not predicted to increase the amount of SO₂ reaching putative underlying receptors. Using the model a wide variety of experiments demonstrating an exacerbation of SO₂ pulmonary effects in normal and asthmatic subjects could be interpreted as resulting from the 3 to 4-fold enhancement in pulmonary dose on exercise. The time to reach steady state concentrations of SO₂ byproducts during continuous SO₂ inhalation is 14 days, so most human exposures never reach steady state. Simple relations between calculated pulmonary dose and pulmonary function could then be found for both asthmatic and normal subjects. While exercise may account for some differences in effect in both normal and asthmatic subjects, factors other than increased dose due to exercise are probably responsible for the sensitivity of asthmatic subjects to SO₂. Supported by grants from the Electric Power Research Institute and the ILSI Risk Sciences Institute.

DIRECT MEASUREMENTS OF PERCHLOROETHYLENE IN THE BLOOD AND EXHALED BREATHS OF RATS DURING AND FOLLOWING INHALATION EXPOSURE. C E Dallas, R Ramanathan, S Subrahmanyam, M Gallo, R C Manning, and J V Bruckner. Depts. of Pharmacology & Toxicology and *Pharmacaceutics, College of Pharmacy, University of Georgia, Athens, GA.

The pharmacokinetics of perchloroethylene (PER) was studied in male Sprague-Dawley rats to characterize the rate of uptake and respiratory elimination by direct measurements of the inhaled compound. Fifty or 500 ppm PER was inhaled for 2 hr through a one-way breathing valve by unanesthetized rats of 225-375 g. Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected during and following PER inhalation and analyzed by gas chromatography. PER exhaled breath and alveolar levels increased rapidly after the initiation of exposure to near steady-state within about 60 min. They were then directly proportional to the exposure concentration. Uptake of PER in the blood was also rapid, but blood levels continued to increase progressively over the course of the 2-hr exposure at both dose levels. Cumulative uptake, or total absorbed dose, was proportional to the inhalation exposure level. The observed values for PER blood and exhaled breath levels and cumulative uptake were employed in the validation of a physiologically-based pharmacokinetic model for PER inhalation in rats. (Supported by Air Force AFOSR 87-0248 and EPA CR812267).
CHARACTERISTICS of 2,4,4-TRIMETHYL-2-PENTANOL (TMP) BONDING TO α2u-GLOBULIN AND OTHER COMPOUNDS THAT CAUSE PROTEIN DROPLET NEPHROPATHY. S J Bardonoff, P D Upton, JA Swennes, CIT, Research Triangle Park, NC.

The nephrotoxicity of 2,4,4-trimethylpentane (TMP) in male rats is characterized by an increase in protein droplets and renal concentration of α2u-globulin (α2u). TMP has been identified as the metabolite of TMP that binds reversibly to α2u isolated from TMP-treated male rat kidney cytosol. Preliminary evidence suggests this binding affects the digestibility of α2u leading to protein droplet formation. Studies were carried out in vitro to characterize binding using kidney cytosol as the source of α2u along with [3H]-TMP. The binding affinity (Kd) of [3H]-TMP to α2u was calculated to be in the order of 10^{-6} M using male rat kidney cytosol. Compounds that have been shown to cause protein droplet nephropathy in male rats compete in vitro with [3H]-TMP for binding to α2u. The relative affinities of each compound for α2u were compared using their apparent inhibition constant values (Ki) determined from their IC50 values and the Kd for the [3H]-TMP-α2u complex. Ki values for D-limonene, 1,4-dichlorobenzene, and 2,5-dichlorophenol were all in the range of 10^{-7} M, whereas the Ki for isophorone and 2,4,4- or 2,4,4-trimethyl-1-pentanol were determined to be 10^{-8} and 10^{-9} M, respectively. TMP, 2,4,4- and 2,2,4-trimethylpentane and acetic acid did not compete for binding. Although retinol was found to compete for binding in vitro (Ki = 10^{-7} M), unlike the other chemicals that bind, when [3H]-retinol was administered to male rats there was an increase in α2u or protein droplets. However, retinol derived radiolabel did not co-elute with the protein fraction in cytosol containing α2u. This suggests that protein droplet nephropathy may not depend on whether α2u is present or not but rather on whether the chemical causes a contornal change in the protein.

LYSOSOMAL DEGRADATION OF α2u-GLOBULIN (α2u): ROLE OF CYSTEINE AND ASPARTIC ACID PROTEINASES AND EFFECT OF d-LIMONENE BINDING. MI Rivera Torres, D Caudill and H Lehman-McKeeman, Miami Valley Laboratory, Procter & Gamble Co., Cincinnati, OH.

d-Limonene causes α2u to accumulate in lysosomes of male rat kidney cells. The objectives of this study were to identify the cathepsins responsible for initiating α2u degradation and to determine whether α2u degradation is altered by d-limonene metabolite binding. Male rat renal cortical lysosmes, isolated by differential centrifugation, were incubated with α2u. Pepstatin, an inhibitor of aspartic acid proteinases and leupeptin, an inhibitor of cysteine proteinases, reduced α2u degradation to 56±4 and 36±17% of control, respectively. Addition of both inhibitors decreased α2u degradation to 8.5±2% of control values. Under the incubation conditions used, 19.5±3% of native α2u was degraded. Binding of d-limonene to α2u did not alter degradation (17.5±2%), whereas binding of d-limonene-1,2-oxide, the major d-limonene metabolite that binds to α2u, reduced α2u degradation to 3.5±2%. In summary, both cysteine and aspartic acid proteinases contribute to α2u degradation. Binding of d-limonene and 1,2-oxide had different effects on α2u degradation. However, a decrease in lysosomal degradation of α2u bound to 1,2-oxide is probably involved in d-limonene-induced accumulation of α2u.

RENEAL ALPHA-2-MICROGLOBULIN DEPOSITION FOLLOWING EXPOSURE OF MALE F-344 RATS TO 2,2,4-TRIMETHYL-1-PENTANENE, TRICHLOROETHYLENE, TETRACHLOROETHYLENE OR CHLOROFORM. CJ Potter, A B DeAngelo and F B Daniel, U.S. Environmental Protection Agency, Cincinnati, OH.

The ability of four nephrotoxic chemicals to produce hyaline droplet formation (HDF, alpha-2-microglobulin accumulation) in rat kidney tubules was determined. Male F-344 rats (200g) were gavaged on days 0, 1 and 2 with trichloroethylene (TCE, 1000 mg/kg), perchloroethylene (PCE, 1000 mg/kg), chloroform (CHCl3, 500 mg/kg) or toluene (TOL, 500 mg/kg) served as positive control. Animals from each group were killed on days 3 and 7. Kidney sections were stained with H&E and Mallory's Heidenhain's trichrome. Sections were evaluated for histopathological changes and HDF, ranked on a scale of 1 to 5 in the proximal convoluted tubules. Nephropathy marked by flattened squamous tubular cells, thickened basement membranes and tubular regeneration occurred in the treated groups. At 3 days after treatment PCE and TMP increased HDF in the proximal tubules with average scores of 3.0 and 2.7, respectively. Moderate recovery from HDF was evident at 7 days. TCE-treated rats exhibited HDF (1.3) similar to controls (1.3) and CHCl3-treated rats exhibited no (0.0) HDF at either time despite the presence of nephropathy. These results indicate HDF was not a factor in TCE or CHCl3 nephropathy, but may have been involved in TMP and PCE nephropathy. HDF with the alpha-2-microglobulin protein as the main component appears to be sex and species specific, it has not been found in mice or female rats. Nephropathy that only occurs secondary to HDF might reflect an idiosyncratic response in the male rat and could be considered a potential interfering factor in renal toxicity studies.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

COMPARATIVE MOLECULAR WEIGHT DISTRIBUTION OF RAT AND HUMAN MALE URINARY PROTEINS. M J Olson, J T Johnson and C A Reidy, Biomedical Science Dept., GM Research Labs., Warren, MI.

α2u-Globulin (α2u), a rat urinary protein (UP), is a key determinant of susceptibility to hyaline droplet nephropathy (HDN) induced by a variety of hydrocarbons in male rats. To aid assessment of human risk of HDN, UP of male F344 rats (3 mo. old) and normal human males were compared after separation and partial identification by cation exchange, gel filtration, SDS-PAGE and Western blotting. We observed that: 1) the protein content of human urine is only 3% of male rat urine, 2) human UP is primarily of high (258 kD) molecular weight (MW) with minor components of 12-42 kD, 3) male rat urine has little high MW protein, but is rich in α2u (18.5 kD), 4) at pH 5, the cationic fraction comprised only about 8% of total human UP while the analogous fraction of rat urine, containing α2u, contained 33% of total UP, 5) cationic human UP, isolated by the same method as male rat α2u, included small amounts of proteins, e.g. albumin, α2-acid glycoprotein, and α2-microglobulin, some of which are products of the gene family coding for α2u in rats. Thus, although humans excrete trace amounts of proteins similar to α2u, the very low relative proportion of cationic protein to total proteins and especially high MW of human UP form a biological basis for suggesting that humans are not at risk for fuel and solvent hydrocarbon-induced HDN.
LYSOSOMAL CHANGES IN RENAL TUBULAR EPITHELIAL CELLS OF MALE SPRAGUE-DAWLEY RATS FOLLOWING DECAVIN EXPOSURE. T E Eurell *, R D Parker, and CL Alden. *College of Vet. Med., Univ. of IL, Urbana, IL and Procter & Gamble, Cincinnati, OH.

The hydrocarbon-induced nephrotoxicity of male rats is characterized by the formation of large hyaline droplets in renal tubular epithelial cells. The hyaline droplets are believed to represent an accumulation of lysosomes which may contribute to the nephrotoxic response. The concentration of acid phosphatase in renal tubular epithelial cells of control and decalin-exposed male rats was determined by a diazoinum salt-based stain. Quantitative histology and morphometric analysis were used to determine acid phosphatase stain intensity and the size of cytoplasmic lysosomes in renal tubular epithelial cells. Decalin exposure reduced acid phosphatase stain intensity when compared to controls. Lysosomes of exposed rats were fewer but significantly larger than control animals. All acid phosphatase stain reaction product appeared to be contained within intact lysosomes.

(Supported by AFSOR grant #88-0033 and Procter & Gamble.)

TOXICITY, TRANSPORT AND METABOLISM OF N-ACETYL S-(1,2-DICHLORO-VINYL)-L-CYSTEINE (NAC-DCVC) IN RABBIT RENAL CORTEX SLICES. GHI Wolfgang, AJ Gandolfi, JL Stevens and AW Brendel. Dept Pharmacology/Toxicology, University of Arizona, Tucson, AZ and W. Alton Jones Cell Science Center, Lake Placid, NY.

Dichlorovinyl cysteine (DCVC) is a potent, specific nephrotoxin. DCVC is acetylated in vivo to NAC-DCVC prior to being delivered to the kidney. An in vitro system was utilized to determine if NAC-DCVC itself was toxic. Cultured precision-cut renal cortical slices from male NZW rabbits were incubated (up to 12 hr) with NAC-DCVC (10⁻¹⁹ M). NAC-DCVC produced time and dose-dependent decreases in slice P and LDH. Histopathology revealed an initial S lesion which progressed to include all proximal tubules (similar lesion as DCVC). NAC-DCVC apparently is transported via the organic anion system since it competes with RAH for entry into the cell, and probenecid decreases the uptake (80%) and toxicity of NAC-DCVC. Although NAC-DCVC is not a substrate for β-lyase, the β-lyase inhibitor amnoinooxycetic acid inhibited both covalent binding and toxicity of NAC-DCVC implying that NAC-DCVC is deacetylated to DCVC before being metabolized. Even though DCVC is acetylated in vivo, NAC-DCVC itself is toxic, expressing similar toxicity to DCVC. Using an in vitro system it was determined NAC-DCVC is less potent than DCVC, it is transported by a different system, and must be deacetylated to DCVC before being metabolized. (NIH GM 38290)

THE EFFECT OF GENTAMICIN ON LYSOSOMES OF HUMAN PROXIMAL TUBULAR CELLS. A L Trifillitis, B P Trump, and A L Regec, Department of Pathology, University of Maryland School of Medicine, and MFINSS, Baltimore, MD. Sponsor: T W Jones

Gentamycin (G) treatment results in changes in lysosomal morphology and enzyme activity in renal tubular cells both in vivo and in vitro. Therefore, the effect of G on lysosomal function and integrity were studied. Cultured human proximal tubular cells (PTC) were treated with G (0, 0.01, 0.1 and 1.0 mg/ml) for 3, 7, 10 and 14 days and the endocytotic activity, pH, and membrane fragility of the lysosomes were examined. G treatment caused a slight increase in intralysosomal pH, estimated by endocytosis of fluorescein isothiocyanate-labeled dextran. Significantly increased fragility, estimated by N-acetyl-β-glucosaminidase release, was seen after 10 days treatment with 1.0 mg G/ml. PTC accumulated 0.47, 2.05 and 10.30 μg G/mg protein with 10 days exposure to 0.01, 0.1 and 1.0 mg G/ml, respectively. G treatment did not affect the endocytotic activity of lysosomes in cultured PTC. Prolonged exposure (14 days) to G increased the pH and fragility of lysosomes. Increased numbers of morphologically altered lysosomes with increased fragility and pH were not associated with significant in vitro cytotoxicity. Thus, it seems these lysosomal alterations are not directly responsible for the in vivo nephrotoxicity. [Sponsored by NIH grant AT-24179.]

INFLUENCE OF AMINOXYCETIC ACID (AOA) AND PROBENECID ON THE NERPHOTOXICITY DUE TO N-ACETYL-L-CYSTEINE CONJUGATE OF STYRENE. S Chakrabarti, A Mallick and C Denuel, Med. trav. hyg. mali., Fac. médecine, Univ. Montréal, Montréal, Quebec, Canada.

We have previously shown that styrene and its N-acetylcycteine conjugate (NAC-ST) are potent nephrotoxins (Toxicology 46:343-356, 1987; Toxicologist 8:134-1988). This study examines the effects of inhibitors of renal cysteine conjugate β-lyase (AOA) and organic anion transport system (probenecid) on the nephrotoxicity due to NAC-ST. Male Fischer-334 rats (180-200g b.w.) were treated i.p. with either AOA (0.13 mmole/rat) 2 h before, or probenecid (80 mg/kg) 36 min before and 60 min after a single i.v. injection of 0.13 mmole NAC-ST per rat. The nephrotoxicity due to NAC-ST was not reduced due to AOA as verified by characteristic biochemical parameters of renal injury. However, a reduction of its nephrotoxicity was observed due to probenecid treatment as verified by reduction in the urinary activities of NAC, LDH and GPDH. These results suggest an important role of renal organic anion transport system, but an insignificant role of metabolism by cysteine conjugate β-lyase in NAC-ST induced nephrotoxicity (MRC grant, MA-9705).

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Our previous studies have shown that tissue glutathione (GSH) concentrations and corresponding conjugating activity appear to be important determinants of streptomycin nephropathy (Toxicology 40:245-50, 1987). Furthermore, we have reported previously both in vitro and in vivo nephrotoxicity of cystine derivatives of streptomycin. In this study, we have therefore examined the in vivo nephrotoxic potential of GSH-conjugate of streptomycin (GSH-S-7T). GSH-S-7T was administered single i.p. injections of GSH-S-7T in saline (0, 0.023, 0.046 and 0.092 ml per kg). Biochemical parameters of renal injury were monitored at 24 and 48 h after the administration. Significant increases in the urinary excretions of γ-glutamyltransferase (brush border), N-acetyl-β-D-glucosaminidase (lysosomal), lactic dehydrogenase (cytosolic) and glutamate dehydrogenase (mitochondrial) were observed at the highest dose level at 24 h. GSH-S-7T was not hepatotoxic. These results combined with our previous studies suggest that GSH-S-7T is a potent nephrotoxin and its renal metabolism is required for the expression of its toxicity (NIH grant MA-9785).

EFFECT OF BUTHIONINE SULFOXIMINE ON ACUTE N-(3,5-DICHLOROPHENYL)SUCINIMIDINE-INDUCED NEPHROTOXICITY. G. D. Rankin, V. J. Teets, D. W. Nicoll, and P. I. Brown. Marshall University School of Medicine, Huntington, WV.

Previous studies from our laboratory using diethyl maleate have demonstrated that glutathione might play a role in mediating the nephrotoxicity induced by the agricultural fungicide N-(3,5-dichlorophenyl)succinimide (NDS). The purpose of this study was to determine if pretreatment with the glutathione synthesis inhibitor buthionine sulfoximine (BSO) would alter the nephrotoxicity induced by NDS. Male Fischer 344 rats (4 rats/group) were administered BSO (50 mg/kg, i.p.) in 0.9% saline (10 ml/kg) 2 h prior to NDS (0.4 or 1.0 mmol/kg, i.p.) or sesame oil (2.5 ml/kg), and renal function monitored at 24 and 48 h. BSO pretreatment markedly attenuated all NDS (0.4 or 1.0 mmol/kg)-induced changes in renal function and morphology. NDS-induced diuresis, increased proteinuria, blood urea nitrogen (BUN) concentration and kidney weight, decreased organic ion accumulation by renal cortical slices, and proximal tubular necrosis were all prevented or markedly attenuated in BSO-pretreated rats. These results support our earlier observations and demonstrate that glutathione plays a role in mediating NDS-induced nephropathy. Supported by NIH grant DK 31210.


The agricultural fungicide 3,5-NDS was shown to be nephrotoxic in rats, but little is known about the relationship between its metabolism and toxicity. In this study the nephrotoxicity of 3,5-NDS was compared to that of 3,5-NDS-OPH, a potential metabolite. Male Sprague-Dawley rats were divided into six groups (N=6) and three of these groups were treated with either compound (1.6 mmol/kg) or the protein vehicle (i.p.). The three remaining groups received the same treatments following pretreatment with phenobarbital (PB) at 80 mg/kg (i.p.) on all 3 days prior to dosing. Renal function was monitored over 6 hour periods at 0, 24, and 48 hours following dosing. Both compounds caused similar elevations in blood urea nitrogen (BUN), urine volume, kidney weight, proteinuria, and glucoseuria without PB induction. Morphological changes were similar although 3,5-NDS-OPH affected the distal tubules to a greater extent. Following PB induction significantly greater increases in BUN and urine volume were noted in only 3,5-NDS treated animals. Thus if 3,5-NDS-OPH is indeed a metabolite of 3,5-NDS, it may contribute to its toxicity. Supported in part by P.H.S. grant ESO4753.


Exposure of rabbit renal proximal tubules to BHQ results in mitochondrial dysfunction, adenine nucleotide alterations, and cell death (Schellman et al., TAP 90; 420, 1987). The purpose of this study was to examine the toxicity of BHQ to RCM. After a 5 min. 37°C exposure to 0.2 and 0.5 mM BHQ, state 3 respiration of RCM respiring on pyruvate/malate decreased 19% and 34%, respectively. Under the same conditions, there was no effect on state 4 respiration or (ascorbate-TPMD)- and FCCP-stimulated respiration. (Ascorbate-TPMD) directly stimulates electron flow through cytochrome c-cytochrome oxidase. FCCP uncouples oxidative phosphorylation allowing maximum flow of electrons through the electron transport chain. After 5 min of exposure, BHQ (0.2 mM) had no effect on state 3 or 4 respiration of RCM respiring on succinate, but 0.5 mM BHQ increased state 4 respiration (19%) and decreased state 3 respiration (17%). These results show that RCM can bioactivate BHQ and that an early and somewhat selective toxic effect of BHQ is the inhibition of NADH-linked respiration. The qualitative and quantitative effects of BHQ on proximal tubules and RCM are similar. (Supported by NIH ES-04146 and PMA Fdn.).
325 Role of Cytochrome P450 in Carbon Disulfide (CS₂)-Induced Renal Toxicity. R B Kroll and R J Rubin, Johns Hopkins University, Baltimore, MD.

CS₂ is toxic to the liver following P450-mediated metabolism to a reactive intermediate. Induction of a specific P450 by isopropanol (ISO) pretreatment has been shown to result in increased hepatotoxicity. We have previously shown that CS₂ (ip) is also renotoxic in the rat. Experiments were undertaken to evaluate the role of P450 in this toxicity by determining the effect of ISO pretreatment. Eighteen hour ISO pretreatment in rats had no further effect on CS₂-induced alterations in BUN, glucose clearance or renal concentrating ability. However, ISO was able to completely prevent the inhibitory effect of CS₂ on organic anion (PAH) uptake. In previous studies it was shown that the specific isopropanol inducible P450 responsible for CS₂ activation also metabolizes aniline (aniline hydroxylase [AH]). Renal AH activity was measured and found not to be increased by ISO pretreatment under conditions where hepatic AH was increased 2-fold. Thus, it is concluded that (1) the renotoxicity seen with CS₂ is not due to the formation of an hepatic metabolite; (2) the specific renal P450 for CS₂ metabolism (as assessed by AH activity) is not inducible by ISO; and (3) it is possible that the increased hepatic metabolism of CS₂ leads to a decrease in the amount of CS₂ delivered to the kidney.

Supported by NIEHS ES02927.


Studies were conducted to determine the effect of soil adsorption on the bioavailability of pollutants via the skin. ¹⁴C-naphthalene alone (P) or with sandy (S) or clay (C) soil was administered dermally to rats. C produced the highest plasma concentration of radioactivity followed closely by P with the lowest value exhibited by S. Increased bioavailability of C was evidenced by a statistically increased area under the plasma concentration time curve versus P. Both soils doubled the time to peak and significantly increased the half-lives of absorption versus P. Urine was the primary excretion route for radioactivity in all groups with significantly higher amounts in the urine of C versus P. A significant decrease in radioactive was observed in the expired air of S versus P. Tissue concentration of radioactivity was highest in skin application sites, duodenum and ileum in all groups.


328 VAGINAL ABSORPTION OF POLYVINYL ALCOHOL IN FISCHER 344 RATS. J M Sanders and H B Matthews. NIEHS, Research Triangle Park, NC.

Polyvinyl alcohol (PVA), has recently been used as a component of spermicide formulations administered intravaginally. As a prerequisite to studies of PVA toxicity, the disposition of this compound has been studied following i.v., oral, and intravaginal administration. A ¹⁴C PVA mixture of molecular weights < 100,000 administered iv to female rats was concentrated primarily in liver, spleen, and kidneys 24 hr after a single dose. Liver contained 17% of the dose at 24 hr, 12% of the dose at 72 hr, and 3% of the dose 10 days following injection. Within one day, 66% of the dose was excreted in urine and 24% in feces. Excretion of PVA-derived radioactivity in feces was 6% at 72 hr and 13% at 10 days. PVA administered by gavage was < 0.05% absorbed. On intravaginal administration there was an increasing concentration of PVA-derived radioactivity in major tissues following 1, 3, or 10 daily doses of 3 mg/kg. The peak concentration in liver reached 1750 ng equivalents/g tissue 24 hr following 10 daily doses. Over 300 ng equivalents/g tissue were still present in the liver 30 days following the last dose. This work indicated that PVA was virtually unabsorbed from the GI tract, but small amounts were absorbed from the vaginas of female P344 rats. Bioaccumulation of vaginally absorbed PVA-derived radioactivity was observed in tissues, primarily liver and kidney. However, there have been no reports of toxicity by this route of administration.


Triclopyr [3,5,6-trichloro-2-pyridinyl]oxyacetic acid] is the active component of CARLON® brand herbicide. Five rats/sex were orally dosed with 3 or 60 mg/kg 14C-triclopyr or 14 daily 3 mg/kg doses (unlabeled) followed by 3 mg/kg 14C-triclopyr on day 15 and sacrificed 72 hrs post-dosing. Additionally 5 rats/sex were administered a single 3 mg/kg i.V. dose. The 14C plasma time-course was determined in 3 male rats (p.o.) at 3 and 60 mg/kg. Over 96% of the dose was recovered and the principle route of excretion was via the urine (92%). The feces, expired 14CO₂ and cage wash contained <5% of the dose. The tissues and carcasses accounted for <2% of the dose. 14C-Triclopyr was rapidly and completely absorbed after oral administration. The plasma radioactivity was eliminated mono-exponentially, with a first-order half-life of 3.5 hr for both doses. At the 60 mg/kg dose, the plasma clearance was saturated through 0-12 hr. 14C-Triclopyr was primarily excreted unchanged in the urine (85%), although partial metabolism was noted. Aside from the initial saturation of plasma clearance of triclopyr at 60 mg/kg, there were no appreciable differences in the absorption, disposition, or metabolism, based on sex, or prior exposure.

*Trademark of The Dow Chemical Company.

Triclopyr, the active ingredient in GARLON® brand herbicide, was given as a single oral dose of 0.1 and 0.5 mg/kg to 6 male volunteers and as a single 3.7 mg/kg dermal dose to 5 volunteers. Triclopyr was given orally as the acid dissolved in apple juice. The dermal dose was applied to the forearm as GARLON® 4, a formulation containing 482 grams/liter acid equivalent of the butoxyethyl ester of triclopyr. From the amounts of triclopyr found in the blood and urine, orally administered triclopyr was rapidly absorbed (t½ = 8 min) and over 80% of both oral doses was excreted unchanged in the urine with an average half-life of 5.0 hr. By comparison, triclopyr was slowly absorbed through the skin (t½ = 16.8 hr) and from the levels of triclopyr found in the blood and urine only 1.65% of the triclopyr applied to the forearm was absorbed. These data demonstrate that triclopyr is rapidly excreted and thus has little potential to accumulate during prolonged or repeated exposures. In addition, since triclopyr is poorly absorbed through skin, it is unlikely that acutely toxic quantities will be absorbed by this route.

*Trademark of The Dow Chemical Co.

PHARMACOKINETIC STUDIES ON METHYL TERTIARY BUTYL ETHER (MTEB) IN RATS. A E Chin, J P Matlock, D R Peterson, and L D Twitty. Exxon Biomedical Sciences, E Millstone, NJ. Sponsor: R A Scala

MTEB is a gasoline octane enhancer that has been approved by the EPA for use in motor fuel at concentrations up to 11 percent. The relative uptake and elimination of MTEB were studied in male Fischer 344 rats following oral gavage at 40 mg/kg and intravenous administration at 10 and 40 mg/kg. The oral bioavailability of MTEB was approximately 37 percent. MTEB was observed to distribute rapidly and extensively outside the blood compartment. Terminal blood half-lives ranged from 56 to 133 min. A rapid elimination of MTEB in expired air occurred with greater than 40 percent of the administered dose eliminated within 90 min following oral dosing. The elimination of t-butyl alcohol in expired air indicated a rapid metabolism of MTEB to this alcohol. A time-dependent decline in MTEB concentration in perirenal fat was observed indicating a redistribution of MTEB out of the fat compartment. These findings clearly distinguish the "applied" and "systemic" dose of MTEB, an important consideration in the hazard assessment of MTEB.

DISPOSITION AND METABOLISM OF 14C-THIOPHENE IN MALE FISCHER-344 RATS FOLLOWING NOSE-ONLY INHALATION EXPOSURE. A A Romeir, P M Markham and M Chadwick. Arthur D. Little, Inc., Cambridge, MA.

Following nose-only inhalation as a vapor for 1 hr at ca. 8000 ppm, 16.3% (493 umol) of the inhaled thiophene was retained in the body of the rat. Within 72 hr following exposure, 99% of the retained 14C were excreted, 73% was in the expired air (identified as chloroform), 25% was in urine, 0.6% was in feces, 0.8% was in the cage wash, while 1% (5.1 umol) remained in the tissues. Elimination of thiophene equivalents from plasma was monophasic with a half-time of 3.6 hr, while elimination from blood cells was biphasic with half-times of 2.9 hr and 9.1 days. The blood/plasma concentration ratios of thiophene derived radioactivity ranged from 3 to 13, with the higher ratio observed at the later time point. At 72 hr after exposure blood cells contained the highest concentration of thiophene equivalents (320 nmol/g), approximately four fold higher than liver (75 nmol/g). Kidney, heart, and lung contained similar but lower concentrations than liver (30-55 nmol/g), while brain, fat and skeletal muscle contained the lowest concentrations (11-17 mmol/g). This study indicates a significant amount of the inhaled thiophene is absorbed from the respiratory tract. (Supported by the Contract NOI-ES-66138).

TOXICOKinetics of 14C RDX (CYCLOTriMethylene-triNITRAMINE) IN RATS AFTER INTRATRAChEAL ADMINISTRATION. C Reddy, R Eisenhut, S A Morrison, and J A Kelly. US Army Biomedical Research and Development Laboratory, Fort Detrick, Frederick, MD.

Toxicokinetic studies on the explosive RDX were conducted to develop environmental and health effects criteria. The absorption, distribution and elimination of 14C RDX were studied in rats following intratracheal administration. Rats were treated with 14C RDX (15 mg/kg, 5-6 µCi) in 1% carboxymethylcellulose suspension and placed in glass metabolism cages. Urine and feces were collected at 6-12 hr and 24 hr intervals, respectively, and radioactivity determined in a liquid scintillation counter. About 10% of the radioactivity appeared in the urine in 24 hr. In the first 4 and 6 days, respectively, females and males had eliminated 23% and 26% of the radioactive label via urine; in the same period, excretion via the feces was 3% and 5%, respectively. After sacrifice of the rats at 4 or 6 days, the plasma levels of radioactivity were 0.02%/ml. The radioactive residues were 0.15%/g in liver, kidneys and lung, whereas brain and adipose tissue showed only 0.02%/g. The results indicate that a sizable fraction of RDX is eliminated in the urine, although considerable radioactive residues were detected in the liver, kidney and lung tissues after 4-6 days.
THE PHARMACOKINETICS OF TRIETHANOLAMINE IN C3H/HeJ MICE AND FISCHER 344 RATS FOLLOWING DERMAL ADMINISTRATION. J. M. Waeckerl, Jr. and D. L. Rick, Mammalian & Environmental Toxicology Research Laboratory, The Dow Chemical Company, Midland, MI. Sponsor: A. M. Schumann

14C-Triethanolamine (TEA) was administered to C3H/HeJ mice intravenously (1 mg/kg; in water) or dermally, (1000 mg/kg; in acetone; 2000 mg/kg; undiluted or in water) and to Fischer 344 rats dermally (1000 mg/kg; undiluted). TEA was extensively and rapidly absorbed following dermal application to C3H/HeJ mice. The data suggest much slower rate of dermal absorption of TEA for rats. Radioactivity was rapidly eliminated from the blood of mice by an apparent first-order process with a half-life ranging from 10 to 18 hours. About 65% of the administered dose of TEA was excreted unchanged in the urine of mice, with about 25% excreted via the feces. The pharmacokinetic fate of TEA in mice did not appear to be markedly dose-dependent or route dependent in the range of doses administered, nor did the use of water as a vehicle markedly change the dermal absorption in mice. Thus, the absence of toxicity to the liver and kidney in recent dermal subchronic toxicology studies of TEA in mice was not due to a lack of systemic availability of TEA following dermal administration.

DOSE-DEPENDENT URINARY EXCRETION OF ACRYLONITRILE (ACN) METABOLITES IN F-344 RATS AND B6C3F1 MICE. G. L. Kedderis, S. D. Held, R. Batra, M. J. Turner Jr., and A. E. Roberts, Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

Although ACN is carcinogenic in rats, the dose-dependence of ACN metabolism has not been reported for any species. Therefore, groups of three male rats and five male mice were given [2,3-14C] ACN p.o. at 1, 2, 4, 10, or 28 (rats only) mg/kg and urine was collected for 24 h. Forty to 70% of the 1 mg/kg dose and 80% to 100% of the higher doses were excreted in the urine of both species. Reversed phase HPLC analysis indicated four major components (A through D in order of elution) which accounted for 85% to 90% of the total urinary radioactivity of both species. Component C was isolated and identified as N-acetyl-S-(2-cyanoethyl) cysteine by mass spectrometry. This compound and component D were the major metabolites in rat urine. In mouse urine, each metabolite was present in similar quantities. The excretion of each metabolite was a linear function of dose in both species: saturation of metabolism was not observed. The apparent rate constants for the formation and excretion of A and B were 2 and 4 times greater in the mouse than in the rat, respectively, while those for C and D were similar. These data demonstrate quantitative species differences in the formation and excretion of ACN metabolites.

COMPARATIVE METABOLISM AND DISPOSITION OF 1,2,3-TRICHLOROPROPANE (TCP) IN RATS AND MICE. N. A. Mahmood and L. T. Burke, NIH, RTP, NC. Sponsor: H. B. Matthews

TCP has been used as a solvent and degreasing agent and as an intermediate in pesticide manufacture. In the NTP chronic toxicity study, F344 rats developed tongue and skin tumors; B6C3F1 mice were found to have an increased incidence of liver and stomach tumors. The present study was undertaken to investigate the metabolic basis for these differences. Following an acute oral exposure to male and female F344 rats and male B6C3F1 mice, TCP was rapidly absorbed, metabolized and excreted. The major route of excretion of TCP was in the urine. By 60 hr postdosing rats had excreted 50% and mice 65% of the administered dose by this route. Exhalation as 14C carbon dioxide and excretion in the feces accounted for 20 and 20% of the total dose in 60 hr in rats and 20 and 15% in mice. No apparent sex-related difference was observed in the ability of the rats to excrete TCP. At 60 hr, TCP-derived radioactivity was most concentrated in the liver, kidney and forestomach in both rats and male mice. Two urinary metabolites were isolated and identified by NMR, mass spectroscopy and comparison with the synthetic standards, as N-acetyl- and S-(3-chloro-2-hydroxypropyl) cysteine. The metabolites were present in the urine of both rats and B6C3F1 mice indicating the role of glutathione in the biotransformation of TCP.

BIO DISTRIBUTION OF 2-14C METHYL 2,3-14C ACRYLONITRILE (MeAN) IN RATS. M. Y. H. Faroogui, R. Cavazos, M. I. Villarreal, E. M. S. and A. Castillo, Div. of Environ. Toxicol. Dept. of Biology, Pan American University, Edinburg, TX.

MeAN is a constituent of plastic elastomers and coatings. Previous studies in our laboratory have shown that it is a potent neurotoxin, is metabolized to cyanide and causes significant depletion of tissue glutathione in various organs of rat. It has also caused abortions and damage to reproductive organs in female rats. In this study we have studied the biological fate of MeAN in male Sprague Dawley rats. The rats were given an oral dose of 100 mg/kg (0.5 ID50, 8 uC/rat) and the urine, feces, expired air, blood and all other organs were collected at different time intervals for up to 5 days post administration. Approximately 43% of the administered dose was excreted in the urine, 15% in the feces and only 2.5% via lungs as 14CO2. MeAN was extensively absorbed through the gastrointestinal tract and distributed in all the tissues of the rat. The major depots of radioactivity were the blood, liver, skin, fat, muscle and gastrointestinal tract with up to 25% of the administered dose at certain time periods. The retention of significant amount of radioactivity indicates extensive interaction with biological macromolecules and may be involved in the manifestation of its toxicity. (Supported by NIH S09RR08038).
DISPOSITION OF THREE GLYCOL ETHERS ADMINISTERED IN DRINKING WATER TO MALE F344/N RATS. M.A. Medinsky, C Singh, W E Bechtold, J A Bond, F J Sabouria, L S Birnbaum*, and R L Henderson. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM, and *NIHES, RTP, NC.

The glycol ethers, 2-methoxyethanol (ME), 2-ethoxyethanol (EE), and 2-butoxyethanol (BE) are widely used solvents in industrial and consumer applications. The reproductive, teratogenic, and hematotoxic effects of the glycol ethers are due to the glycolic acid metabolites of these compounds. The effect of alkyl group length on disposition of these three glycol ethers was studied in rats allowed access for 24 hrs to 2-butoxy[1-14C]ethanol, 2-ethoxy[1-14C]ethanol, and 2-methoxy[1-14C]ethanol in drinking water at three doses ranging from 40 to 310 umoles/rat. Elimination of radioactivity was monitored for 96 hrs. The majority of the 14C was excreted in urine or exhaled as CO2. Less than 1% of the dose was excreted as unmetabolized glycol ether. Distinct differences in the metabolism of the glycol ethers as a function of alkyl chain length were noted. For BE 72% of the dose was eliminated in the urine as butyric acid and 19% as CO2; for ME 52% was eliminated as ethanoic acid and 20% as CO2; for BE 34% was eliminated as ethanoic acid and 21% as CO2. Ethylene glycol, a previously reported metabolite of these glycol ethers, was excreted in urine, representing 45, 31 or 17% of the dose for ME, EE, and BE. Formation of ethylene glycol suggests that deylation of the glycol ethers occurs prior to oxidation to aliphatic acid and as such may represent a detoxification pathway. (Research supported by NIHES through Interagency Agreement ES2092 with U.S. DOE/GER Contract No. DE-AC04-767 EVEL2103.)

PHARMACOKINETICS, DISPOSITION AND METABOLISM OF ETHYLENE GLYCOL MONOETHYL ETHER (EGE) AFTER CUTANEOUS ADMINISTRATION TO FISHER 344 RATS. C B Jensen, S W Frantz, C M Grosse, J L Beckett, and B Ballantyne, Bushy Run Research Center/Union Carbide Corp., Export, PA.

The fate of EGE (CAS #112-25-4) was characterized after IV and cutaneous doses containing 14C-EGE were given to male Fisher 344 rats. Data from IV studies provided a plasma pharmacokinetic (PK) description, while percutaneous studies expanded the PK description and profiled EGE disposition. Intravenous doses of 25 and 2.5 mg/kg EGE showed very rapid distribution, a Vd of ca. 1.0 L., an elimination half-life of ca. 14 hrs, and no indication of capacity limitation of elimination processes. After cutaneous exposure to 25 mg/kg EGE, both sexes showed very rapid absorption of 14C-EGE, with apparent bioavailability of ca. 75%. The elimination half-times were comparable to those observed in the IV studies, with the majority of 14C recovered in urine, feces and by volatile traps; however, little 14C remained in selected organs or the carcass after 48 hrs. Greater than 95% of the 14C was recovered after cutaneous doses in both sexes. Chromatographic analysis showed that EGE, as opposed to total 14C, was rapidly cleared from plasma and only metabolites of EGE were found in urine. These results are consistent with a two-compartment PK model showing rapid first-order absorption and elimination processes. Copyright © 1988 Union Carbide Corp.

COMPARISON OF ETHYLENE GLYCOL PHARMACOKINETICS AND DISPOSITION BY THREE ROUTES IN SPRAIGE-DAWLEY RATS. J WFrantZ, C B Jensen, C M Grosse, M J Tallant, J L Beckett, and B Ballantyne, Bushy Run Research Center, Export, PA, and *Union Carbide Corporation, Danbury, CT.

The pharmacokinetics and material balance of [1,2-14C]-Ethylene Glycol (EG) were evaluated in Sprague-Dawley rats by the intravenous (IV), peroral (PO) and percutaneous (PC) routes following doses of 10 and 100 mg/kg; additional doses of 400, 600, and 800 mg/kg were evaluated for the peroral route. Exhaled CO2 was the major metabolite for all three routes at the 10 mg/kg dose, and urine the secondary elimination route. A dose-dependent shift in the routes of excretion was observed following IV and PO 1000 mg EG/kg, with urine being the major route of elimination. For the peroral route, 14CO2 exhalation was inversely related to increases in dose and urinary 14C output was directly related to dose. 14C accounted for the largest 14C recovery fraction following the PC route of dosing. The plasma pharmacokinetics were different for unchanged EG vs. total radioactivity for all three routes studied. Comparison of parameter values from both doses (10 and 100 mg/kg) for total clearance, apparent Vgs, terminal T1/2, and mean residence time demonstrated that a first-order plasma time-course exists for unchanged EG and total urinary EG was consistent with this first-order behavior. This plasma kinetic pattern is not consistent with excreta profiles, which were dose-dependent, particularly for urinary 14C glycolate. In summary, unchanged EG demonstrates an apparent first-order pharmacokinetic behavior by all three routes for disposition in and elimination from plasma, but dose-dependent changes occur in urine and 14CO2 excretion after single doses for the intravenous and oral but not percutaneous routes.


THE TOXICOKINETICS OF 1,3-BUTYLENE GLYCOL VERSUS ETHANOL IN THE TREATMENT OF ETHYLENE GLYCOL POISONING. SK Cox, E Ferslow, and L J Boelen. Section of Toxicology, Depts. of Pharm. and Path, East Tennessee State Univ. and Veterans Administration Medical Center, Johnson City, TN.

Ethylene glycol (EG) is a toxic chemical found in antifreeze and heat exchangers. Standard therapy for EG intoxication is administration of ethanol (EToH) to inhibit its metabolism by alcohol dehydrogenase (ADH). Studies indicate 1,3-butyleneglycol (BG) binds to ADH more efficiently than EG and is orally less toxic than EG or EToH. Male rats were divided into 3 groups of 6 animals. Groups received either: a single dose of EG; BG initially and every 6 hours up to 72 hours; EToH initially and every 6 hours up to 72 hours; or EG initially and then either BG or EToH every 6 hours up to 72 hours. EToH produced hepatotoxicity and pulmonary pathology as indicated by clinical chemistry, urinalysis, and histopathology, while EG did not. ETOH produced atria, lethargy and CNS depression while EG did not. Neither EToH nor BG produced any apparent nephrotoxicity. BG produced a higher concentration of urinary EG and a lower concentration of EG metabolites than ETOH, indicating a better inhibition of ADH metabolism of EG. Ethanol produced a higher EG blood concentration than BG, which may be attributed to dehydration and decreased urine output. The EG/EToH combination produced mortality quicker than either agent alone due to additive toxicity of the combination. Lack of any significant toxicity produced by BG and the production of significant toxicities by EToH indicates that BG is potentially a better antidote than ETOH.
This study and one on 2-CNB were initiated to aid in the design of toxicology studies under the NTP. 

\[ ^{14}C \] in 2-CNB was administered by gavage at 2, 20, or 200 mg/kg. Radioactivity was determined in urine and feces up to 72 hr and in tissues at 24 and 72 hr. Urine was analyzed by HPLC. At all dose levels, 45-74% of the dose was excreted in urine and 10-12% in feces. 

\[ ^{14}C \] was excreted more slowly at 200 mg/kg, such that 35% was in the 24-hr and 71% in the 72-hr tissues, versus 23% in the 24-hr and 5% in the 72-hr tissues at the lower doses. All tissues except fat, 4-CN equivalent concentrations were proportional to dose. At 200 mg/kg concentrations in fat were disproportionately higher. Unlike other tissues, concentrations in blood cells (BCs) and spleen were the same at 72 as at 24 hr. At 24 hr after all doses the highest concentrations were in fat, followed by BCs, kidney, liver, and spleen. At 72 hr they were in BCs followed by fat and spleen. At least 12 metabolites were in urine. In summary over the dose range the pattern of excretion and 4-CN equivalent concentrations in most tissues were linear. Rate of excretion and equivalent concentrations in fat were linear at 2 and 20 mg/kg, but not-linear at 200 mg/kg. (Supported by NEIHS Contract NO1-ES-66138).

This study and one on 4-CNB were initiated to aid in the design of toxicology studies under the NTP. 

\[ ^{14}C \] in 2-CNB was administered by gavage at 2, 20, or 200 mg/kg. Radioactivity was determined in urine and feces up to 72 hr and in tissues at 24 and 72 hr. Urine was analyzed by HPLC. At 2 and 20 mg/kg 60% of the dose was excreted in urine, 25-30% in feces, primarily during the first 24 hr, 6% was in 24-hr and 3% in 72-hr tissues. At 200 mg/kg 70% was in urine and only 7% in feces and it was excreted more slowly with 20% in 24-hr and 4% in 72-hr tissues. At 2 and 20 mg/kg 2-CN equivalent concentrations in tissues were proportional to dose. At 200 mg/kg they were disproportionately higher in all tissues, especially fat, and disproportionately lower in liver. At all doses the highest concentrations were in liver and kidney and at 200 mg/kg in fat. Up to 23 metabolites were in urine. At the lower doses there was a major metabolite XXI, 75-80% of the dose, and a less major, XIX, 8%, others were 3% or less. 200 mg/kg, XXI, again 25%, was excreted more slowly, XIX, 4%, decreased and XI increased to 23%. In summary, pattern and rate of excretion, relative amounts of urinary metabolites and concentrations in tissues were linear at 2 and 20 mg/kg, but non-linear at 200 mg/kg. (Supported by NEIHS Contract NO1-ES-66138).

Elimination and brain distribution of 

\[ ^{14}C \] TRIS(2-CHLOROPHENYL)PHOSPHATE in RATS. 

D W Herr and W B Matthews. NEIHS, Research Triangle Park, NC.

Tris(2-chlorophenyl)phosphate (TCP) is a flame retardant which produces a dose-related hippocampal lesion in female rats. Experiments were performed to determine the fate of TCP in rats with particular emphasis on its regional distribution in brain following single or repeated doses. TCP was administered by gavage (0, 175, 350, or 700 mg/kg) to female Fischer 344/N rats. Urine, feces, exhaled volatiles, CO, and selected tissues were collected. Regional brain distribution of TCP was determined 2, 24, and 72 hr after a single dose, and 24 hr after 14 daily doses of TCP. Data indicate TCP is absorbed from the gut, distributed to brain regions, and that excretion is near complete in 72 hr. Most of the TCP-derived radioactivity was excreted in urine (up to 85%) with feces, volatiles and CO2 combined accounting for less than 10% of the dose. Two major metabolites were observed in urine. Predominant signs of toxicity associated with TCP administration (350 and 700 mg/kg) were seizures within 2 hr of dosing, and only TCP was extractable from cortical and liver tissues. Traces of inextractable TCP were detected at later times, but had no particular affinity for brain. No regional brain distribution of TCP was observed in any experiment, indicating that the hippocampal lesion is due to factor(s) other than selective accumulation of TCP by this brain region.

In a chronic toxicity study on TRCP under the NTP, lesions were observed in the hippocampus of the brain of Fischer-344 rats. One objective of our study was to determine whether there was localization of TRCP-derived TCP in the hippocampus. TCP was administered by gavage at 88 mg/kg. Plasma, blood cell and excretory kinetics of TCP were determined radiochemically and tissue distribution of TCP was determined by whole-body autoradiography. In 72 hr 90% of the dose was excreted in urine, primarily in first 24 hr, 7% in feces and 1% as TCP. TRCP was well absorbed. Peak concentrations of TRCP equivalents in plasma and blood cells were at 0-3 hr. TCP was similar to total body water volume. Elimination from plasma and blood cells was biphasic, initial t1/2s 3.4 and 3.0 hr, and terminal t1/2s 1.8 and 10.8 days, respectively. At 0.5 and 4 hr TRCP-derived TCP was evenly distributed throughout most tissues including brain, higher concentrations were found in liver, kidney, fat, and in the GI contents. At 72 hr concentrations in liver, kidney, and fat were again higher and there was retention of radioactivity in blood cells, GI mucosa, skin, bone marrow, spleen, thymus and salivary glands. In addition there was evidence for possible retention of TCP in regions of the brain. (Supported by NEIHS W61-ES-66138).
345 IN VIVO METABOLISM OF CIS AND TRANS 3,7-DIMETHYL-2,6-OCYDIOINOL (CITRAL) IN RATS. J J Biliberto, P Grinivas, L T Butka, L S Birnbaum. NIEHS, Research Triangle Park, NC.

Citril belongs to the aliphatic aldehydes of the terpenes series. It is the main component (~80%) of lemon grass oil, found in all citrus fruits, and used extensively in the food, cosmetic, and detergent industries. In this study, metabolism of citral by male F344 rats was studied. For metabolite identification, urine was collected over dry ice for 24 h after a single po 500 mg/kg dose of 14C-citral. Elimination in urine was rapid with 30% of the dose excreted within 24 h. The urine was fractionated using a C-18 Sep-Pak followed by reverse phase HPLC. Both the radioactivity and UV spectra were monitored. The chromatographic profile of the urine showed -10 peaks. Synthetic standards were prepared as follows: oxidation by Soret for the stereospecific oxidation of citral and citronellal at the C-8 position, by MnO, (for the dialdehydic compounds), and by NaClO4 to obtain cis and trans 3,7-dimethyl-2,6-octadienoic acids and 3,7-dimethyl-6- octenedioic acid.

3,7-Dimethyl-2,6-octadienoic acid was made by oxidation of citral with NaClO4-DSMO. Four urinary metabolites compared well, by both their HPLC retention times and UV spectra, with these standards. Separation and identification of these metabolites are in progress. Glucuronic and sulfonic conjugates were prepared, as well as several unidentified polar metabolites. Citral appears to undergo further carbon loss by both β- and ω-oxidation.

346 THE METABOLISM AND NEPHROTOXICITY OF INDIAN IN FISCHER 344 RATS. M P Serac*, M A Ferry, G McDonald, K O Yu, C T Olson* and D W Hobson*.

Department of Chemistry, Wright State University, Dayton, OH and N.C. Armstrong Aerospace Medical Research Laboratory, Wright-Patterson AFB, OH.*

The Qc cyclic hydrocarbon Indian, when administered by gavage to Fischer 344 male rats over a 14 day period yielded only moderate bile acid droplet formation in kidney proximal tubules. The renal damage produced by Indian was less than the nephrotoxicity induced by other fused ring systems.


Research sponsored by U.S. Air Force Grant No. AFOSR 87-0108.


The metabolic fate and disposition of 14C-ETH were studied following oral, 96 hr occluded dermal or iv administration. Pharmacokinetic parameters were derived for the total 14C in blood. After iv dosing (1 mg/kg), the blood 14C concentration declined. After oral dosing of 0.1 mg/kg, the mean peak blood level of 14C was 85.1 µg equiv EHA/g was reached at 15 or 30 min. After dermal application of 0.1 mg/kg, the mean peak blood level of 8.5 µg equiv EHA/g was attained at 3.7 hr. The bioavailability of 14C after dermal application was 60-70% that after iv dosing. The terminal half-lives of 14C after iv, oral and dermal administration were 1.7 hr, 9 hr and 32 hr, respectively. 14C was eliminated in the urine and feces, primarily by 24 hr after dosing. At the 0.1 and 1 mg/kg single oral dose levels, 79.3% and 82.3%, respectively of the 14C was excreted in the urine and 12.4% and 6.7%, respectively, was excreted in the feces. After repeated oral dosing at 0.1 mg/kg, 60.6% of the 14C was excreted in the urine and 14.9% was excreted in the feces. After dermal application of 0.1 mg/kg, 41.7% and 60.6%, respectively, of the 14C was excreted in the urine and 7.5% and 7.1%, respectively, was excreted in the feces. Whereas recovery was 80.2% after prompt washing of the skin, after iv dosing, 66.6% of the 14C was excreted in the urine and 3.6% was excreted in the feces. The major urinary metabolites of EHA were the glucuronide of EHA-2-ethylhexanoic acid, isomers of hydroxy-2-EHA and two isomeric N-AcEHA metabolites, proposed to be lactones. Evidence for the metabolism of EHA via 8-oxidation was also found, consistent with the incorporation of 14C into 14C labeled cellular metabolism. (Supported by the Chemical Manuf. Assoc. EHA Program Panel).

348 BODY DISTRIBUTION, BIOTRANSFORMATION AND EXCRETION OF 4-NITRO-N-METHYL-PHTHALIMIDE (4-NPI) IN RATS. J J Coffey, W F Mueller, Toxicology Program New Mexico State University, Las Cruces, NM and L W Smith, G E Plastics, Pittsfield, MA

4C-labeled 4-NPI was studied in rats after single oral administration of 25 mg/kg. Within 48 hours after dosing, 33 - 79% of the dose was excreted in urine and 13 - 20% in feces. 1.2 - 2.3% of the dose remained in the body. This confirmed results of earlier studies using 1 and 100 mg/kg of 4-NPI in rats (Toxicologist 6, 252, 1986). Current studies show highest concentrations in the contents of the large intestines and cecum, and in liver, adrenal and kidneys. The lowest levels were measured in brain, spleen, adipose tissue and the reproductive organs. No significant differences were seen in body distribution or elimination between male and female rats. Radio-HPLC of urine samples showed that 14C is extensively metabolized; at least 10 different biotransformation products were found, ranging in polarity from slightly more polar than the parent compound to highly polar, probably conjugated metabolites.
The enterohepatic circulation of T-2 toxin or its metabolites may be responsible for the protracted signs following exposure. Bile duct-cannulated, male rats were administered [3H] T-2 toxin intraduodenally (id). The rats eliminated 44.65% (4 hr) and 57.25% (8 hr) of the administered dose in the bile. TLC profiles of the T-2 metabolites were similar after iv and id administration. The major metabolites detected were 3-OH HT-2, glucuronide conjugates (GCon), TOL, 4-DN and HT-2. Tritium-labeled GCon obtained from the bile of rats administered [3H] T-2 toxin iv were extracted and purified using C-18 and silica column chromatography. Enzymatic deconjugation studies and TLC indicated that the GCon were composed of conjugates of 3-OH HT-2, HT-2, 4-DN, and TOL. Following id administration of the GCon, the rats eliminated 6.01% (4 hr) and 11.86% (8 hr) of the dose in the bile. No free metabolites of T-2 toxin were detected in the bile of any animals administered the purified conjugates. The β-glucuronidase inhibitor, saccharic acid lactone, did not have a significant effect on biliary excretion. These studies substantiate the enterohepatic circulation of T-2 toxin metabolites in the rat.

AGE RELATED CHANGES IN DISPOSITION OF BENZYL ACETATE (BA); A MODEL COMPOUND FOR GLYCINE CONJUGATION. S F Mithen, J J Diliberto, and L S Birnbaum. NIEHS, RTP, NC.

BA, a rodent carcinogen, was used to assess age-related changes in glycine conjugation. Male Fischer 344 rats aged 4, 9, and 25 mo were administered 5 mg/kg BA. Urine, feces, and bile were collected for 96 hr. Plasma levels of BA metabolites were also investigated. The glycine conjugate hippuric acid (HA) was the major urinary metabolite detected by HPLC with minor amounts of benzyl mercapturic acid (BMA) also present. Minor differences were observed with age in urinary elimination of BA-derived radioactivity (RA) and RA formation. BMA excretion was significantly greater in 25 mo rats than in 3 mo rats. Fecal and biliary excretion of BA-derived RA declined significantly in 25 mo rats. A significantly higher plasma level of HA and benzyl glucuronide was found in 25 vs. 3 mo rats. Similar experiments in C57BL/6N mice revealed no changes with age except for a significant decline in 24 hr urinary excretion and an increase in fecal excretion in 25 mo mice. The results of this study indicate that changes in minor routes of BA excretion and metabolism occur with age. However, aging has no significant effect on glycine conjugation in rats or mice as measured by formation of HA from BA.

F, released during metabolism of several anesthetics, causes nephrotoxicity at high serum concentrations. Following anesthesia, the significantly higher serum F concentrations of obese compared to non-obese subjects could result from either increased F production via metabolism or decreased F elimination. This study was designed to determine if F disposition and elimination are altered in a rat model of human hypertrophic obesity. F disposition was examined in diet-induced obese rats (483, 596, 691 g) and non-obese rats (380, 402, 430 g). Arterial blood was withdrawn post injection at: 0.5, 1, 2, 4, 6, 9, 13, 20, 30, 45, 75, 120, 180, 240, 300, 480 and 1400 min. An array of pharmacokinetic parameters of serum F disposition was determined. Serum F disposition curves for the two groups were superimposable and the area under concentration-time curves were similar. The steady state volume of distribution was significantly decreased in obese rats (6083 ± 1726 vs 17962 ± 3875 ml/kg; P<0.05). F clearance was not significantly altered (35.3 ± 4.5 vs 45.0 ± 3.7 ml/min/kg). For obese rats, the 50% reduction in terminal half-life (191.2 ± 56.1 vs 367.7 ± 133.6 min) and a 45% decrease in F mean residence time (182.3 ± 78.2 vs 408.6 ± 112.3 min) were not statistically different. Thus, nephrotoxicity in obese rats does not appear to result from inherently prolonged F elimination.

METABOLISM OF 14C-FURAN IN THE MALE FISCHER 344 RAT. K D Washburn, L T Burk, and V C Dauterman. Toxicology Program, North Carolina State University, Raleigh, NC and "NIEMS, Research Triangle Park, NC.

Furan is a toxic five-membered oxygen containing heterocycle with limited aromaticity. Substituted furans are found in nature and in the anthropogenic environment. The furan ring of many substituted furans appears to be important for toxicity. To investigate the mechanism of toxic action of the furan ring, male Fischer 344 rats were dosed with 14C-Furan orally (8 or 100 mg/kg) for the collection of urinary metabolites or intravenously (8, 25 or 50 mg/kg) for the collection of biliary metabolites. High pressure liquid chromatography analysis of urine and bile from treated animals showed the presence of at least five radiolabeled metabolites. Treatment of bile samples with gamma-glutamyl transpeptidase suggested the presence of a glutathione conjugate. Bile from animals treated with diethyl maleate prior to 14C-furan dosing further supported the presence of a glutathione conjugate. The presence of butyraldehyde semicarbazone was evidence for oxidation of furan in vitro. Urinary and biliary metabolites were purified by preparative HPLC and partially characterized by 1H nmr.

Chemically tritiated microcystin-LR ([3H]MCYST-LR, sp. act. 194 mCi/mmol), a cyclic hepatotoxin produced by Microcystis aeruginosa, was purified >95% by C-18 reverse-phase HPLC. [3H]MCYST-LR exhibited the same HPLC and UV absorption profile as unlabeled toxin. Acid-hydrolyzed [3H]toxin yielded tritiated glutamate and 3-methylaspartate by HPLC analysis. Stability of the radiolabeled toxin in urine and saline was >95% after 30 days stored at either 23°, 4°, or -20°C. There was a temperature- and time-dependent breakdown of toxin in blood (63% at 23°C, 30 days). The LC50 (mice IP) of [3H]MCYST-LR and unlabeled toxin were the same (75 µg/kg (65-90) and 65 µg/kg (53-80), respectively). Tissue distribution, determined either at death or 6 hr post-injection, was similar for all doses (3-15 µg/kg). At the 101 µg/kg dose, liver contained 56±1%, intestine 7±1%, kidney 0.9±0.2%, and cecum 0.01±0.01%. Heart, spleen, lung, and skeletal muscle contained <1% of the radiolabel. The [3H]MCYST-LR studied in this investigation had the same toxicity and physiochemical properties as unlabeled toxin, making it an excellent probe for studying the mechanism of action and metabolic fate of microcystin.


Rat hepatocytes are highly sensitive to the necrogenic effects of aflatoxin B1 (AFB1) but toxicity is independent of reduced thiol status. N-acetyl cysteine (4mM) or diethylmaleate (160µM) had negligible influences on the lethal effects of AFB1. We assayed the protective activity of GSTs against DNA binding by 3H-AFB1 activated in vitro by rat hepatocyte microsomes. Dialedyzed cytosol and S-hexyl-glutathione-affinity-purified GSTs from normal F344 rat liver had limited inhibitory activity against AFB1-DNA binding in this system. Dialedyzed cytosol from carcinogen-generated liver nodules and carcinomas from F344 rats had increased inhibitory activity on AFB1-DNA binding but this protective effect was also observed in the absence of added GST. Pured GSTs from rat liver nodules and GST-P, the GST isoenzyme most induced in carcinogen-generated rat liver neoplasms, had little effect on AFB1-DNA binding. At equivalent concentrations of CDNB-conjugating GST activity, cytosol and purified GSTs from mouse liver greatly reduced AFB1 binding to DNA. This supports the hypothesis that F344 rat hepatocytes are more sensitive to the genotoxic effects of AFB1 because their constitutive GSTs have a relatively low affinity for AFB1 intermediates.

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THE USE OF RABBITS IN MALE REPRODUCTIVE TOXICOLOGY STUDIES. J Williams, P E Chapin, J L Phelps and B C Gladen. Developmental and Reproductive Toxicology, NTP, NIEHS, RTP, NC.

We are interested in evaluating the rabbit as an animal model for human exposure to male reproductive toxicants. The rabbit has the advantage of being the smallest lab species from which semen can be collected longitudinally. In addition, fertility can be evaluated using artificial insemination. As part of our evaluation we assessed the variability of some physical and biochemical semen parameters (including sperm number, fructose, citric acid and acid phosphatase), and its influence on reproductive study design. The most variable parameter measured in single weekly ejaculates was sperm number. However collecting 4 ejaculates per male each week reduced the cv within buck from 71% to 23%. In addition, introducing a 6 week pre-treatment collection period for all groups into the study design (15 weeks exposure) increased the power of detecting a 20% change in sperm number from 28% to 81%. We also determined that one million motile sperm was the minimum number of sperm that produced litters (6.95 ± 0.71 kits/litter, mean ± sem n=10) that were not different from those produced with excess sperm (8.86 ± 1.02 n=16). Hence, it will be possible to measure seminal characteristics and the fertilizing capacity of sperm from toxicant treated males. Ethylene dibromide, which alters human semen characteristics, is being evaluated in this model.


Rat hepatocytes are highly sensitive to the necrogenic effects of aflatoxin B1 (AFB1) but toxicity is independent of reduced thiol status. N-acetyl cysteine (4mM) or diethylmaleate (160µM) had negligible influences on the lethal effects of AFB1. We assayed the protective activity of GSTs against DNA binding by 3H-AFB1 activated in vitro by rat hepatocyte microsomes. Dialedyzed cytosol and S-hexyl-glutathione-affinity-purified GSTs from normal F344 rat liver had limited inhibitory activity against AFB1-DNA binding in this system. Dialedyzed cytosol from carcinogen-generated liver nodules and carcinomas from F344 rats had increased inhibitory activity on AFB1-DNA binding but this protective effect was also observed in the absence of added GST. Pured GSTs from rat liver nodules and GST-P, the GST isoenzyme most induced in carcinogen-generated rat liver neoplasms, had little effect on AFB1-DNA binding. At equivalent concentrations of CDNB-conjugating GST activity, cytosol and purified GSTs from mouse liver greatly reduced AFB1 binding to DNA. This supports the hypothesis that F344 rat hepatocytes are more sensitive to the genotoxic effects of AFB1 because their constitutive GSTs have a relatively low affinity for AFB1 intermediates.

Supported by NSERC (Canada), and MRC (Canada).

SUBLTE TOXICANT-INDUCED CHANGES IN RAT SPERM VELOCITY ARE DETECTED BY COMPUTER-ASSISTED MOTION ANALYSIS. V L Slott, J D Suareze1, L F Strader, R M Poss1, J E Simmons, R Linder, S W Penneault. Reproductive Toxicology Branch, NIEHS, USEPA and NSF1, RTP, NC.

The ability of computer-assisted motion analysis to detect toxicant-induced alterations in rat sperm motility parameters was evaluated using epichlorohydrin (EPI), a compound known to selectively affect sperm motility. Male F-344 rats were exposed to 100 ppm EPI via inhalation for 4 hours on day 0 and sacrificed immediately (n=12) and on days 1, 2 (n=12), 6 and 14 (n=6) postexposure. Videotapes of cauda epididymal sperm were analyzed (300-350 sperm/sample) with a Hamilton Thorn Motility Analyzer (HFM-2000, Hamilton Thorn Research, Danvers, MA) set to evaluate 13 frames at 19 frames/sec. EPI did not affect the percentage of motile sperm at any time. However, transient changes in sperm velocity were found: on day 0 path (curvilinear) velocity was significantly decreased to 92% of control, and on day 1 both path and straight line velocity were reduced to 76 and 83% of control, respectively. Sperm velocity was unaffected at later times. Other endpoints (testis and epididymal weights, testicular sperm head counts and cauda epididymal reserves) were unaltered by EPI. The HFM-2000 was able to detect relatively subtle, toxicant-induced changes in rat sperm motility.


**357 EFFECT OF A SINGLE DOSE OF 2,5-HEXADICLINE (2,5-HD) ON SPERM MORPHOLOGY AND MOTILITY IN THE RAT. L F Strader, R E Linder, and S D Perreault. Reprod Toxic Br, HERL/USEPA, RTP, NC. Sponsor: L F Gray, Jr.**

2,5-HD is a neurotoxicant and testicular toxicant thought to alter microtubule assembly. Adult male rats were gavaged with a single dose of 0 or 2,000 mg/kg of 2,5-HD and killed 2, 16, 22, or 28 days posttreatment for evaluation of testicular and epididymal effects. On d 16, 22, and 28, the percentage of misshapen sperm heads in the caput epididymis of treated rats was 41, 31, and 20% vs 3, 6, and 4% in controls. On d 28 the percentage of misshapen heads in the cauda was 48% vs 1% for controls; many sperm with this defect were actively motile. When sperm with misshapen heads were stained with a DNA specific fluorescent dye their nuclei appeared normal in shape. Therefore, the atypical head shape is probably due to changes in sperm head structures other than the nucleus. On d 28 the number of cauda sperm with flagellar defects was increased to 36% vs 1% for the controls. On d 22 and 28 the percent of cauda sperm with progressive motility was decreased to 35 and 25% of controls, and straight-line velocity was decreased to 34 and 38% of controls. By d 16, retained condensed spermatids were seen in the testis. Thus, a single subneurotoxic dose of 2,5-HD can produce changes in sperm morphology and motility that may alter fertilization potential.


Previously we have reported that paraxon (50 uL), inhibited fertilization of mouse gametes in vitro. Addition of acetylcholin to the medium decreased the inhibitory effect of paraxon. Using the chlorotetracycline fluorescence assay confirmed that paraxon inhibited sperm capacitation, and that in the absence of both paraxon and acetylcholine (50 uL), sperm capacitated as in controls. In this study, the possible involvement of cholinergic mechanisms in sperm capacitation or the acrosome reaction was examined by challenging the paraxon toxicity with triacetin (a substrate of cholinesterase), and carbacol (active in both the muscarinic and the nicotinic systems), and carbachol (active only in the muscarinic system). The profiles of the capacitated and uncapsulated sperm populations indicated that paraxon delays the progress from capacitated sperm to acrosome reacted sperm. This progress in both paraxon-triacetin and paraxon/bethanechol treated sperm populations was similar to that in the controls up to 95 min. In the paraxon/carbacol treated sperm, a sharp decrease in capacitated sperm population, from 63%±7 to 20%.±7, was observed between 55 and 75 min. Decreased sperm motility was also observed in this treatment group.


EGME is a known testicular toxin. Previous testis morphology studies showed dose-dependent decreased tubular diameter, presence of vacuoles in tubules, and decreased spermatids and spermatocytes. This study characterizes early morphological events of EGME testis toxicity. Male C57L mice received p.o. a single EGME dose from 100 to 500 mg/kg. At various times (6 to 168 hours) after dosing, the animals were injected i.p with heparin, perfused through the heart with saline, and fixed with glutaraldehyde. The testes were removed, embedded in epoxy, and sectioned on an ultramicrotome. Sections stained with Mallory's methylene blue were viewed under a light microscope. Spermatogonia were present at all time-points. All effects were dose-dependent. Beyond 48 hours post-dosing, effects included major disruption of tubule architecture, and spermatocyte damage and degeneration. The effects decreased in magnitude but were limited to stages with late spermatocytes at 24 hours post-dosing. A time-dependent testis damage was seen as early as 6 hours after a single dose. Future work aimed at identifying the initiating events of EGME testis toxicity should focus on a short post-dose time scale.

**360 COMPARATIVE TESTICULAR TOXICITY OF BIS(2-METHOXYETHYL) ETHER (DICYME) AND 2-METHOXYETHANOL (2-ME) IN RATS. S. Lee, L A Kinney, R Valentine, AND M. C. Carakostas E. I. du Pont de Nemours & Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, Newark, DE.**

Rats were exposed to 0, 110, 370, and 1100 ppm diglyme or 300 ppm 2-ME for 2 ws. Rats were killed after 2 ws exposure and 14, 42, or 84 days post-exposure (PE). Minimal to moderate testicular atrophy was found at 110 and 370 ppm diglyme. At 110 ppm diglyme, spermatocytes in phase of pachytene and melotic division at spermatogenic stages XII-XIV were mainly affected. At 370 ppm diglyme, affected germ cells were similar to those seen at 110 ppm diglyme, but round spermatids at stages I-VIII were also affected. The testes regained normal structure by 84 days PE. At 1100 ppm diglyme or 300 ppm 2-ME, marked testicular atrophy was observed but stage-specific damage was not discernible due to a wide range of cell types affected. Testicular atrophy seen at 300 ppm 2-ME was more severe than that seen at 370 ppm diglyme but less severe than 1100 ppm diglyme. The damaged seminiferous tubules were lined with regenerating pachytene spermatocytes at 14 days PE and spermatocytes with round spermatids by 42 days PE. Most but not all testes from 300 ppm 2-ME and 1100 ppm diglyme groups had normal morphology at 84 days PE. Reversibility of damaged germinal epithelium was inversely proportional to severity of damaged stem cells.

Previous work demonstrated that limiting mating of rats to one ejaculation increased sensitivity of fertility testing to the spermatotoxic effects of ethoxethanol (EE). The present study examined the dose-response effects of EE on measures of sperm production, sperm quality and fertility using single ejaculation mating. Male rats (12 weeks old) were treated for seven weeks p.o. with 0, 225, 300, 375, or 450 mg/kg/d EE. After receiving mating experience during weeks 5 and 6, males were mated to receptive, ovariectomized females during week 7 to recover ejaculates for examination. During week 8, each male was allowed a single ejaculation with a female in estrus and necropsied. Mated females were examined two weeks after mating. Significant dose-response effects were observed on tests and epididymis weights as well as all measures of sperm production. Cauda epididymal sperm number was most sensitive, showing significant reductions with 225 mg/kg/d. Number of morphologically normal sperm was reduced to 71, 35, 15 and 2% of control with increasing dose level. Fertility was affected significantly only at the highest dose (300 pregnant vs 86% for controls), a dose level at which no effect on fertility was seen previously with the standard breeding protocol.

363 POTENTIATION OF ETHYL METHANESULFONATE (EMS) DOMINANT LETALITY BY PHORONE DEPLETION OF GLUTATHIONE (GSH). H K Bates*, R D Habrison and I Candy. Pathology Associates, Inc./NCTR, Jefferson, AR and Division of Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR

Studies in our laboratory have shown that phorone (dilisopropylidene acetone) can deplete reproductive tract GSH. Phorone (250 mg/kg i.p.) depleted GSH in the liver, testis, caput and cauda epididymides to 6%, 61%, 14% and 9% of control values, respectively, by four hours after administration (n=4). EMS is a reactive electrophile known to undergo GSH-dependent metabolism and a well established dominant lethal mutagen. Phorone was tested for its ability to potentiate the dominant lethal effects of EMS. Male Sprague Dawley rats were exposed to either corn oil, phorone (250 mg/kg), EMS (50 mg/kg), or phorone (250 mg/kg) plus EMS (50 mg/kg) challenge four hours later. One and two weeks after exposure the males were bred to naive female rats which were sacrificed between 15 and 19 days of gestation. The females were examined for number of corpora lutea and fetal implantation status. No significant differences were observed in the pre- and post-implantation rates between the oil, phorone, and EMS controls. The combined phorone-EMS group caused 48% of the implants to die in utero during week 3 compared to 13%, 12% and 16% of the controls (Significant, p<0.05). Therefore, these results indicate that depletion of GSH pools prior to EMS administration potentiates EMS-induced dominant lethal mutations. (NIOSH Grant OH102258)

362 THE ENHANCEMENT OF GLUTATHIONE AND ITS PROTECTIVE ROLE IN GERM CELL MUTATIONS. L A Buchler, R D Habrison and J Candy. Division of Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR

It has been shown that depletion of glutathione in the male reproductive tract will cause potentiation of germ cell mutations on exposure to alkylating agents such as ethyl methanesulfonate (EMS). In order to determine the level of protection afforded by GSH, we evaluated various compounds for their ability to enhance male reproductive tissue GSH levels in vivo. The dominant lethal effects of EMS were then compared between male rats with normal or enhanced GSH tissue levels. Compounds evaluated for enhancing GSH included N-Acetyl Cysteine (NAC) (250 mg/kg, every 2 hrs), L-2-oxovalerioic acid (OCA) (1mM/kg), or AT-125 plus NAC (ANAC) (20 mg/kg AT-125 with 250 mg/kg NAC given every 2 hrs) in male Sprague Dawley rats, ip, followed by measurement of hepatic and reproductive tract GSH. Of the compounds tested, ANAC was the most efficacious. Increases in tissue GSH levels were liver-26.4%, caput-89.7%, cauda-33.6%, testis-10.4%. Therefore, normally mature Sprague Dawley rats were treated with ANAC and challenged with EMS (100 mg/kg) to determine if protection from mutation was possible in a dominant lethal assay. ANAC significantly decreased the germ cell mutations as determined by a decreased fetal resorption rate from 23.2% with EMS alone to 38.6% with the ANAC pretreatment. These results suggest that germ cell mutations may be minimized by enhancing the GSH level in the reproductive tract. (NIOSH Grant OH102258)

364 INDUCTION OF TESTICULAR TOXICITY IN RATS BY 23-2895: TIME COURSE OF MORPHOLOGIC CHANGES. T Borsowsky, A A Lavine, and S K Durham. Dept. of Toxicology and Pathology, Hoffmann-La Roche, Nutley, NJ. Sponsor: EA Pfizer

Subchronic administration of retinoids at high doses causes testicular degeneration in rodents, but no studies have detailed the morphologic progression of changes. Male CD rats, 8 weeks of age, were treated with 80 mg/kg/day of a synthetic retinoid, Ro 23-2895. Treated rats (n=3) were sacrificed at 3, 7, 11, and 21 days of dosing. Control rats (n=3) received the vehicle (Tween 80) and were sacrificed at 3 or 21 days. Thin plastic sections of formalin-fixed testes were stained with PAS and hematoxylin. Microscopic examination did not reveal any morphologic changes in any rats after 3 days. After 7 days, occasional tubules (stages I-VIII) had delayed release of mature sperm, often observed near the basement membrane undergoing absorption by Sertoli cell cytoplasm. The severity of these changes was increased after 11 days of treatment. Early stage tubules (stages I-III) showed mild numbers of mature spermatids, occasional round spermatids with clear-centered nuclei, and often disorganization of the germinal epithelium. After 21 days, testes weight was significantly reduced and the germinal epithelium showed marked degeneration up to complete desquamation. In tubules with less advanced degeneration, multinucleated giant cells, germ cells with clear-centered nuclei, and moderate to severe oligospermia were noted. Most degenerated tubules also had swollen Sertoli cell nuclei with luteinized, vesiculated nucleus. These changes were similar to those reported for vitamin A-deficient rats.
Gossypol decreases sperm production and motility in man and several animal species. Zinc and selenium are essential elements in the production of spermatozoa and are closely associated with sulfhydryl content. We have investigated the effect of gossypol on zinc, selenium and sulfhydryl content of the testes and spermatozoa. Mature male hamsters were treated and castrated orally with vehicle, 15 mg/kg or 30 mg/kg of gossypol daily for 3 weeks. Animals were euthanized on day 22, the testes and epididymides removed and weighed. Caudal spermatozoa(CSZ) were obtained, counted and evaluated for motility. Zinc and selenium content of a testes homogenate(TH) and CSZ were performed utilizing atomic absorption spectroscopy. Sulfhydryl content was determined in TH and SZ using 5,5'-dithio-bis(2-nitrobenzoic acid) and 14C-Jodacetamide methods, respectively. The body weight and sperm motility of both treated groups decreased compared to control. Sperm count was decreased only in the 30 mg/kg group. Selenium content in TH was decreased in both treated groups while selenium content in CSZ was increased only in the 30 mg/kg group. No significant changes in zinc and sulfhydryl content was observed either in TH or CSZ. These data demonstrate the ability of gossypol to alter the level of selenium in the testes and epididymal spermatozoa. This alteration may be related to the male antifertility effects of gossypol.

CO-OXYGENATION AS ALTERNATIVE PATHWAY TO CYTOCHROME P-450 FOR BENZ0(a)PYRENE ACTIVATION IN HUMAN TERM PLACENTAL MICROSOMES. J Z Byczkowski and A P Kulkarni. Florida Toxicology Research Center, College of Public Health, University of South Florida, Tampa, FL.

The link between lipid peroxidation and benzo(a)pyrene (BP) activation was studied in microsomes isolated from human term placenta. Lipid peroxidation was initiated in the presence of NADPH by potassium superoxide, paraquat, menadione and/or partially chelated iron. Covalently bound and soluble metabolites of BP or BP-7,8-diol were quantitated by radiometry and HPLC respectively. Peroxidative conditions increased the amounts of BP-trans-anti-tetrol from BP-diol, BP-diones from BP and protein-bound metabolites from both. Reactive oxo-complex of iron and superoxide is proposed as an ultimate species initiating lipid peroxidation. It is believed that the same complex catalyzes co-oxygenation of BP and BP-diol in placental microsomes under mild peroxidative conditions. The peroxidative reaction may represent the pathway for bioactivation of benzo(a)pyrene, alternative to cytochrome P-450.

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

PEROXIDASE: A POTENTIAL PATHWAY FOR XENOBIOTIC OXIDATION IN HUMAN TERM PLACENTA. D C Cook and A P Kulkarni, Florida Toxicology Research Center, College of Public Health, University of South Florida, Tampa, FL.

Peroxidase mediated generation of highly reactive xenobiotic radicals has been linked to target organ toxicity. In human term placenta, peroxidase activity was found to be associated with different membrane fractions and was extractable with 0.5 M Ca2+. In the presence of H2O2, the reaction exhibited dependency upon enzyme concentration, time and substrate concentration. A significant stimulation of peroxidase activity was observed in the presence of either Ca2+ or N-ethylmaleimide at pH 7.4. Epinephrine, 2-methoxyphenol, uric acid, indole-3-acetic acid, bilirubin, benzidine, pyrogallol p-phenylenediamine, tetramethylbenzidine, ABTS, dimethoxybenzidine, thiobenzamide, catechol and tetramethylphenylenediamine all appeared to be oxidized by the enzyme in the presence of H2O2. It appears from the results of this study that peroxidase may represent a alternative pathway to cytochrome P-450 for xenobiotic metabolism in human term placenta. Supported by Grant from The Council for Tobacco Research USA, Inc.

BIOTRANSFORMATION OF BENZO(a)PYRENE-7,8-DIOL IN RAT UTERUS IN VITRO. A P Kulkarni and J Z Byczkowski. Florida Toxicology Research Center, College of Public Health, University of South Florida, Tampa, FL.

The metabolic fate of fetotoxic chemicals of environmental origin in the mammalian uterus is largely unknown. In this study, the effects of NADPH and/or hydrogen peroxide on the metabolism of (+)-trans-7,8-dihydrobenz(a)pyrene-diol (BaP-7,8-diol) in the extracts of rat uteri were studied. Covalently bound and soluble metabolites of BaP-7,8-diol were quantitated by radiometry and HPLC, respectively. The addition of hydrogen peroxide to uterine extracts increased the generation of BaP-trans-anti-tetrol, BaP-dione, protein bound metabolites and three other products. Addition of NADPH to the incubation mixture containing H2O2 and uterine extract decreased the protein binding. Biotransformation by uterine peroxidase is proposed as pathway for activation of BaP-7,8-diol to dihydrodiol epoxide which binds to the macromolecules. In the presence of NADPH the epoxide may be reduced to the corresponding BaP-triols.
BIOTRANSFORMATION OF ETHYLENE DIBROMIDE (EDB) BY HUMAN FETAL LIVER AND TERM PLACENTAL GLUTATHIONE TRANSFERASE (GST). J K Edwards and A P Kulkarni. Florida Toxicology Research Center, College of Public Health, University of South Florida, Tampa, FL.

EDB is a mutagenic and carcinogenic compound used as a gasoline additive and soil fumigant. The conjugation of EDB with reduced glutathione (GSH) yields a reactive episulfonium ion that irreversibly binds to DNA. GST activity in fetal liver cytosol was tested with EDB as substrate. The average specific activity for 10 samples was found to be 3.07 nmoles/min/mg protein. The dependency of the conjugation on time and the concentrations of enzyme, EDB and GSH suggests an enzymatic nature of the reaction. Placental GST was purified by affinity chromatography and HPLC as reported previously (Thomas and Kulkarni: BBRC.128:75,1985). The specific activity of the purified placental GST with EDB as substrate ranged from 300-600 nmoles/min/mg protein. EDB appears to inhibit purified placental GST in a noncompetitive manner with respect to 1-chloro-2,4-dinitrobenzene (CDNB). Therefore, it appears that human fetal liver and placenta are capable of activating EDB to a mutagen.

USE OF IN VITRO FERTILIZATION METHODS TO ASSESS OOCYTE FUNCTION IN THE HAMSTER: EFFECT OF CARBENDAZIM (MBC) AND OTHER MICROTUBULE POISONS. S D Perreault, R R Barbee, and P M Poes. Reproductive Toxicology Branch, NIEHS, USEPA, and NSI, RTP, NC. Sponsor: L F Gray, Jr.

MBC is a systemic fungicide with antimitotic activity due to its tubulin-binding ability. We tested whether MBC might perturb meiotic events that depend on tubulin polymerization in hamster oocytes. Mature oocytes were fertilized in vitro and cultured for 3.5 hours in the presence of MBC or vehicle (0.5% DMSO). MBC had no effect on the percentage of oocytes penetrated by sperm. However, the incidence of normally fertilized oocytes (i.e. with 2nd polar body and two pronuclei) declined from 94% in controls to 79%, 38% and 9% in 0.8, 4, and 6µg/ml MBC. The most common abnormality was failure of 2nd polar body extrusion and fragmentation of the female pronucleus. Male pronucleus formation was unaffected. Two other tubulin-binding agents were tested in this system as positive controls. Podophyllotoxin (0.4µg/ml) produced female pronucleus fragmentation similar to that seen with MBC while colcemid (0.4µg/ml) not only prevented polar body extrusion, but also blocked formation of both pronuclei in fertilized eggs. These in vitro findings indicate that MBC may have the potential to interfere with meiotic events in vivo and thereby cause very early pregnancy loss.

CHANGES IN A FETAL LYMPHOCYTIC SURFACE ANTIGEN AFTER GESTATIONAL EXPOSURE TO DIETHYLSTILBESTROL (DES). P Lindstrom, C Comment, M Luster, R Morrison, NTP, NIEHS, Res Tri Fk, NC.

We hypothesized that changes in expression of fetal Thy 1,2 antigen (T cell differentiation antigen) after in utero exposure to immunotoxins might be a predictor of impaired immune function observed in the offspring. To test this hypothesis, DES (immunotoxicant in young adult mice exposed in utero) was injected daily s.c. (0.0.1, 1.0, or 10.0 µg/kg in corn oil) into dams from gestation day (gd) 6-14 or 10-14. Splenocytes were obtained from fetuses on gd 17. The avidin-biotin immunocytochemical technique (ICC) was applied to frozen tissue for visualization of Thy 1,2 antigen. Fluorescence activated cell sorting (FACS) was used to quantitate the concentration of cell surface Thy 1,2. While 10 µg/kg DES given from gd 6-14 was fetotoxic, mice receiving 1 µg/kg DES from either gd 6-14 or gd 10-14 had an increased number of spleen cells expressing Thy 1,2 antigen. Spleen morphology and distribution of cells carrying Thy 1,2 within the spleen were similar in all groups. No effects were observed in fetal thymus and maternal spleen and thymus in any dose group. In conclusion, treatment of the dam with 1 µg/kg DES from gd 6-14 or gd 10-14 modulated expression of Thy 1,2 antigen.

MONO(2-ETHYLHEXYL) PHTHALATE (MEHP) INHIBITION OF FSH-STIMULATED cAMP ACCUMULATION IN CULTURED GRANULOSA CELLS. K A Treinen, A James and J J Heindel, DEVELOPMENTAL AND REPRODUCTIVE TOXICOL. NTP/NIEHS RTP NC. Sponsor: L A Dostal.

MEHP is both a male and female reproductive toxicant as determined in the NTP Reproductive Assessment by Continuous Breeding protocol. In the male, MEHP has been shown in vivo and in vitro to be a Sertoli cell (SC) toxicant. In vitro MEHP inhibited FSH-stimulated cAMP accumulation in cultured SCs. This inhibition occurred after a 6 hr preincubation period, with maximal inhibition (50%) by 24 hrs. Half-maximal inhibition is seen at 12.15 µM of MEHP. Since MEHP is also a female reproductive toxicant, and granulosa cells are thought to be the female counterpart to SCs, we examined the effect of MEHP on FSH-stimulated cAMP accumulation in cultured granulosa cells (gcs). GCs were harvested by ovarian puncture of DES-primed immature (19-22 d) F-344 rats and 300,000 viable cells were plated onto fibronectin-coated plates. FSH, forskolin, and isoproterenol were shown to stimulate cAMP accumulation. MEHP inhibited gc cAMP accumulation in a dose- and time-dependent manner. Significant inhibition (30-58%) of gc cAMP accumulation occurred with 200 µM MEHP after a 15 hr exposure, with maximal inhibition at 30 hrs (48% at 100 µM, 78% at 200 µM). These results indicate that, like SCs, the FSH-stimulated cAMP accumulation in GCs is inhibited by MEHP which may play a role in its reproductive toxicity.
CHEMICALLY INDUCED GROWTH INHIBITION AND CELL CYCLE PERTURBATIONS IN CULTURES OF DIFFERENTIATING RODENT EMBRYONIC CELLS. P L Ribeiro and E M Faustman. Depts of Environ. Health and Pathology, Univ. of Washington, Seattle, WA.

Ethynitrosourea (ENU) is a proven animal teratogen, although the mechanism of its toxicity is unknown. The micromass rat embryo midbrain (CNS) and limb bud (LB) culture systems (O P Flint 1983) were applied in an effort to determine potential mechanisms by which ENU exerts its teratogenic effect. When cultured at high cell densities, both cell types undergo several rounds of replication while differentiating into discrete bodies of neuronal cells and chondrocytes, respectively. Differentiation was monitored after 5 days by staining with hematoxilin (CNS) and Alcian blue (LB). Our objectives were to 1) define the population kinetics of the micromass cultures (cell proliferation, cell cycle compartmentation and differentiation) and 2) determine how ENU disrupts the normal growth and differentiation of these cultures. Dose dependent decreases in cell attachment and viability were observed in the first 24 hours after ENU exposure. Exposed cultures also exhibited dose dependent growth inhibition as determined by cell counts. Flow cytometric cell cycle analysis of affected cultures revealed an accumulation of cells in the G1 phase. CNS cultures exhibited a greater sensitivity to ENU than LB. Our examinations suggest that the effects of ENU on cell differentiation are related to its early effects on cell attachment, cell cycling, and cell proliferation. Studies will be conducted to compare these effects to those of other structurally related N-nitroso compounds. (NIH ES-03157)

374 STRUCTURE-ACTIVITY RELATIONSHIPS OF T-2 TOXIN AND MAJOR METABOLITES USING EMBRYO CULTURE AND HYDRA AS INDICATORS OF DEVELOPMENTAL TOXICITY. K Mayura, M S Bean, E E Smith, B A Clement, and D Phillips. Texas A&M University, College Station, TX.

T-2 toxin (a trichothecene mycotoxin) has been implicated as a food and feedborne factor in disease in man and animals. The developmental toxicities of T-2 and major de-acetylated metabolites (HT-2 and T-2 tetraol) were evaluated using cultures of extrapolantly maintained whole rat embryos (WEC) and Hydra attenuata (HA). Results from the WEC assay indicated a reduction in yolksac diameter, crown-rump length, somite number count, and protein and DNA content with T-2 > HT-2 > T-2 tetraol; only HT-2 resulted in malformed embryos. The HA assay supported these findings; i.e., T-2 exhibited the greatest toxicity to ha. Results are in order by HT-2 and T-2 tetraol, respectively. HT-2 resulted in a higher index of developmental hazard (A/D ratio) than T-2 or T-2 tetraol. These results are in agreement with previous toxicity studies in vivo. Both WEC and HA confirm that T-2 is a more potent teratogen. The combination of WEC and HA may facilitate the prioritization of teratogenic mycotoxins and the delineation of structure-activity relationships (Supported by USAID CRSP 02-50305-2 and TAES H6215).

375 PRENATAL TOXICITY OF NAPHTHALENE MONITORED VIA PREIMPLANTATION RODENT EMBRYO CULTURE. J E Martin, P Iyer, and T R Irvin. Laboratory of Toxicology, Veterinary Anatomy Department, Texas A&M University, College Station, TX. Sponsor: A Ray.

We have characterized the in vitro developmental toxicity of napthalene via preimplantation mouse embryo culture. Whole mouse embryos were collected 72 hours after conception and cultured in serum-supplemented medium NCTC 109 (Gibco) for 96 hours. During the culture period, developmental effects examined for each agent included: embryo hatching, attachment, and development of one and two layer inner cell mass. Over a 10-fold dose range, napthalene alone exhibited minimal embryotoxicity; after culture supplementation with rodent hepatic S-9 fractions, dose dependent embryotoxicity and embryolethality were observed. These findings implicate napthalene as an important prenatal toxicant in complex mixtures of polycyclic aromatic hydrocarbons and support the application of this culture system for identifying principle toxic components of chemical mixtures suspected as prenatal and abortive agents.

376 PRENATAL TOXICITY OF LEAD MONITORED VIA PREIMPLANTATION RODENT EMBRYO CULTURE. L Kurosky, P Iyer, J E Martin, and T R Irvin. Laboratory of Toxicology, Veterinary Anatomy Department, Texas A&M University, College Station, TX. Sponsor: A Ray.

We have characterized the in vitro developmental toxicity of lead via preimplantation mouse embryo culture using lead acetate. Whole mouse embryos were collected 72 hours after conception and cultured in serum-supplemented medium NCTC 109 (Gibco) for 96 hours. During the culture period, developmental effects examined for each agent included: embryo hatching, attachment, and development of one and two layer inner cell mass. Dose dependent embryolethality and embryotoxicity was observed at exposure levels of 1.6 ppm (80μM) and 4.0 ppm (20μM). Attachment and trophoblastic outgrowth was retarded in both groups of embryos exposed to lead. The results implicate lead exposure as a possible cause of early embryonic mortality. The study suggests that the preimplantation culture system is an effective means of screening chemicals for prenatal toxicity.
TCDD is a by-product of herbicide and bactericide industry. Recent reports indicate concentrations of TCDD up to 2,200 parts TCDD per billion parts soil (2.2 μg/g soil) in residential, recreational and commercial areas. Cleft palates and kidney malformations have been reported in young delivered to mice dosed with TCDD during their pregnancy. The objective of this study was to investigate effects of TCDD in a mouse embryo culture system. Embryos (8.5 days of gestation) were incubated in buffered Dulbecco's Modified Eagles/Ham's F12 medium for 24 hours in the presence of 0 to 1 μg/ml TCDD. The embryos were evaluated for cranial facial anomalies, heart rate and crown-rump length. Embryos treated with 0.005 and 0.001 μg TCDD/ml appeared the same as control embryos. Concentrations of 0.05 μg and 0.01 μg TCDD/ml produced defects in the head fold and mandibular arch (1). Despite these defects, the embryos appeared healthy with a heart rate similar to control embryos. The addition of 1 μg and 0.1 μg TCDD/ml to the culture media was lethal to the embryos within minutes. These data demonstrate the ability of low levels of TCDD to cause cranial facial abnormalities in a mouse embryo culture system.


Early-life stages of fish and amphibians have been proposed as model systems for evaluating the teratogenicity and developmental toxicity of environmental contaminants. They have also been advocated for use in screening assays to identify and rank potential mammalian teratogens for further testing. This study summarizes the comparative developmental toxicity of a series of metals (As, B, Cd, Cu, Cr, Hg, Hg, Se, and Zn) to four fish species (channel catfish, goldfish, black bass, and rainbow trout) and three amphibians (leopard frog, Fowler's toad, and narrow-mouthed toad). Observed anomalies included defects of the head, jaws, and vertebral column, dwarfed bodies, partial twinning, absent or reduced eyes and fins, and edema among others. Developmental toxicity is compared using three or more indices: 1) a Teratogenicity Index (TI) where TI = EC50/EC50 (concentration producing 50% terata in survivors), 2) a threshold index (e.g. LC10/EC10), and 3) the slope generated when percentages of malformed embryos are plotted against metal concentrations, expressed as percentages of the concentration inducing 50% embryonic mortality (Teratology 35: 439-437). The use of these indices for ranking teratogens and identifying sensitive species appropriate for screening systems is discussed.


In view of the known teratogenic activity of a number of synthetic retinoids it was considered pertinent to investigate the potential teratogenic activity of a series of new synthetic retinoids at an early stage in their development. Compounds were initially selected for pharmacological activity using the F9 teratocarcinoma assay and topical rhino mouse comedolytic model. Teratogenic potentials of selected compounds were subsequently assessed using rat whole embryo culture assay. 9 1/2 day old embryos were explanted and incubated for 48 hours. Novel and reference retinoids were added directly to the culture media at various concentrations in the presence of an 59 metabolic activating system. The results indicated that the teratogenic potential of synthetic retinoids does not necessarily parallel the pharmacological activity. It has, therefore, been possible to select compounds for further investigation based upon high pharmacological activity and low teratogenic potential.


Methymercury (MeHg) induced cytotoxicity was investigated in suspension cultures of logarithmically growing murine erythroleukemic cells (MELC) by flow cytometry (FCM) and cytogenetic methodology. FCM parameters investigated included propidium iodide (PI) uptake (viability), 90° light scatter, fluorescein isothiocyanate (FITC) fluorescence (protein content), and cell cycle progression. At MeHg concentrations above 5 μM, MELC exhibit a dose-related increase in 90° scatter, decrease in volume and, ultimately, PI uptake. At/above 5 μM, MeHg perturbs the cell cycle in a dose-dependent manner. Treatment with Colcemid during the final 1 hr of 90° scatter, 90° light scatter, and FITC fluorescence measurements in a dose-dependent manner. These observations suggest fixation (protein denaturation, crosslinking, etc.) as a mode of MeHg toxicity. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)
METHYL MERCURY INCORPORATION INTO THE HAIR OF NEONATAL MICE. C Shi, A T Lane, and T W Clarkson. Environmental Health Sciences Center, School of Medicine and Dentistry, University of Rochester, Rochester, NY.

The incorporation of methymercury into hair of neonatal mice was investigated and compared with methymercury distributions in other organs. Newborn mice received i.p. injections of radiolabeled 203Hg-methylmercury. The dosing times were selected at stages of beginning, maximum, and minimum hair growth according to the growth cycle of mouse hair. Animals were sacrificed two days after the injection and the total radioactivity in the pelt, liver, kidney and brain was counted in a gamma-counter. Our results revealed that the amount of methylmercury found in the pelt was significantly higher than that in other organs during active hair growing period. The greatest incorporation into the pelt occurred when the hair growth was maximum. However, uptake into the pelt was dramatically decreased after the hair ceased to grow, while the liver and kidney burdens of methylmercury were elevated. These results suggest that growing hairs of neonatal mice concentrate methylmercury and constitute a major body pool of methylmercury. The switch to the resting phase of hair growth decreases methylmercury uptake by the hair and has significant impact on its distribution to other organs. Additional autoradiographic experiments with tritium labelled methylmercury demonstrated that methylmercury was concentrated in hair follicles in the skin. Within hair follicles and hair shafts, methylmercury was accumulated in areas that are rich in sulfhydryl groups. (Supported by NIEHS ES-07026, NIH ES-01247 and ES-01248).

POST-TRANSLATIONAL MODIFICATION OF NEURONAL MICROTUBULES ALTERS SUSCEPTIBILITY TO METHYL MERCURY. K R Reuhl, Neurotoxicology Lab, Rutgers University College of Pharmacy, Piscataway, NJ. Sponsor: H E Lowndes

The influence of post-translational modifications of microtubules (MTs) on susceptibility to disassembly by methylmercury (MM) was studied. Embryonal carcinoma cells (EC1 Line P19) were induced to differentiate into neurons by exposure for 5 d to 5 x 10^-7 M retinoic acid. Day 10 neurons were treated for 4 h with 3uM MM. MT stability was assessed by immunofluorescence using MAb to acetylated (AC), tyrosinated (TYR), and detyrosinated α-tubulin (GLU), as well as MAPs 1a, 1b, 2, and tau. MTs of neuronal perikarya stained primarily with TYR, and were highly sensitive to MM. Virtually all perikaryal Mts disappeared, with only a few GLU Mts remaining. Mts in neurites stained primarily with GLU and AC. MAb exposure to TYR Mts, while AC Mts remained relatively stable. MAP 1a, MAP 2 and tau co-localized with stable Mts. MT staining in neurites persisted even after disassembly of perikaryal Mts. Stable neurite Mts disappeared and neurites retracted with continued MM exposure. Post-translational modifications of α-tubulin and decoration of Mts by MAPs confers greater resistance to MM-induced disassembly. (Supported by ES-04976).

EFFECTS OF IN VITRO MERCURY ON RAT BRAIN MICROSONAL Mg2+ ATPase. B Rajanna, C S Chetty and S Rajanna. Selma University, Selma, AL. Sponsor: Prasada Rao S Kodavanti.

Mercuric Chloride (Hg) in micromolar concentrations inhibited Mg2+ dependent ATPase activity in rat brain microsomes. Hydrolysis of ATP with 15 and 50 ug of microsomal protein for 45 min without and with (2.0 x 10^-7/M) Hg showed linear rates for 15 - 20 min. Altered pH versus activity demonstrated comparable inhibitions by Hg in buffered (neutral or acidic > basic) pH ranges. Inhibition of enzyme activity by Hg was found to be greater at 37°C than at lower temperatures (37 > 27 and 32 > 17 and 22°C) suggesting positive correlation trend. Cationic-Substrate activation of Mg2+ ATPase in presence of Hg showed significant changes in the kinetic constants. Inhibition was partially restored by repeated washings. The results indicate that inhibition of microsomal Mg2+ ATPase by Hg was pH, temperature and incubation time dependent but independent of the enzyme concentration. (Supported by NIH/MBRS Grant #RR-08169-10).

METHYL MERCURY (MeHg) ALTERS IONIC SELECTIVITY OF SYNAPTOSOMAL Ca CHANNELS AND BLOCKS DIHYDROPYRIDINE (DHP)-SENSITIVE 45Ca INFLUX INTO PC12 CELLS. T J Shafer and W D Atchison. Michigan State Univ., E. Lansing, MI.

The ability of MeHg to alter neuronal Ca channel inactivation rate and ionic selectivity were assessed in rat forebrain synaptosomes, and the ability of MeHg to block DHP-sensitive Ca channels was examined using rat pheochromocytoma (PC12) cells. To measure the rate of Ca channel inactivation, synaptosomes were predepolarized in Ca-free, high K+ (77.5 mM) solution for 1-10s intervals prior to measuring Is of K+-induced 45Ca uptake. MeHg (100 μM) did not alter the rate of inactivation when compared to control; I50 for inactivation of 45Ca influx was 1-2s in both control and MeHg-treated synaptosomes. Effects of MeHg on ionic selectivity of Ca channels in synaptosomes were investigated by comparing entry of 45Ca, 85Sr, and 3H-Ba following 1s of depolarization in 77.5 mM K+ solution. Influx of each isotope was blocked in a dose-dependent manner by MeHg with estimated IC50 values of 75, 150 and 150 μM for 45Ca, 85Sr and 133Ba, respectively. In undifferentiated and nerve growth factor-differentiated PC12 cells, 45Ca uptake induced by 50 mM K+ was maximal after 2 min of depolarization and was blocked by the DHP nifedipine with an approximate IC50 of 5 mM. MeHg reduced 45Ca uptake with an estimated IC50 of 50 μM, and 125 μM MeHg reduced uptake by ~90%. These results indicate that MeHg: 1) alters synaptosomal Ca channel ionic selectivity but not inactivation rate and, 2) blocks Ca uptake via DHP-sensitive Ca channels. (Supported by NIH grant ES03295.)
Hg produces a concentration-dependent (2, 3, 5, 10 μM) increase in spontaneous DA release from rat brain striatal synaptosomes that is independent of extracellular Ca²⁺. Cobalt (2 mM) blocks spontaneous DA release by 2 μM Hg. Up to 5 μM Hg has no effect on high-K⁺-evoked DA release. Using the Ca²⁺-specific fluorescent probe Fura-2, resting Ca²⁺ in striatal synaptosomes in nominally Ca²⁺-free and 1.2 mM Ca²⁺-containing media was 176±5 nM and 474±2 nM respectively and was not significantly altered by 5-min exposures of up to 8 μM Hg. Hg (4 and 8 μM) increases MnCl₂ (Mn) quenching of extracellular fura-2 (p < 0.01). To determine if increases in Mn quenching were due to increases in fura-2 release from synaptosomes or increases in membrane permeability to Mn, synaptosomes were centrifuged (7000 x g; 2 min) after 5-min exposures to 1, 2, 4, or 8 μM Hg. The supernatant fura-2 signal increased as a function of [Hg] independent of extracellular Ca²⁺ (p < 0.001) and was not accompanied by an increase in LDH release. It is hypothesized that Hg acts directly on the synaptosomal plasma membrane to increase the release of fura-2 and DA. Supported by ES-03992.

The effect of potassium channel blocking agents in demyelinated CNS axons has not been investigated. Segmental demyelination, confirmed by electron microscopy, was produced using a developmental and continuous Pb exposure model (0.2% PbAc to dams and weanlings) resulting in blood lead values of 60 μg/dl at 21 and 90 days of age. Our in vivo studies examined the frequency-dependent effects of vehicle (lactated Ringer's: pH 7.40, 310 mM), 4-aminoypyridine (4-AP), and 4-AP + tetraethylammonium (TEA), injected into the optic tract (OT), on compound action potential (CAP) characteristics and excitability properties in fast (t1) and middle (t2) conducting OT axons following optic chiasm stimulation. Pb decreases conduction velocity and amplitude, and increases rise time, fall time, CAP duration, and chronaxie in both t1 and t2 axons. In t2 axons, Pb increases absolute and relative refractory periods, decreases frequency following and produces a subnormality. Compared to vehicle, 4-AP effects are generally larger in t2 than t1 axons. The effects of TEA are similar to those produced by 4-AP, however, with increasing stimulus frequency they are larger in t1 than t2. Thus, large and medium diameter OT axons exhibit a differential frequency-dependent sensitivity to 4-AP and TEA following Pb exposure. Supported by ES 03183 (DAF).

A mechanism underling pororheasias in methyl mercury (MeHg) poisoning was investigated by examining electrophysiological and morphological changes induced in dorsal root ganglion (DRG) neurons. In MeHg treated rats, Intracellular recordings from L4 DRG were obtained from greater than normal numbers of types A6 and A6 which had decreased conduction velocities but enhanced action potential amplitudes and overshoot. Those cells with an initial segment-soma inflexion had a prolonged conduction time but a significantly (p<0.05) lower threshold. Multiple discharge, usually characterized by triple spikes upon single stimulation, occurred in 23% treated animals compared to 6% in controls. Hyperpolarization eliminated the third spike, indicating a possible somal origin. Morphology at the LM and EM levels revealed early degeneration of DRG neurons. These results reveal that all DRG neuron types are affected to some extent in MeHg neuropathy, with a preferential involvement of larger cells. Increased excitability and frequent multiple firing in smaller neuron types may underlie the paresthesias in MeHg neuropathy. Supported by NS-23325.
A total of 52 monkeys (Macaca fascicularis) were dosed orally from birth with 1.5 mg/kg/day of lead on one of four dosing regimes (13 monkeys/group): Group 1, vehicle only; Group 2, lead from birth onward; Group 3, lead from birth to 400 days of age and vehicle thereafter; Group 4, vehicle from birth to 300 days of age and lead thereafter. This dosing regimen allowed evaluation of differential infant vulnerability as well as reversibility of the behavioral toxicity of lead. Blood lead concentrations averaged 3-6 μg/dl when monkeys were not being exposed to lead, and 32-35 μg/dl during lead exposure. When monkeys were 5-6 years old, they were tested on a series of non-spatial discrimination reversal tasks: form, form with irrelevant color cues, color with irrelevant form cues, and alternating form and color. Group 2 exhibited the greatest degree of impairment compared to controls. Group 4 also exhibited impaired performance, although less marked than that of Group 2. Group 3 was marginally impaired at most. These results confirm findings observed in other monkeys exposed continuously to lead, and suggest that while exposure beginning after infancy produces impairment, exposure during infancy as well exacerbates the effect.

**Microanalysis of drinking behavior following lithium challenge in two strains of rats postnatally exposed to lead.** P M Martin and R B Mallman, Biological Sciences Research Center, Departments of Psychiatry and Pharmacology, Chapel Hill, NC

We have previously shown that administration of lead acetate (200 mg/kg/day) to neonatal Long-Evans rat pups results in a permanent increase in lithium-induced polydipsia (LIP) during adulthood, although 24 hr water consumption of these animals did not differ from controls without lithium challenge (Science, 201, 637-639, 1978). These results have now been generalized to Sprague-Dawley rats, and a comparison has been made of the effects of lead on the drinking patterns of the two strains. Animals were given either lead or sodium acetate on days 2-9 of age, and water consumption and licking patterns were measured following ten days of lithium administration at 60 days of age. Lead exposure enhanced LIP in both strains. More importantly, however, we found that lead altered several components of the motor response of licking, for example, number of licks per bout, even in the absence of lithium challenge. These data are consistent with the hypothesis that lead administered to developing rodents permanently perturbs dopaminergic neuronal systems. These alterations were seen primarily following lithium challenge in Sprague-Dawley rats, while effects of the same dose of lead were not found independently of lithium treatment in Long-Evans rats, possibly reflecting strain differences in lead sensitivity. (Supported, in part, by Grants ES01104 and ES07126).

**Lead-induced alteration of retinal mitochondrial respiration mediated by calcium?** C J Medrano, M J Weil, S D Rubinstein and D A Fox. U. Houston, Coll. Optometry, Houston, TX.

Developmental Pb exposure produces long-term selective rod deficits and degeneration (Exp. Eye Res. 46: 597-611; 613-625, 1988). To determine the mechanism of action, we are examining the effects of Pb on retinal metabolism since Pb exposure produces an inhibition of oxygen uptake in isolated whole rat retinas (SOT, 1987). The present study, using ion:EGTA buffers, examined the effects of Pb and Ca on mitochondrial respiration (State 4 and 3: S4 and S3; S3/S4 ratio: respiratory control ratio or RCR; ADP/O) and on the activity of activated pyruvate dehydrogenase (PDH), one of three Ca-sensitive intramitochondrial enzymes that regulate oxidative metabolism. Preliminary studies reveal doses below 10 nM of Pb and Ca slightly increase S3 and PDH activity. At 20-500 nM Pb, S4, S3, RCR, ADP/O and PDH are decreased in a dose-response manner. At 20-500 nM Ca, mitochondrial respiration also shows dose-response inhibition while PDH activity reaches its peak. Above 5 μM Ca, PDH activity is decreased. Our preliminary findings reveal the Pb-induced pattern of respiratory stimulation and inhibition in retinal mitochondria is paralleled by similar changes in PDH activity and both these changes are reproduced by Ca. The inhibition of respiration by Pb may contribute to the rod deficits and degeneration and both may be mediated by Ca. Supported by ES 03183 (DAF).

**Lead-induced aggregation of α2u-globulin in vitro.** G E Duval, D A Jett, D A Fowler. University of Maryland Toxology Program and Department of Pathology, University of Maryland School of Medicine, Baltimore, MD.

Examination of kidneys from lead-poisoned male rats has shown the presence of lead-protein aggregates as inclusion bodies (Chole and Richter: Science 177:1194, 1972). Recently, α2u-globulin (α2u) has been demonstrated to be the highest affinity soluble low molecular weight lead-binding protein in male rat kidney (Duval et al.: submitted for publication). Reported here are gel filtration and Western blot experiments which demonstrate an aggregation of α2u in both male rat kidney cytosol and purified solutions of α2u that have been incubated with lead acetate. The higher molecular weight aggregates are not dissociated by 5 minute incubations in 1% SDS at 90°C prior to SDS polyacrylamide gel electrophoresis. With purified solutions of α2u, a 50,000 dalton aggregate was detected after treatment with lead by Western blot analysis. This form corresponds to the molecular weight of a lead-binding protein complex previously identified in 203Pb-labeled male rat kidney cytosol (Oskarsson et al.: BBRC 104:290, 1982). In lead-treated cytosol, a series of higher molecular weight bands were recognized by anti-α2u to purified α2u which were not observed in either purified α2u solutions, or kidney cytosol that were not treated with lead.
INDUCTION OF STRESS PROTEINS BY GALLIUM, IODIDE AND ARSENITE IN CULTURED RAT KIDNEY CELLS. Y. Aoki, M. M. Lipsky, and B. A. Fowler. University of Maryland Toxicology Program and Department of Pathology, University of Maryland School of Medicine, Baltimore, Md.

In order to develop new biological indicators for assessing exposure to chemicals used in the manufacture of III-V semiconductor devices, we observed the induction of cellular markers following exposure to GaCl₃, InCl₃ and Na³⁺. After incubation in Earle's balanced salt solution (EBSS) containing these chemicals for 20 hrs, de novo synthesized proteins were labeled with 35S-methionine for 1 hr. Treatment with 35S-labeled proteins were detected by fluorography. Exposure to GaCl₃ (100-300 μM) caused induction of a 30K Dalton (Kd) protein and two 51Kd proteins. Increased synthesis of several 25-30K proteins and a 72Kd protein was also observed. Similar results were obtained by exposure to 3-10 μM Na³⁺. No significant changes in synthesis patterns were produced by InCl₃ treatment. Activity of lactate dehydrogenase (LDH) in the EBSS was examined as an indicator of overt cell injury, and did not significantly increase after GaCl₃ exposure. The induction patterns of these proteins may be useful as early indicators of Ga exposure before cell injury.


Children with elevations of blood Pb (BHP 25-55 μg/dl) account for most patients with Pb toxicity. Because the majority of the body burden of Pb is in the skeleton, direct measurement of cortical bone may provide a more exact index for treatment. LXRF was used to measure Pb in XRF from the tibias of 51 untreated Pb-toxic children. 51 children (<7 years) were followed for 6 months. LXRF was performed sequentially at enrollment and 6 wk. LXRF was performed sequentially at each time point. Group 1 (n=25): BHP (μg/dl): enr 29; 6 wk 25; 6 mos 22. LXRF (net counts): enr 123; 6 wk 127; 6 mos 115. Group 2 (n=14): BHP: enr 39; 6 wk 28; 6 mos 26. LXRF (net counts): enr 202; 6 wk 157; 6 mos 151. Group 3 (n=12): BHP: enr 42; 6 wk 29; 6 mos 25. LXRF (net counts): enr 234; 6 wk 160; 6 mos 122. LXRF measurements were higher in children with the greatest Pb burdens, who required multiple courses of chelation treatment. This was accompanied by a marked decline in the mean LXRF net counts. We conclude that serial LXRF measurements may provide an effective technique to determine the persistence of skeletal Pb burdens. Moreover, LXRF may provide a quantitative assessment of the effectiveness of reducing the body burden of Pb after intervention with CaNa₂EDTA. (Group 1: no Rx; Group 2: Rx X 1; Group 3: Rx X 2.)


Flyash from MSW incineration escapes into the atmosphere. Due to high temperatures within the incinerator, the ash becomes coated with high concentrations of cadmium and organic compounds. The potential inhalation or ingestion could result in acute and/or chronic cadmium and lead poisoning. Experiments measuring the bioavailability of cadmium and lead from flyash in laboratory rats have been carried out to produce dose-response relationships. Grab samples of incinerator flyash were taken from two incinerators, separated according to particle size and then ground to less than three microns. Gavage was employed to determine absorption from the gastrointestinal tract based upon particle size. Bioavailability was quantified by kidney levels of cadmium and lead. Dose-response of lead seems to indicate a linear correlation with approximately 1% of extractable lead (which is 5% of total lead) being absorbed. (Funded by the NJ CTR for Haz. Substance Management).


Lead (Pb) has been shown to interfere with cellular Ca homeostasis and Ca mediated cell processes. The present studies have lead to the hypothesis that Pb toxicity is mediated through Pb-induced alterations in intracellular Ca ion concentrations ([Ca²⁺]ᵢ). [Ca²⁺]ᵢ was measured in the osteoblastic bone cell line, ROS 17/2.8, using the intracellular divalent cation indicator, 1,2-bis(2-amino-5-fluorophenoxo)ethane-N,N,N',N'-tetracetic acid (SF-BAPTA) and fluo-19 nuclear magnetic resonance spectroscopy (¹⁹F NMR). This method provides for the simultaneous identification and measurement of [Ca²⁺]ᵢ and a variety of heavy metals including [Pb²⁺]. Using this method, the [Ca²⁺]ᵢ of untreated ROS 17/2.8 cells was measured to be 128 ± 6 nM (mean ± SD). Treatment with Pb (500μM) produced a nearly two fold increase in [Ca²⁺]ᵢ within the first half hour and a prompt return to pretreatment levels. Treatment with Pb (500μM) produced a 50% increase in [Ca²⁺]ᵢ without a return to basal levels. Treatment with Pb beginning 1 hour before addition of PTH inhibited the PTH-induced elevation of [Ca²⁺]ᵢ. These findings demonstrate that Pb alters [Ca²⁺]ᵢ and inhibits changes in [Ca²⁺]ᵢ in response to hormonal stimulation by PTH.
EFFECT OF LEAD TOXICITY ON HEPATOCYTOULAR IRON HOMEOSTASIS J.G. Pounds and G.J. Long. Brookhaven National Laboratory, Upton, NY.

The liver is the organ responsible for most aspects of iron metabolism and storage. Lead intoxication has diverse and profound effects on the utilization of iron, mainly through effects on the biosynthesis and degradation of heme. The impaired utilization of iron ultimately results in altered cellular energetics, altered steroid metabolism, and electron transport in lead intoxicated tissues. To more fully understand the nature of Pb$^{2+}$ - Fe interactions, experiments were conducted to define the effects of lead intoxication on the cellular kinetics of $^{55}$Fe.

Primary cultures of rat hepatocytes were labeled with 1 μCi/ml $^{55}$Fe (as 30 μM FeCl$_3$) in the presence of 0, 3, 15, or 50 μM lead acetate. After 20 hours the efflux of $^{55}$Fe was monitored for 3.5 hr and the pool sizes, rate constants, fluxes and half-times describing the steady-state metabolism of Fe obtained from the washout curves. Three kinetic pools of intracellular Fe totaling 18 nmol/mg cell protein were observed. 5% of intracellular Fe was in a rapidly exchanging pool with a half-time of <5 min; 20% was with a half-time of 50 min, and 75% exchanged with a half-time of 2000 min. Total hepatocellular Fe, and all three kinetic pools were reduced in size by exposure to lead concentrations as low as 3 μM. Thus, Fe homeostasis is very sensitive to lead exposure. The relationship of these kinetic pools to cellular heme, ferritin, and iron proteins remains to be determined. (Supported by NIH ES04040).

COMPARATIVE EFFECTS OF LEAD ON GROWTH IN RATS. J. Hamilton and E.J. O’Flaherty. Environmental Health, Univ. of Cincinnati, Cincinnati, OH.

Lead is a developmental toxicant that affects the growth of young animals and children. The purpose of this study was to examine the effects of lead on postnatal growth in rats. Female weanling rats received 0, 250, or 1000 ppm lead in drinking water for 7 weeks. The control rats were pair-fed, and food consumption, tail length and body weight were measured daily. The rats (dams) were mated after 7 weeks of treatment. At birth, half of the control dams were given lead-treated water (250 or 1000 ppm), and half of the lead-treated dams were removed from lead treatment. Lead had no effect on body weight and tail length gains in the dams prior to mating in this pair-fed control design. Pup birth weight, body calcium and phosphorus content at birth were also unaffected by lead. Dam and pup serum calcium and phosphorus levels were reduced in the 1000 ppm prenatal-postnatal exposure groups. Serum alkaline phosphatase was reduced in all groups exposed to lead during lactation. Suckling and weanling pup body weights were decreased in the 250 and 1000 ppm prenatal-postnatal exposure groups and in the 1000 ppm postnatal exposure group. Histomorphometric studies are under way to determine possible effects of lead exposure on the tibial epiphyseal growth plate of the dams and the weanling pups. (Project supported by NIOSH OH02376-02).

LEAD ALTERED LIPID METABOLISM IN BONE MARROW DERIVED MACРОPHAGES. M. Kowolenko and D.A. Lawrence, Dept. Microbiology and Immunology, Albany Medical College, Albany, N. Y.

Our laboratory has recently demonstrated that bone marrow cell (BMC) and macrophage (MO) responsiveness to CSF-1 is inhibited in the presence of Pb. Known modulators of CSF-1 responsiveness are PGE$_2$, which inhibits colony formation and LTC$_4$ and LTD$_4$, which enhance colony formation. These compounds are derived primarily from phospholipids which in turn may serve as a site of Pb binding. Experiments performed with $^{3}H$-acetate loaded MO indicate that Pb (100 nM) enhanced radiolabel incorporation into the chloroform:methanol extracted lipids. LPS-and CSF-1 induced a 183% and 75% increase in $^{3}H$-lipids/phospholipids, respectively, and this lipid biosynthesis was further enhanced by Pb (15% and 70%, respectively). In addition, the 22% inhibition by 100 nM Pb of colony formation of soft agar cultures was reversed partially, by NDGA (9% inhibition). Indomethacin treatment demonstrated no effect on the number of colonies formed in the presence of Pb but did increase colony formation of control cultures. The data indicates that Pb may exert its effect, in part, by modulation of phospholipid metabolism. Supported by NIH Grant ES03179 and NRSF Fellowship ES05416.

DIETARY LEAD ALTERS HEPATIC AND SERUM FATTY ACID COMPOSITION IN CHICKS IN A DOSE-RESPONSE MANNER. L.J. Schmidt and W.E. Donaldson. N.C. State Univ., Raleigh, NC.

Previous work showed that dietary lead (Pb) levels from 500 to 4000 ppm increase relative arachidonic acid (20:4) concentration and decrease the relative proportion of linoleic acid (18:2) to arachidonic acid (18:2/20:4) in chick blood and liver. The purpose of this study was to determine the lowest level of Pb at which these fatty acid alterations occur, and to examine the magnitude of these changes with increasing Pb levels. Additionally, we attempted to correlation between Pb-induced changes with enhanced lipid peroxidation. Chicks were fed diets containing 0, 62.5, 125, 250, 500 or 1000 ppm Pb from day old to 21 days of age. No growth effects were observed; however, Pb lowered the 18:2/20:4 ratio and increased 20:4 concentration in liver and serum in a dose-response manner. Hepatic mitochondrial membrane fatty acids were not altered, nor was there any increase in hepatic lipid peroxidation. Fatty acid chain length and unsaturation are important determinants of membrane fluidity. Changes in these parameters could have profound effects on membrane integrity and function, and thus affect growth. If the latter is correct the data suggest that alteration of fatty acid composition is a more sensitive measure of Pb toxicity than is growth rate.
PEROXIDE FORMATION AND LEAD TOXICITY. W. E. Donaldson, N.C. State Univ., Raleigh, NC.

Lead (Pb) alters membranes in animals, and part of the alteration is ascribed to lipid peroxidation in tissues. However, Pb may also stimulate peroxide (Perox) formation in unsaturated fats in feed. To study the latter effect, an experiment was conducted in which dietary variables of Pb, O vs. 1 vs. 0.03 per cent of fish oil (FO); and antioxidant (Ethoxyquin, EQ) 0 vs. 75 per cent were used in a 2x2 factorial. Diets were stored at 20°C and fed to 4 groups of 10 chicks/treatment for 18 days. Body weight (g) and PX in oil extracted from feed (mg/kg) are shown, PX in ( ). Dietary Pb

- Pb + Pb
- Eq 533(114) 213(341) 407(348) 300(517)
+Eq 520(112) 456(108) 576(57) 443(109)

Decreased growth in all diet combinations and increased PX values above control in all combinations without Eq. Two comparisons suggest an effect of Pb unrelated to Pb formation in feed. First (FO+Pb vs. FO+Pb = Eq E45 diets, growth was lower with CSO + Pb than with FO -Pb despite similar PX values. Second, although PX was the same as CSO control with CSO+Pb=Eq, growth was depressed. Eq addition prevented the increase in PX and partially reversed the growth depression by Pb which suggests that Pb formation in feed plays a role in Pb toxicity.

ERYTHROCYTE PYRIMIDINE 5'-NUCLEOTIDASE LEAD RESISTANCE. C Konantakieti, F. C. Beuthin and R. L. Louis-Ferdinand. Department of Pharmaceutical Sciences, Wayne State University Detroit, MI.

Erythrocyte Pyrimidine 5'-Nucleotidase activity (PSN) is related to blood lead (Pb) levels in acute Pb exposure (Konantakieti, et al., 1986). However after Pb acetate (5-10 mg/kg, i.p.) for 20 days PSN was not related to blood Pb. Acute Pb exposure reduced PSN maximum velocity (Vmax) (23.8±2.5 umol p1/gm Mb/hr) compared with controls (36.02±3.2) but the PSN Vmax (32.22±2.9) of chronic Pb exposed rats was not different (P<0.05) from controls. Acute 24 hr Pb acetate (300 mg/kg, ip) produced similar plasma Pb (0.94±0.2 ug/dl) compared with chronic Pb exposed animals (0.82±0.1 ug/dl). However blood Pb (40.8±3.2 ug/gm Mb) after chronic Pb treatment was 14 times greater than that of rats exposed acutely. Erythrocyte glutathione (GSH) (244.9±12 ug/gm) after acute Pb exposure was not elevated whereas GSH of chronic Pb exposed animals (450.5±16.2) was 33% higher (P<0.05) than controls. The observed differences in PSN inhibition are attributable to GSH dependent erythrocyte Pb sequestration.

(Supported by USPHS Grant ES01638)

EFFECTS OF LEAD ON HEME BIOSYNTHESIS DURING ERYTHROID DIFFERENTIATION IN VITRO. W. W. Ku, D Sloweyko, L. Bestervelt and W. R. Pinder. Toxicology Program, Dep. of Env. and Ind. Health, University of Michigan, Ann Arbor, MI.

The biosynthesis of heme in bone marrow following lead exposure and its relationship to the development of anemia has not adequately studied. Marine erythroleukemia cells (MELC) are erythroid precursor cells which undergo erythroid differentiation in the presence of the inducer hexamethylene-bisacetamide (HMBA). The effects of lead on heme biosynthesis in MELC following HMBA-induced differentiation was studied. MELC were induced with HMBA in the presence of 20, 40, and 80 uM lead acetate. Cell density, heme content, and the Activities of the enzymes delta-aminolevulinic acid dehydratase (ALA-D), porphobilinogenase (PBGase), and ferrochelatase (Ferro) were determined following induction. MELC exposed to 80 uM lead showed significant erythroid hypoplasia (40-50%) and a significant decrease (30-50%) in heme content at 2, 4, and 6 days post-induction compared to controls. Significant inhibition of ALA-D, the most sensitive index, was noted at 20 uM lead, and showed a 60-70% depression at 80 uM. PBGase and Ferro showed significant decreases only at 80 uM lead. The results suggest that the impairment of heme formation by lead is coincident with the production of severe erythroid hypoplasia. Furthermore, this system should be useful for studying the role of hormonal and nutritional factors in the development of lead or drug-associated anemia. (Supported by ES-07426 and ES-07602)

METHO-2,3-DIMERCAPROUSYXOLIC ACID (DMSA) IN HUMAN BLOOD. G M Malorino, J M Akins, and H V Apshian. Dep. Med. Molecular & Cellular Biology, University of Arizona, Tucson, AZ.

DMSA is an orally useful drug for the treatment of chronic lead intoxication. Very little is known about its absorption and distribution. A fasted, normal male human was given 10 mg (0.05 emmol) DMSA/Kg p. Blood from the left forearm vein was collected in heparinized vacutainers over a 4 hr period. Unlabeled DMSA was not found in whole blood or plasma at any of the time points. Only after reduction with thioglycosidol (TDT) was DMSA found. DMSA is in disulfide linkage. The blood and plasma concentrations of altered DMSA were the same. This suggests that DMSA was confined to the plasma and did not penetrate red blood cells. After TDT reduction the predominant endogenous thiol found in plasma was cysteine. This suggests that DMSA is converted to DMSA by disulfides with cysteine. Four hr after DMSA administration, 3.5% of the dose was found in the blood as altered DMSA. The results of a further study with 3 male volunteers will be reported. The methodology used was as follows: Whole blood and plasma samples were treated with bromobimane followed by ultrafiltration to remove protein. The samples were also treated with TDT to reduce disulfides followed by ultrafiltration and bromobimane treatment. The 5,6-dibromo derivative of DMSA was then determined by HPLC separation and fluorescence detection. (Supported in part by ES03356).

A decrement in body weight gain is frequently observed in classic subchronic toxicity studies and is often used as the criteria in establishing a ceiling on dose levels. Oishi et al. (Toxicol & App. Pharmacol. 57(1), 15-22, 1979) reported that food restriction altered common toxicity parameters in rats. Body weight gains, organ weights, and some clinical chemistry values decreased in diet restricted animals. Since behavioral observations are a part of subchronic testing in rats, it was proposed to evaluate the effects of food restriction on the FOB and motor activity. Baseline measurements were obtained prior to placing female rats on a 2-week food restriction diet (5 and 10 gms/day). Rats receiving 10 g of food per day lost weight (approximately 10 grams) during the first 5 days, then maintained their weight. Rats receiving 5 g of food per day lost weight (approximately 60 grams) over the 2-week period. No differences in motor activity measured as total counts or ambulatory counts (Oto-Varix) were recorded. Changes in FOB observed in rats receiving 5 g of food per day included hunched posture and walking on tip toes. No changes in FOB were observed in rats receiving 10 g of food per day.

DEVELOPMENT OF A MOTOR ACTIVITY TEST SYSTEM IN RODENTS FOR GUIDELINE NEUROTOXICITY SCREENING. J. P. Van Miller, M. W. Gill, and R. E. Wilson. Bushy Run Research Center, Export, PA.

The sensitivity and reliability of an automated motor activity test system were evaluated in Sprague-Dawley® rats placed in trios of each of thirty 16”x 9”x 7” cages on a standard 36-unit cage rack fitted with an array of 12 photocell beams designed to measure ambulation, fine movement, rearing, and total motor activity (San Diego Instruments). The effect of experience, diurnal cycle, sex, and chemical treatment on total activity and habituation rate was determined. In positive control experiments, male and female F-344 rats received (p.o.) d-amphetamine sulfate (AMP, 0.0, 0.5, 1.0, or 5.0 mg/kg) or chlorpromazine HC1 (CPZ, 0.0, 1.0, 5.0, or 10.0 mg/kg) 1 hour prior to evaluation, and female Sprague-Dawley® rats received acrylamide (AC, 0.0, 5.0, 20.0, or 35.0 mg/kg/day) or iminodipropionitrile (IDP, 0, 50, 100, or 200 mg/kg/day) for 14 consecutive days prior to evaluation on day 15. Previous experience of animals to the test system (repeated tests over a 15 day period) and test sessions performed later in the day generally resulted in decreased total session activity and decreased latency to habituation. Positive controls produced treatment-related increases (AMP) or decreases (CPZ, AC, and IDP) in motor activity and latency to habituation. Females tended to be more sensitive to AMP, CPZ, experience, and time of day.

VALIDATION OF A FUNCTIONAL OBSERVATIONAL BATTERY FOR GUIDELINE NEUROTOXICITY SCREENING. M. W. Gill, R. E. Wilson, and J. P. Van Miller. Bushy Run Research Center, Export, PA.

A behavioral screening battery designed to detect gross alterations of central and peripheral nervous system function was evaluated in the rat. Female Sprague-Dawley® rats received either a single treatment (p.o.) with d-amphetamine (AMP) or chlorpromazine (CPZ) one hour prior to testing or 14 consecutive treatments with acrylamide (AC) or iminodipropionitrile (IDP) prior to testing on day 15. Dose-response relationships were used to evaluate the sensitivity of the test system following exposure to AMP at 0, 1, 5, or 10 mg/kg, CPZ at 0, 5, 7.5, 10, or 15 mg/kg, AC at 0, 5, 20, or 35 mg/kg/day, or IDP at 0, 50, 100, or 200 mg/kg/day. Alterations in multiple test parameters (7 to 13) were observed for each test material. Dose-related findings in these studies were consistent with the published literature. Agreement between observers was evaluated by comparing the concurrent, independent evaluations for 160 test animals. Observers agreed (>80% of the time) on 26 of the 27 test parameters (exception; respiratory pattern). The CPZ and IDP protocols were repeated after six months to evaluate the stability of the test system across time. The results of these repeated protocols demonstrated reliability across time. Based on these results, this test battery can be a sensitive and reliable method for detecting neurobehavioral deficits in the rat.
A neurotoxicological 'test battery' capable of detecting nervous system damage in animals acutely or chronically exposed to test substances has been developed which combines clinical, electrophysiological and neuropathological methods. The sensitivity of this approach has been examined using known neurotoxic chemicals, selected on the basis of their ability to produce damage in different parts of the central or peripheral nervous system. Hence, a peripheral neurotoxicant, 2,5-hexanediol (2,5-HD) and two central nervous system toxins, trimethyltin (TMT, forebrain lesion) and difluoroenzene (DNB, hind brain lesion) have been examined. Groups of young adult rats received oral doses of the chemicals (300mg/kg x 5 days/week x 8 weeks, 2,5-HD; 4mg/kg x 1 day/week x 4 weeks, TMT; 15mg/kg x 3 days/week x 3 weeks, DNB). The animals were then monitored using a functional observational battery, motor and sensory nerve conduction velocity measurement and neuropathology. Results demonstrate that this combination of techniques can successfully identify specific functional and neuropathological deficits in both peripheral and central nervous system within the framework of short-term sub-acute studies.

Monkeys were divided into three groups and exposed to either 0.0, 0.15 or 0.35 mg/ml of caffeine in drinking water (equivalent to 0, 10 to 15 or 25 to 35 mg/kg/day of caffeine, respectively) prior to and throughout pregnancy. Dose-related reproductive failure in the form of stillbirths and miscarriages was observed (J. Pharmacol. Exp. Ther. 245, 1048-1053, 1988). The surviving infant monkeys were separated from their mothers at birth. Infants were trained to push a button for delivery of milk, and were then tested on a series of behavioral tasks. One of these, a non-spatial form discrimination reversal task, required the monkey to respond to a particular form irrespective of position. Once the task was learned, the incorrect and correct stimuli were reversed, for a total of 24 reversals. Impaired performance was observed in the treated groups. These results indicate that in utero caffeine exposure may adversely affect the performance of infant monkeys.

A positive control study was conducted as part of an ongoing validation program for behavioral teratology testing. Sprague-Dawley rats received 10mg/kg diazepam (DZ) or 20mg/kg methimazole (MET) by gavage from gestation Day 15 (DZ) or 17 (MET) through lactation Day 10. A group of control animals remained untreated. Offspring were assessed for growth and survival, developmental landmarks, and behavior. Both drugs effected change in the same direction for maternal body weight gain (suppressed), horizontal activity in an open field (increased) and rotorod performance (decreased, males only). In contrast, DZ and MET effected change in different directions for neonatal and postweaning body weight gain (suppressed, MET only), incisor eruption (delayed, MET only), acoustic startle reflex (DZ diminished, MET enhanced, the response), habituated acoustic startle (DZ animals demonstrated habituation, MET animals did not), cage emergence (DZ decreased, MET increased, emergence time), platform location in a water tank (DZ enhanced, MET diminished, learning) and active avoidance (MET enhanced avoidance responding). Results indicated that the test battery detected enhancement or suppression of behavior, and that DZ and MET were appropriate positive control agents.

The effects of lesions in nucleus basalis (NBM) or medial septal area (MSA) alone or in combination (COMB) on learning and memory are not well studied. Fischer-344 rats received 4 ug colchicine into the MSA, 1 ug colchicine bilaterally in the NBM or a COMB dose. Three weeks after surgery, locomotor activity was not affected by treatment. Four weeks after surgery, rats in the MSA, NBM and COMB groups were impaired in the acquisition and retention of a passive avoidance task, as measured by decreased latencies to crossover upon retest 48 hrs after training. Rats with COMB lesions were affected the most. Five weeks after surgery, a significant difference between treatment groups was found in acquisition in the Morris water maze. Mean total time per session was higher with NBM and COMB groups. In the free swim (no platform), only COMB group was different from control. Histological examination indicated lesion damage was limited to the NBM and MSA injection sites. These experiments indicate that colchicine-induced lesions to either the NBM or MSA results in deficits in behavior and COMB lesions can produce a greater effect on learning and retention than either lesion alone. (M.B. supported by T32 ESO7126).

The effects of high dosages of the organophosphate acetylcholinesterase (AChE) inhibitor paraoxon (P) on FR10 performance was determined at 1 and 2 days. Brain AChE inhibition was about 65 and 45% on these 2 days. While the centrally acting atropine sulfate (AS) yielded a small transient depression in performance, both non-centrally acting methyl atropines (bromide and nitrate) yielded severe performance deficits, with incomplete recovery on day 2. Performance was also depressed by a high sub-lethal dose of P. Performance was more greatly depressed at the lethal dose, with the greatest effects and the poorest recovery with the non-centrally acting antidotes. A low dose of AS with the lethal dose of P resulted in severe, non-recovering deficits. A lethal dose of the non-persistent anti-AChE eserine sulfate, antidoted with a low dose of AS, yielded no deficits. No alterations in striatal muscarinic receptors were noted at 2 days after any treatment. Thus a high level intoxication with the persistent anti-AChE P yields severe performance deficits which are attenuated by high levels of a centrally acting muscarinic antagonist, and whose recovery do not correlate directly with AChE inhibition or with receptor densities. (Supported by EPA R-881295).

THE EFFECT OF 1,1,1-TRICHLOROETHANE DELIVERED BY CERAMIC-GLASS RESERVOIR SYSTEMS ON SELECTED BEHAVIORAL TESTS IN THE FISCHER 344 RAT. D E Hollembach, P X Baupai, R B Draughn, D R Mattie and J C Cooper, Armstrong Aerospace Medical Research Laboratory, Toxic Hazzards Division, Wright-Patterson AFB OH, Sponsor: B R Kinead.

The purpose of this study was to determine the behavioral effects of 1,1,1 trichloroethane (TCE) following continuous low level exposure in the rat. Ceramic glass reservoir systems (CGRS) fitted with rubber injection ports were surgically implanted subcutaneously into the anterior thoracic area of 225-250 gram male Fischer 344 rats. Four days following surgery, the animals were randomly assigned to a control or one of three treatment groups composed of 13 animals each. The CGRS of each treatment group was then injected with 0.25, 0.5, or 1.0 ml of TCE. Forty-eight hours after injection of TCE, 10 rats from each group underwent the following behavioral tests: open field activity, figure eight maze activity, and acoustic startle reflex. Blood samples were collected from the remaining 3 animals in each treatment group and analyzed for TCE using gas chromatography. The blood levels (mean ± S.E.M.) of TCE were 1.2 ± 0.1, 2.0 ± 0.5, and 4.5 ± 1.0 µg/ml respectively. Results of the behavioral tests revealed that rats receiving 1.0 ml of TCE displayed a significant (P<0.05) decrease in performance as compared to the control animals.

THE EFFECTS OF 2,4-DITHIOBIURET (DTB) ON SENSORY AND MOTOR FUNCTION. K F Dean, R C Hamrick, and R H Crofton. Neurotoxicology Division, US EPA, RTP, NC and NC Technology Services, RTP, NC. Sponsor: V Bailey

DTB exposure causes a delayed onset muscle weakness in rats that has been attributed to depressed neuromuscular transmission. The present study compares the effects of DTB on sensory and motor function in rats. Adult male Long Evans hooded rats were exposed to saline, 0.25, 0.5, or 1.0 mg/kg DTB, ip, for 5 consecutive days (Days 1-5). Body weights were monitored throughout the experiment. Motor activity was measured for 1-hr in figure-eight mazes on Day 0, 6, 13, and 27. Forelimb and hindlimb grip strength were assessed on Day 6, 13, and 27. Auditory thresholds were determined for 5 and 40 kHz tones using reflex modification of the startle response on Days 0, 7, 14, and 28. All endpoints except auditory thresholds were decreased in a dose- and time-dependent manner. Decreases in body weight were maximal on Day 9 at 1.0 mg/kg/day (20% from control), but recovered by Day 22. Motor activity was suppressed on Day 6 only, whereas grip strength measures were decreased on both Days 6 and 13. Auditory thresholds were not significantly altered; however, baseline startle amplitude was decreased at the highest dosage on Days 7 and 14, but recovered by Day 28. These data demonstrate that DTB produces a reversible impairment of motor function, without altering sensory function.

INCREASED RUNNING WHEEL AND LOCOMOTOR ACTIVITY DURING ACUTE TOLUENE OR m-XYLENE EXPOSURE. J F Graefe and R M Wood, Institute of Environmental Medicine, NYU Medical Center, New York, NY.

To maximize the sensitivity of unlearned motor behaviors to solvent exposure, rats were exposed in two 1.3 m³ stainless steel chambers for 6 hr during the dark phase of the light/dark cycle, the period when rats are most active. Moderate food restriction also increases activity; adequate food was provided following testing to maintain 0.3 kg bodyweight. Six exposures to air or one of 6 concentrations of toluene (300 to 4000 ppm) occurred on Tuesdays and Fridays in an ascending-descending series, three exposures per step. Rats were exposed to m-xylene (178 to 3000 ppm) using the same design. Infrared detectors adjacent to each wheel or cage counted activity in successive 10 min intervals. Both solvents increased locomotor activity, producing activity decreases and ataxia at higher concentrations. Increased ventilation and cardiac output associated with exercise enhanced solvent uptake; thus wheel running was more sensitive to toluene exposure than sedentary locomotor activity in cages. m-Xylene is more lipophilic than toluene, and increased cage activity at lower concentrations than toluene. m-Xylene is more irritating than toluene; wheel-running displayed a complex time course, suggesting simultaneous processes of irritancy, adaptation, and biphasic effects on the nervous system. Support: DA04438, DA00117.

Rats and mice were exposed either to n-octane or n-hexane (7000 ppm for 4 hours) and examined for behavioral impairment of 1) open field locomotor activity (a general measure of coordination and well-being); 2) acoustic background modulation of the startle reflex (a measure of arousal); and 3) acoustic prepulse inhibition of the startle reflex (a measure of auditory functioning). Exposure of rats to n-hexane did not produce reliable behavioral changes in the tests used in this series, whereas the mice exposed to n-hexane failed to show the normal, expected reduction in locomotor activity with repeated testing. Similarly, exposure of rats, but not mice, to n-octane reduced the amplitude of the startle reflex and eliminated the normal startle-facilitating effects of background noise. In neither species was acoustic prepulse inhibition impaired by the chemical exposures. The results are discussed in terms of possible species differences in the behavioral response to the exposures, as well as differences in the effectiveness of n-hexane and n-octane to impair the behaviors studied.


No treatment-related neurologic or morphologic changes were observed in male or female Fischer 344 rats after subchronic exposure to dichloromethane (DCM, methylene chloride). Rats were exposed to DCM for 6 hrs/day, 5 days/wk, for 13 weeks. Since oxidative metabolism of DCM to CO and CO2 is a saturable process, DCM exposures were selected to be clearly below saturation (50 ppm), just below saturation (200 ppm), and well above saturation (2000 ppm). Post-exposure tests included a functional observational battery, grip strength, and electrophysiologic evaluation of visual, auditory, somatosensory, and peripheral nerve pathways. After functional testing, rats were perfused and multiple areas from brain, spinal cord and peripheral nerve were specially stained and were examined by light microscopy. Although some miscellaneous functional and morphologic variations were recorded, none were related to treatment.

OPERANT BEHAVIORAL SCHEDULE ACQUISITION AND PERFORMANCE IN BABOONS SURVIVING TWICE THE LD50 OF SOMAN OR SARIN. J L Orr, Southwest Research Institute, San Antonio, TX. Sponsor: J A Dellinger.

Transient disruptions in normal home cage behavior of baboons were observed following inhalation exposure to twice the LD50 of soman or sarin. The baboons received the organophosphate or vehicle (isopropyl alcohol) by inhalation while under pentobarbital anesthesia and received atropine and ventilatory support. There were five baboons in each of the groups: vehicle control, soman, and sarin. Behavioral training began on the one-year anniversary of exposure. The baboons progressed through a series of stages toward a multiple schedule of Fixed Ratio (FR) 30 and Differential Reinforcement of Low Rate (DRL) 20 seconds. Repeated-magnetic analysis of variance revealed statistically significant (p < 0.05) differences in performance related to the behavioral schedule (FR or DRL), the training stage, and the sessions within training stages, but no statistically significant effects or interactions involving treatment group (vehicle control, soman, or sarin) were detected. The baboons dosed with organophosphate are indistinguishable from the vehicle controls in the acquisition and performance of the behavioral tasks examined. This work supported in part by the US Army Medical Research and Development Command, Contract No. DAMD17-85-C-5159.

EFFECTS OF ELECTRIC FIELDS ON NONHUMAN PRIMATES. W R Rogers, J L Orr and A C Coelho Jr. Southwest Research Institute and Southwest Foundation for Biomedical Research, San Antonio, TX. Sponsor: J A Dellinger.

Because concern exists that electric fields associated with transmission and distribution of electric power might have adverse effects, baboons (Papio cynocephalus) were used as human surrogates in studies of operant and social behavior. Following a six-week baseline, the exposed groups received electric fields (60 kV/m or 60 kV/m in separate experiments) for 12 hours/day, 5 days/week followed by a six-week post-exposure period. Performance on FR30 and DRL20 schedules by six experimental and six control animals was compared: introduction of the electric field produced response suppression, usually lasting one to three days, followed by normal performance. The social behavior of groups of eight experimental and eight control monkeys was systematically observed. Introduction of the electric field produced changes in the mean rates of occurrence of several behaviors including Passive Affinity and Tension; after the third week, mean rates for the two groups no longer differed. Preliminary experiments established that (1) the mean threshold for detection of electric fields by baboons is 12.6 kV/m, and (2) baboons will not reliably respond to terminate electric field exposure, suggesting that exposure is not highly aversive. Exposure appeared to have no important effects on common clinical chemistry measures. Although it seems reasonable to assume that the transient behavioral changes, which were similar at 30 and 60 kV/m, were associated with detection of electric fields, the mechanism is not understood. This research was sponsored by the Central Research Institute of the Electric Power Industry (Japan) and managed by the Office of Energy Storage and Distribution of the Department of Energy.

Rats prenatally exposed to phenytoin exhibit a spectrum of behavioral alterations, one of the most obvious being a rotational movement seen in some rats. A similar behavior has previously been observed by Erway and others in some inbred strains of mice which is associated with a congenital defect of otocoria in the utricle and/or saccule. We hypothesized that phenytoin exposed offspring might have a similar otocional deficit. Pregnant Sprague-Dawley rats were gavaged on days 7-18 of gestation with 200 mg/kg of phenytoin in propylene glycol or with propylene glycol alone. Following postnatal testing, the olfac capsules were dissected and preserved in buffered formalin, fixed in alcohol, and cleared in methyl salicylate. In an initial test of the hypothesis we found that approximately 21% of the offspring born to dams treated with phenytoin demonstrated this rotational behavior and was associated with a deficit of otocorial crystals in their inner ears. In a subsequent experiment the extent to which these otocional deficits could account for the behaviors seen was examined.


Thalidomide (TH) is a recognized structural teratogen for humans and many animals, although rats appear to be resistant. However, the behavioral teratogenicity and/or effect of TH on rat CNS is not known. To explore these possibilities 12 pregnant Sprague-Dawley rats were orally dosed once daily on gestational days 7-18 with 100 mg/kg of TH suspended in propylene glycol (PG) at a volume of 2 ml/kg. Ten control dams were gavaged with PG at the same volume. No indications of maternal toxicity were noted. At birth, litters were weighed, sexed and culled to 8 (4 each sex). Body weights were recorded weekly for 20 weeks. Prior to weaning (d.21), the onogeny of locomotion, olfactory orientation and air righting were assessed. Postweaning tests included Cincinnati water maze acquisition and reversal, auditory and tactile startle, Morris water maze acquisition and reversal, and spontaneous alternation. Preweaning mortality was significantly increased for TH offspring. Preweaning TH pups showed a small but consistent decrement in body weight which continued throughout the study. Deficits in Cincinnati maze learning were found for males but not females.

NICOTINIC AND MUSCARINIC INTERACTIONS WITH D1 AND D2 DOPAMINERGIC SYSTEMS IN AFFECTING COGNITIVE BEHAVIOR OF RATS. ED Levin, JE Rose, SR McGurk and LL Butler, Nicotine Research Lab, VA Medical Center and Dept of Psychology UCLA, Los Angeles, CA. Sponsor: AChO.

Chronic acetylcholinesterase inhibition has been found to cause a down regulation of both muscarinic and nicotinic acetylcholine receptors (Overstreet et al., 1974, Pharmacol Biochem Behav 2:45-54), but little is known about the relative functional roles that these different cholinergic receptors play. We have found that the effect of nicotinic and muscarinic receptor blockade is additive in impairing choice accuracy in a radial-arm maze. However, we later found that nicotinic and muscarinic blockade have differential involvement with dopamine D1 and D2 receptors. The cognitive impairment caused by muscarinic blockade is reversed by the D1 antagonist SCH 23390 and is unaffected by the D2 antagonist ractopride. On the other hand, the cognitive impairment caused by nicotinic blockade is unaffected by SCH 23390 and is potentiated by ractopride. In the current study, using female albino rats (N=10), we found that the D2 agonist LY 171555 (0.05 mg/kg, IP) was effective (p<.01) in reversing the radial-arm maze choice accuracy impairment caused by the nicotinic blocker mecamylamine (10 mg/kg, IP). The D1 agonist SKF 38393 (3 mg/kg, ip) was ineffective. Thus, we have found selective dopaminergic D1 and D2 treatments which counteract the adverse cognitive effects of either nicotinic or muscarinic blockade. A combination of these treatments may be useful in treating the cognitive effects of generalized cholinergic underactivation. (Supported by NIDA grant DA 02665 to JER. AND NS 10928 TO LBB)

SPECIFICITY OF NMDA-INDUCED FUNCTIONAL DEFICITS. B C Rogers and H A Tilson. Curriculum in Toxicology, University of North Carolina, Chapel Hill, NC and NIEHS, Research Triangle Park, NC.

We have previously reported that the bilateral intrahippocampal administration of N-Methyl-D-Aspartate (N-MDA) produces dose-dependent functional deficits and hippocampal cell loss which can be prevented by preadministration of the non-competitive N-MDA antagonist MK-801. Here we report that the preadministration of intrahippocampal (5) 2-amino-7-phosphonooxonic acid (2-AP) (2.5, 5.0 and 10.0 ug/site), a competitive N-MDA antagonist, prior to the administration of N-MDA (10 ug/site) attenuates N-MDA-induced hyperactivity, water maze acquisition deficits and hippocampal cell loss. These effects were stereospecific since preadministration of the isomer (L) 2-APH (10 ug/site) had no effect. Further studies showed MK-801 had no effect on neurotoxicity produced by a non-N-MDA agonist. MK-801 (0.1, 1.0 and 10.0 mg/kg) was unable to significantly attenuate the behavioral deficits and cell loss induced by intrahippocampal kainate (0.33 ug/site). These results support the hypothesis that N-MDA-induced deficits are a consequence of the overactivation of N-MDA receptors. (B.C.R. was funded by E507126).

The 90-day oral subchronic toxicity of nitroguanidine (NG), a primary component of US Army triple-base propellants, was evaluated in male and female Sprague-Dawley rats. NG was administered in the diet at dose levels of 0, 100, 316, and 1000 mg/kg/day for 90 days. NG produced a transient reduction in food consumption and a dose-related increase in water consumption. A significantly reduced rate of weight gain was observed for females administered NG at a rate of 1000 mg/kg/day. Blood samples taken at necropsy for hematological and serum chemistry exhibited no significant NG-associated alterations. Microscopic examination of tissues from control group and 1000 mg/kg/day group animals revealed no lesions attributable to administration of NG. These findings indicate that NG is nontoxic in rats when administered at doses as high as 1000 mg/kg/day for 90 days. The findings of increased water consumption suggest that NG, which is excreted unchanged in the rat’s urine, may be acting as an osmotic diuretic.

ACUTE TOXICITY OF 2-METHYLPIPERAZINE

The acute toxicity of 2-methylpiperazine (2-MP), a nonproprietary chemical used in drug synthesis, was studied orally in CrlCDBR rats, dermally in NZW rabbits and for dermal sensitization in Hartley guinea pigs. In the oral study, 5 rats/sex were dosed at 1.708, 2.066 and 2.5 g/kg 2-MP in denitrogenated water at 10 ml/kg and observed for 14 days. Three, 4 and 8 rats died, respectively. The 2.066 g/kg level was repeated and 6 rats died. Most (19/21) rats that died were found dead on the day after dosing. The po LD50 was calculated to be 2.03 g/kg. Few clinical signs of toxicity were observed. Significant gastric irritation was present in all rats that died. Day 14 gross necropsy examinations were unremarkable. 2-MP applied neat at 0.5 g/site (moistened with a small amount of H2O) to intact rabbit skin induced severe dermal irritation; however dermal doses of 0.1, 0.5 and 1.0 g/kg did not cause systemic toxicity. Skin irritation was also evaluated using 10% aqueous 2-MP. Neutral buffered with HCl 10% 2-MP caused no irritation; unbuffered solution induced mild irritation. 2-MP was found to be non-sensitizing in a guinea pig maximization study. The severe skin irritation caused by neat 2-MP was apparently due to its vesicant and alkaline properties. Hence, 2-MP has only slight dermal or oral toxic potential. The finding of most concern (severe irritation) was not due to intrinsic biological properties but to chemical and physical properties.

COMPARATIVE ACUTE TOXICITY AND IRRITATION PRODUCED BY ALKYL AND AROMATIC AMINES:
R C Myers and B Ballantyne, Bushy Run Research Center, Union Carbide Corp., Export, PA

Various alkyl and aromatic amines were evaluated for acute toxicity and irritancy. High oral toxicity was produced in rats by short-chain (C6) aliphatic amines (I), long-chain (C12) aliphatic amines (II) and aromatic amines (III), with median LD50s ranging from 0.62 to 1.07 mg/kg. Cyclic amines (IV) were moderately toxic (7.59 mg/kg). Oral toxicity was generally inversely proportional to the number of nitrogen substituents in Groups I and II. Necropsy revealed hemorrhage and inflamed GI tracts. Most amines were more toxic by the percutaneous route: Amine Groups I, II and III produced high toxicity in rabbits (0.26-0.71 ml/kg); Group IV was moderately toxic (1.26 ml/kg). Signs of oral/dermal toxicity included dyspnea, incoordination, tremors, convulsions and prostration. Inhalation exposure to vapor of most amines was without effect in rats except for Groups I and III which caused blepharoconjunctivitis, dyspnea, incoordination, tremors, convulsions, narcosis and death. Gross lesions included lung hemorrhage and pneumonia. In irritation tests, Group III was moderately irritating to rabbit skin; all other groups were severely irritating. All groups produced severe eye injury.

COMPARATIVE TOXICITY OF ACETYLATED SCRIPENOL MYCOTOXINS IN CHICKENS:
A A Ademeyer and P B Hamilton, Toxicology Program and Department of Poultry Science, North Carolina State University, Raleigh, NC. Sponsor: H E Donaldson.

Scripenol (STO) and its mono (MAS), di (DAS) and triacetoxyl (TAS) derivatives have been implicated in natural outbreaks of toxicity in poultry and other farm animals. These toxins were compared by feeding (0, .5, 1, 2, 4, 8, 16 and 32 ug/g diet) 4 groups of 10 birds per treatment until 3 weeks of age. The minimum effective dose (MED) on body weight was 4 ug/g for DAS, MAS and STO and 8 ug/g for TAS. The most sensitive indicators for DAS and MAS were decreased spleen size and oral lesions (0.5 ug/g). MAS caused hemorrhages of the small intestine (0.5 ug/g). Spleen weight was not affected significantly (<.05) by STO or TAS. The crop, proventriculus and gizzard were enlarged by DAS, MAS and STO (4 ug/g) but not by TAS. The duodenum of Fabricius was decreased by DAS (2 ug/g), MAS (8 ug/g), and STO (16 ug/g) but not by TAS. Serum enzymes showed similar specificities. The scripenols had feed refusal activity in this order: DAS > MAS = STO > TAS. Since the scripenols frequently occur naturally in unpredictable mixtures, it appears that the symptoms of scripenol toxicity will have equally unpredictable variations.

Many toxic agents have been reported to affect body core temperature (Tco) and there is increasing evidence which suggests that moderate decreases in Tco may significantly attenuate the toxicity of xenobiotic compounds. This hypothesis has been tested in our laboratory using several different compounds, including chlorodimeform, Nicl, and ozone. In most studies, adult pentobarbital-anesthetized rats were maintained at one of several Tco's (33°C, 35°C, 37°C, or 39°C) using a specially-designed temperature-clamping apparatus which controlled Tco to within ±1°C of the selected set point. Electrocardiogram, heart rate, arterial blood pressure, tail and foot skin temperatures, and lethality were monitored following exposure to identical toxic doses of the test agents over the range of Tco's. In general, the degree of toxicity observed, as reflected by changes in the measured cardiovascular parameters, was diminished at Tco's between 35°C and 37°C, while lethality was increased at Tco's outside this range. The results of these studies suggest that Tco is an important consideration in toxicology research. Even simple studies, such as lethality tests, may be misinterpreted if Tco is not monitored and/or controlled. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.


The acute, and subacute toxicity, as well as the teratogenic, and genotoxic potential of a new fabric conditioner active, ditalomimidazole (D), was evaluated. Data indicate that DI has a low order of acute toxicity. No deaths were observed at the maximum oral dosage administered of 20 g/kg in rats, and there was minimal irritation in low volume eye and standard skin irritation studies in rabbits. DI was not mutagenic in four mutagenicity tests.

91-day oral and dermal studies were conducted in rats and rabbits, respectively. These studies included both gross and histopathologic evaluation. The only test substance related effects observed were in the high dose (400 mg/kg/day) group of the dermal study and consisted of dermal irritation at the site of test substance application. The only observed test material related effect in the oral study was in the high dose (1500 mg/kg/day) male group, consisting of a slight reduction in body weight gain. The studies provided NOELs of 200 mg/kg/day (dermal) and 120 mg/kg/day (oral).

A dermal teratology study was conducted in rabbits. Decreased body weight gain and 5% mortality were observed as signs of maternal toxicity at the highest dose tested (400 mg/kg/day). There were no test substance related external, visceral, or skeletal malformations in fetuses observed at any dose tested. In summary, DI has a low acute and subchronic toxicity profile, and was not mutagenic or teratogenic. This safety profile is similar to that observed with other structurally related materials used safely as fabric softener actives.

These studies were conducted to provide data for establishing occupational health criteria for the organophosphate nerve agents, Sarin (Types I & II) and Soman. Range finding studies established that the Maximum Tolerated Dose (MTD) (highest dose group without lethality) in CD Rats for S was Type I, Sarin Type I and Soman were 300, 300 and 70 ug/kg respectively. In each study four groups (12/group/sex) of 11 to 12 week old CD rats were gavaged with either 0, MTD, MTD/2 or MTD/4 of the agent 5 days/week for 13 weeks. No protective pretreatment was administered. The rats were killed three or four days after the last exposure. The only toxicologically significant reductions (P<0.05) in body weight and liver/brain weight ratio in the males of the Soman MTD group. Males and females for all other groups and agents showed no significant differences. Histopathologically there were no neoplastic changes; the non-neoplastic changes observed were considered to be incidental and not compound related. Estimate of NOELs for the Sarin Type I, Sarin Type II and Soman are 300, 300 and 35 ug/Kg respectively. Hematological and clinical chemistry evaluations are reported in a separate abstract. (Supported by US Army Medical Research & Development Command, contract #DAAG 15-84-K-0088).

A 13-WEEK ORAL TOXICITY STUDY ON SUCINNATE TARTRATES IN RATS. D W Petersen. The Procter & Gamble Company, Cincinnati, OH. Sponsor: J F Griffith.

Succinate tartrates (ST) was given via the drinking water to male and female Fisher-344 rats at concentrations of 0, 0.01, 0.05, 0.1 and 0.5% w/w active for 13 weeks to determine ST's potential oral toxicity. There was no mortality and no pharmacotoxic signs were observed during the study. No treatment related differences were noted in week 13 body weights, or in body weight changes or food consumption. ST administration produced no treatment-related changes in hematochemical, clinical chemistry and urinalysis parameters except for a significant decrease in serum magnesium levels in high dose (0.5%) females. A statistically significant increase was observed in absolute and relative adrenal weights in high mid-dose (0.1%) and high-dose (0.5%) females and in relative liver weights in high dose (0.5%) females vs. controls. However, the overall ANOVA was significantly different only for the relative adrenal weights. No gross or microscopic changes were observed in any organs or tissues including the adrenals and liver. In the absence of histological changes, the organ weight changes were not considered biologically significant. The NOAEL for this study was thus 0.5% ST.
THE INFLUENCE OF HUSBANDRY REGIMENS ON ABSORPTION KINETICS OF ORALLY ADMINISTERED BARBITURATES IN DOGS. MA Collins, NJ Loftus, LPinto, ICI Central Toxicology Laboratory, Macclesfield, Cheshire, UK. Sponsor: PMD Foster.

The dog is frequently used as a non-rodent mammalian species for toxicity testing and test compounds are often administered to dogs orally. This study investigates the effect of preadministration and husbandry regimen on the absorption profile of model compounds of long (phenobarbital) and short (thiopental) elimination half life. Three female beagle dogs were given single doses of 10 mg/kg phenobarbital or thiopental. Dosing methods investigated were by gelatin capsule given either before, with or after feeding, by dietary admixture, by gavage as a solution or by intravenous injection. Treatments were given in a random sequence for each compound. Serial blood samples were obtained and analysed by hplc for barbiturate concentration. Peak plasma phenobarbital concentrations were achieved within 2 hours of dosing by gavage, and following dosing by capsule 4 hours before feeding. When the dose was administered in a capsule at the same time as, or 2 hours after, food or by dietary admixture absorption was delayed and peak plasma concentrations were achieved between 4 and 6 hours. A similar effect was noted with the more rapidly eliminated thiopental. These results clearly demonstrate that husbandry regimen influences barbiturate absorption.

CATALYTICALLY IMPORTANT AMINO ACIDS IN HUMAN AND MURINE LIVER CYTOSOLIC EPoxide HYDROLASE. E C Dietze, D Bonder, and DD Hammack. Departments of Entomology and Environmental Toxicology, University of California, Davis, CA.

The impetus to study CEH lies in its possible importance in human health. The enzymatic mechanism and in vivo function(s) of CEH are unknown. Before the mouse, a convenient system for both in vivo and in vitro studies of CEH, is used it must be determined whether or not the human and murine CEH are similar. Amino acid specific inhibitors were used to determine which amino acids are important for activity of both human and murine CEH. Cysteine, histidine, and aspartate/glutamate were important for activity of both human and murine CEH. Trypsin may be important for activity of murine but not human CEH. Arginine, lysine, serine, and tyrosine were not important for either CEH. Methionine was not tested. Both CEH's had cysteine at a critical site. Modification by methyl methane-thio- sulfonate blocks all activity in either CEH. Human and murine CEH are nearly identical in the amino acids required for activity. Therefore, by this criteria, murine CEH is a valid model for mechanistic studies of human CEH. Additional work will be carried out to confirm the exact identity of the catalytically important amino acids and determine their position and number.

PRETREATMENT WITH 3,3'-DICHLOROBENZIDINE (DCB) ALTERS THE PATTERN OF POLYUNSATURATED FATTY ACIDS IN RAT HEPATIC MICROSOMES. M M Iba, B Lang, and R A Cross. Department of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ.

Pretreatment of rats with the pigment precursor DCB induces hepatic microsomal drug-metabolizing enzymes and enhances lipid peroxidation (MLP) in vivo and in vitro. These studies were carried out to determine whether the enhanced MLP response from DCB-induced increase in microsomal polyunsaturated fatty acid (PUFA) content. In rats, a single 40 mg/kg dose of DCB significantly increased oleic acid content (5%) and decreased arachidonic acid content (11%) in hepatic microsomes. In vitro, added DCB caused a dose- and an NADPH-dependent decrease of linoleic acid (100% at 100 nM DCB), arachidonic acid (90% at 100 nM DCB) and docohexaenoic acid (100% at 50 nM DCB) contents in microsomes. These in vitro changes were not paralleled by increases in malondialdehyde formation. None of the effects was observed with either benzidine or any of its other congeners examined. The data suggest that: (1) DCB enhances MLP not by increasing PUFA content but by decreasing the antioxidant potential; (2) PUFA loss is more sensitive than MDA formation in detecting DCB-induced membrane damage, and (3) DCB may affect the desaturation of fatty acids in vivo. (Supported by EPA R-812459).
437 OMEGA-3 FATTY ACIDS INDUCE VASCULAR ANOMALIES ON CHICKEN CHORIO-ALLANTOIC MEMBRANE (CAM). M I Klibaner, A C Wallstrom, Angio-Medical Corp. Boston, MA. Sponsor: A F Rogers

Chicken CAM represents a large area of peripheral vasculature readily available for live observation using standard light microscopy. Localized application of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in a range of 50–750 micrograms to the developing CAMs of 9 day-old embryos consistently induced focal vascular malformations similar to those caused by diethylstilbestrol in the same experimental model. Vascular anomalies included conversions of normal vessels into multi-channeled vascular structures and formation of aneurysm-like bulbous enlargements. The changes were distinct by 24 hours and remained visible until the end of the experiment (120 hours). These changes were highly reproducible and affected all CAMs treated with DHA or EPA in amounts at or higher than 100 micrograms. These data demonstrate a previously unknown vascular teratogenic effect of DHA and EPA. This model can be used to study early stages of normal and pathologic vascular development.


Various clinical types of leukemia have been reported in benzene-exposed workers, with few studies considering the background risks and distributions of leukemias by clinical type. We have conducted a meta-analysis of the published literature to determine what types of leukemia are found to be in excess among workers with significant occupational benzene exposure. Our analysis shows that only acute myeloid leukemia (AML) is found in excess. A quantitative exposure/response analysis of data from the major benzene leukemia study (Ohio Pliofilm workers) could not be performed, since we newly found that the AML cases came from one factory and the benzene exposure data came from another.

439 THE BEST SOURCES OF DATA FOR CONSUMER PRODUCT EXPOSURE ASSESSMENTS. P J Hakkinen, S Baldwin, and J C Gallender*. The Procter & Gamble Company. Packaged Soap and Detergent Product Development Division and *Corporate Home Performance Testing, Cincinnati, OH. Sponsor: J F Griffith

A thorough understanding of the extent of potential accidental exposure and those exposures resulting from normal product use is needed for well-founded human safety assessments for a consumer product. This presentation describes the best sources of data needed to assess potential product exposure (e.g., the concentration of product used for a particular type of task, how long the task takes, the number of times a day or week the task is performed, and the amount that could be ingested from a product package). Also described are the limitations associated with other sources. Sources of the data include 800-line phone calls, poison control centers, consumer diaries of product use, phone surveys asking consumers how a product is used, and laboratory measurements. Also described is the importance of knowing whether the data collected follow a normal, log-normal, or other type of distribution, and why it is important to consider whether the data are correlated with each other.

440 A PROSPECTIVE SURVEY OF ACCIDENTAL INGESTIONS OF AUTOMATIC DISHWASHING DETERGENTS. S Baldwin, T L Schwab, V H Sublet, Procter & Gamble Co., and Cincinnati Drug and Poison Information Center, Univ. of Cincinnati, Cincinnati, OH. Sponsor: J F Griffith

Nine U. S. poison control centers participated in a 2 year prospective study to assess accidental ingestions that involved Liquid and Granular Automatic Dishwashing Detergents (LADD and GADD). Accidental ingestion of LADD and GADD by children were compared by product source, volume of ingestion, and reported symptoms. Accidental ingestions of LADD or GADD were proportionally greater from the dishwasher (61.5%) than from other sources. More children obtained detergent from the original product package for LADD than GADD (18% vs. 7%). The reported volumes ingested were comparable between LADD and GADD, with 80% of callers reporting "taste/lick" (<1/4 tsp). Higher ingestion volumes (2 tsp.) were greater when product was obtained from the package vs. the dishwasher (13% vs 2%). No symptoms were reported for 80% of the cases and most reported symptoms were minor. A comparison was made between LADDs with and without a child-resistant closure. Notably, 30% fewer accidental ingestions were reported for LADD products marketed in packages with a child-resistant closure.
441 DRUG-INDUCED PHYSIOLOGIC CHANGES IN MATERNAL ARTERIAL BLOOD RELEVANT TO EMBRYONAL DEVELOPMENT IN RATS. K S Khara, Sir Frederick Banting Research Centre, Tunney’s Pasture, Ottawa, Ontario.

Arterial blood gases, acid-base equilibrium, osmolality, and concentrations of hemoglobin, K⁺ and Na⁺ were measured in unanesthetized pregnant rats (control and drug-treated) at repeated post-treatment intervals using cauda surgically implanted in the carotid artery. The control values (±SD) were 7.45±.008 for pH; 32±.5 Torr for PCO₂; 105±1 Torr for PO₂; 22.6±3.3 mEq/L for Na⁺; 131±1 mEq/L for K⁺; 129.4±.5 and 4.99±.11; mEq/L respectively for Na⁺ and K⁺. Ethylmethionine at teratogenic yet nonmaternotoxic doses had no significant effect on these values. Blood values following maternotoxic teratogenic doses of dimethadione, ethylene glycol, cadmium chloride and sodium salicylate suggested varying degrees of metabolic acidosis and respiratory compensation, with or without hyperosmolality or hypokalemia. In a teratology study, sodium salicylate-induced malformations and resorptions were strikingly followed combining dosing of the salicylate with NaHCO₃ (known to correct metabolic acidosis), and markedly increased with NaCl (known to enhance acidosis). The method of blood analysis in this study and the fact that on conventional amino acid analyzers, it coelutes with leucine (LEU). The ability of a reversed phase (RP) HPLC method with pre-column o-phthalaldehyde (OPA) derivatization to separate Hyp-A was studied. The OPA method utilized the following: Column, Pecosphere 15x 0.46 cm 5C C₁₈ RP column; Detector, Fluorescence with excitation and emission wavelengths at 360 and 455, respectively; Buffers, A-80% 0.05 M sodium phosphate, pH 5.5 in MeOH, and B-20% of same buffer in MeOH. The gradient elution profile was: 0-10% B in 10 minutes: 10-85% B in 5 min: 5 min re-equilibration. A ninhydrin-positive isolate of the amino acid known to contain Hyp-A, isoleucine (ILE), and LEU was used. 500 μl of an aqueous solution containing 25 μg/ml of the isolate was thoroughly mixed with 2000 μl of a commercial OPA reagent. After 2 min, 200 μl of this mixture was injected onto the column. The analysis revealed 3 well separated peaks with retention times (min) of 31.2, 31.9 and 32.5. Subsequent spiking with ILE and LEU standards identified the 31.2 and 32.5 peaks as ILE and LEU, respectively.

442 THE EFFECT OF PROLONGED DRYING ON TRANSDERMAL WATER LOSS, MOISTURE AND pH OF HUMAN LABIA MAJORA AND FOREARM SKIN. P Elsen, H I Malbach. Dept. of Dermatology, University of California San Francisco, San Francisco, CA.

To determine if impairment of vulvar skin barrier function is caused by chronic occlusion of the vulvar region, the effect of prolonged drying on transdermal water loss (TWL), moisture and pH of vulvar and forearm skin was studied in several healthy volunteers.- A desiccating chamber absorbing the water evaporating from the skin surface was applied to a defined area of the vulvar and labia majora skin for five days, renewing it every 24 hours. Skin TWL, moisture and pH were measured daily and four days after removal of the desiccating chamber at the site of drying and at a symmetrical control site using standard methods. Under desiccation, TWL both of forearm and of vulvar skin showed a significant increase after one day of drying followed by a gradual decrease. After 5 days of drying, TWL at the desiccated site was 82% and 73% of that at the control site, respectively. Relative reduction of TWL was higher than that of forearm TWL, the absolute value of vulvar TWL (9.47 ± 5.10 g/m²/h) remained significantly higher than that of forearm TWL (4.25 ± 2.68 g/m²/h). Changes of skin moisture were less pronounced. Skin pH was significantly reduced by drying both at the vulva (-0.60 ± 20 pH units) and at the forearm (-0.40 ± 0.19 pH units). Skin pH (5.32 ± 0.45) remained significantly higher than forearm skin pH (4.81 ± 0.28). It is concluded that although changes in physiological parameters during drying are more pronounced in vulvar than in forearm skin, remaining differences that the specific properties of vulvar skin cannot be explained by occlusion alone.


Quantitation of hypoglycin A (Hyp-A), a toxic amino acid found in the popular Jamaican akkee fruit, has been hampered by the fact that on conventional amino acid analyzers, it coelutes with leucine (LEU). The ability of a reversed phase (RP) HPLC method with pre-column o-phthalaldehyde (OPA) derivatization to separate Hyp-A was studied. The OPA method utilized the following: Column, Pecosphere 15x 0.46 cm 5C C₁₈ RP column; Detector, Fluorescence with excitation and emission wavelengths at 360 and 455, respectively; Buffers, A-80% 0.05 M sodium phosphate, pH 5.5 in MeOH, and B-20% of same buffer in MeOH. The gradient elution profile was: 0-10% B in 10 minutes: 10-85% B in 30 min: 85-0% B in 5 min: 10 min re-equilibration. A ninhydrin-positive isolate of the akkee fruit known to contain Hyp-A, isoleucine (ILE), and LEU was used. 500 μl of an aqueous solution containing 25 μg/ml of the isolate was thoroughly mixed with 2000 μl of a commercial OPA reagent. After 2 min, 200 μl of this mixture was injected onto the column. The analysis revealed 3 well separated peaks with retention times (min) of 31.2, 31.9 and 32.5. Subsequent spiking with ILE and LEU standards identified the 31.2 and 32.5 peaks as ILE and LEU, respectively.


The subchronic toxicity of PD 126213, a selective cholinomimetic agent, was evaluated in Mistar rats and cynomolgus monkeys. In rats, PD 126213 was administered in diet for 4 weeks at dose levels of 0, 25, 50, 100 and 200 mg/kg. No deaths occurred during the study. Rough palate and urine scald were noted at 100 and 200 mg/kg. Corneal edema, opacity and neovascularization occurred in both sexes given 100 and 200 mg/kg. Dose-related body weight gain suppression was noted in males at 100 and 200 mg/kg and in females at all dose levels. Gross pathological findings included roughening of the central cornea at 200 mg/kg and staining of the perineum in females given 50 mg/kg or more. Histopathologic lesions were confined to the eye, Harderian, lacrimal and submaxillary salivary glands. No adverse findings were noted at 25 mg/kg. In monkeys, PD 126213 was given by gavage for 4 weeks at daily doses of 0, 0.1, 1 and 2 mg/kg. At 2 mg/kg, clinical signs included anorexia, salivation, miosis, tremors, and soft feces. Decreased food consumption and hyponatremia were observed at 1 and 2 mg/kg. Decreased heart rate was observed frequently postdosing at 1 and 2 mg/kg. Dose-related decreases in body weight occurred by Week 2. No apparent drug-related effects on organ weights, gross pathology or histopathology were noted.

CI-935 (2-B-D-ribofuransonyl-4-phenazolcarboxamide) is anabolized in cells to an analogue of NAD which inhibits IMP dehydrogenase causing decreased guanosine nucleotide biosynthesis and interrupted nucleic acid synthesis. The minimum lethal dose in mice was approximately 700 mg/kg. In subacute studies, male and female Wistar rats were administered doses of 1, 10, 50, 150 or 300 mg/kg intravenously once a day for five consecutive days. Animals were euthanized for clinical and pathological evaluation on Days 8 and 33 of the study. Administration of CI-935 at doses of 50 mg/kg and greater was associated with death and dose-related body weight gain suppression that was reversible in animals surviving to termination on Day 33. Major target organs were bone marrow and lymphoid tissue. Leukopenia characterized by neutropenia and lymphopenia at doses of 10 mg/kg and greater was observed in blood samples taken on Day 8. Histologically, marked hypocellularity of bone marrow and lymphoid depletion primarily in thymus and spleen of animals administered 50 mg/kg and above was recorded on Day 8. Only incidental, non-treatment-related lesions were observed in animals sacrificed on Day 33. The toxicity associated with CI-935 administration was reversible and consistent with the cytotoxic action of the compound.

PRESHOCK TOXICITY STUDIES OF CI-I DIRECT BLUE 15 AND 3,3'-DIMETHOXYBENZIDINE IN F344 RATS. D L Morgan, JH Menneear, NTP/NIHS, RTP, NC; BM Utland, Hazelton Labs, Vienna, VA.

The benzidine congener, 3,3'-dimethoxybenzidinie (DOMB) and CI-I Direct Blue 15 (DB15), a prototype of the DOMB-derived class of dyes, were evaluated in this prechronic study to characterize the toxicity and establish dose levels for subsequent chronic studies. Groups of ten F344 rats of each sex were administered either DOMB or DB15 in drinking water for 90 days. Concentrations of DB15 were 0.063, 0.125, 0.25, 0.5, and 1.0% for males and 0.125, 0.25, 0.5, 1.0, and 3.0% for males. DOMB concentrations were 0.017, 0.033, 0.063, 0.125, and 0.25% for males and females. All treated rats showed dose-related trends in reduced water and food consumption, and body weight gains. All DOMB-treated rats survived the 90 day treatment. There were 7 deaths in male rats treated with 3% DB15. Alkaline-hydrolyzable conjugates were the major urinary metabolites from DOMB and DB15-treated rats. Acetylated derivatives were also detected along with significant levels of DOMB in urine of DOMB and DB15-treated rats. Urinary metabolite levels generally increased in a dose-related manner, and were highest in males treated with DOMB and in females treated with DB15. Histological evaluation revealed that target organs for DOMB-treated rats were the kidney and thyroid. The kidney and liver were identified as target organs for DB15-treated rats.

DETERMINATION OF AMPHETAMINE AND ITS MAJOR METABOLITES BY HPLC UV DETECTOR. W Ruangyuttikarn and D E Moody. Center for Human Toxicology, Dept. of Pharmacology and Toxicology, Univ. of Utah, Salt Lake City, UT.

Simultaneous determination of amphetamine (Amph) and the primary products of its hydroxylation, and oxidative deamination metabolic pathways, p-hydroxyamphetamine (pHA) and phenylacetone (PA) respectively, would facilitate studies on Amph metabolism. Previous techniques require either separate methods for each product, or the use of radiolabeled substrate. We have now investigated using reversed phase high pressure liquid chromatography (HPLC) with UV detection, for this purpose. Substrate and products could be separated with isocratic elution, 60:40 (v/v) acetonitrile:phosphate buffer, pH 6.0, on a reversed phase C8 column (2.0 ml/min), with approximate retention times of 2.8, 3.2, and 5.2 min for PA, pHA, and Amph respectively. Isolation of these basic and neutral metabolites from rat liver microsomes after incubation with Amph and NADPH were performed using a simple rapid extraction based on salt-solvent pair, ammonium carbonate-ethylicacetate. The detection limits of Amph, pHA, and PA (5, 2, & 1 ng respectively) were found to be sufficiently sensitive for the determination of Amph and its metabolites in microsomes at the ng/ml level. These results demonstrate that HPLC is a potentially useful procedure for the nonradioactive measurement of multiple pathways of Amph metabolism.

POSSIBLE ENVIRONMENTAL FATE OF ANATOXIN-A. D K Stevens and R T Krieger*. WOI Regional Program in Veterinary Medicine, Washington State University, Pullman WA and *Department of Food and Agriculture, Worker Health and Safety Unit, Sacramento, CA.

Anatoxin-a, 2-acetyl-2-azabicyclo(4.2.1)non-2-ene, antx-a, is a potent acetylcholine-like produced by some toxigenic strains of Anabaena cyanobacteria (LD50 = 0.25 mg/kg, ip mouse). Ingestion of sufficient cell material of these organisms has resulted in death of livestock, pets and wildlife. To better evaluate the risk posed by antx-a "blooms", stability studies of antx-a have been undertaken by this laboratory. The aims are to identify the breakdown products of antx-a and determine their toxicity, as well as to determine the optimum conditions for laboratory storage of antx-a. Secondary nitrogenous in bicyclic structures analogous to antx-a have been shown to form stable nitroxy radicals as an oxidation product. EPR analyses of some material, laboratory cultures and synthetic simulations have failed to demonstrate the presence of any such moiety. Sunlight, on the other hand, has been shown by FT-NMR and UV spectroscopy to cause loss of antx-a with concomitant loss of toxicity, which is oxygen independent and with a rate which is pH dependent. FT-NMR, FT-IR and GC-MS techniques are being used to identify these photolysis products.
COMMUNITY ASSISTANCE IN TOXICOLOGY: A CASE STUDY. M. A. Kamrin, D. Bennis and L. J. Fischer, Center for Environmental Toxicology. Michigan State University, East Lansing, MI.

The Center for Environmental Toxicology operates a Community Assistance Program in Environmental Toxicology funded by the C.S. Mott Foundation. This Program provides education and advice to small communities with health-related environmental contamination concerns. Assistance is provided with the help of faculty with expertise in the areas of concern. One community serves as an example of how the Program can provide toxicological information and advice to communities. The problem in this community was contamination of drinking water by nitrates. The extent and severity of the contamination was unknown, as was the source. To address the problem, the Program tested all of the drinking water wells in the community (about 250) and provided rapid feedback to residents. Program staff discussed high nitrates values with each affected resident and a pamphlet was produced that explained the nitrate problem in lay terms. In addition, the Program funded hydrogeological tests to help identify the contamination sources(s). The data were analyzed and brought to the community in a town meeting which evaluated present and future risk and suggested options that could be taken to mitigate the risk. Thus, university resources were used to help alleviate fear and lead to a community-based action plan.


The Superfund Amendment and Reauthorization Act requires industry to provide the public with information on the emission of certain chemicals to the environment from chemical plants. To help the public understand the information being processed, Exxon initiated a project to determine "no-effect-levels" (NELs) for the reportable chemicals. NELs represent the atmospheric concentrations of these chemicals to which the general population could be continuously exposed without adverse health effects. A modified version of the EPA Reference Dose method was adopted. No observed adverse effect levels (NOAELS) from human and/or animal studies were identified for each chemical. The adverse effect that first occurs as dose is increased was identified. The NEL was calculated by dividing the NOAEL by standard uncertainty factors. Documentation for each of the evaluated chemicals was written to support the recommended NEL. Comparison of the NELs with community air concentrations from air monitoring or dispersion modeling programs indicate that actual atmospheric levels of chemicals are well below the NELs. Comparison of the Exxon NEL method to other methods of deriving community exposure guidelines indicate that it generates moderately conservative, scientifically supportable values.
RESPONSE PROTOCOLS: A RESOURCE FOR OCCUPATIONAL/AND ENVIRONMENTAL HEALTH EXPOSURE INFORMATION.

The passage of Hazard Communication and Right To-Know laws has increased community awareness throughout the U.S. regarding the hazards of environmental and occupational exposures. As a result, poison centers have begun receiving a significant number of calls concerning these exposures. The reference materials to quickly answer these requests, however, have not been available. The objective of the present research was to design/develop response protocols that provide accurate and complete information about occupational and/or environmental exposures.

Data determined from a national survey of poison centers was used to derive the format and content of these documents. 50 substances were selected to develop as protocols from a list based on previous cases received by the DPIC and NIOSH. Occupational medicine residents from the DEH drafted the protocols. They were subsequently reviewed and finalized by occupational medicine physicians, industrial and environmental hygienists, toxicologists and DPIC staff. The first set of protocols have been completed and will soon become available as an occupational environmental health database to provide information for health professionals, emergency responders and the public.

FRAMEWORK FOR EFFECTIVE HEALTH COMMUNICATION.

Technical personnel in environmental health sciences generally lack formal training in basic communication skills. The public meeting is one of the most important and frequently used mechanisms to communicate health implications associated with exposure to hazardous waste sites. Therefore, ATSDR’s Health Communication Program (HCP) is aimed at preparing technical personnel for public meetings. Involvement of an environmental public health professional in a public meeting is approached as a 3-phase process: “before, during, and after”. To adequately prepare technical staff for public meetings, the HCP encompasses on-camera exercises, including simulated public meetings, and internal and external feedback. Several case studies involving presentations by ATSDR technical staff at public meetings have illustrated the significance of the HCP in the overall favorable outcome of these meetings. Effective health communication was achieved in all instances irrespective of the often significantly different case-specific circumstances. This is confirmed by the positive feedback from the public and other participating entities at the respective meetings.

THE TOXICOLOGY RESOURCE INFORMATION SERVICE. A L Craigmill. Environmental Toxicology, University of California, Davis, CA.

The SOT Committee on Public Communications has developed a computerized registry of educational and informational resources in toxicology. The types of resources listed in the Toxicology Resource Information Service (TRIS) include audiovisuals, publications, teaching materials, computer software, and public and private organizations which offer informational programs about toxicological matters. The focus of these materials is public education and service, however many materials included in TRIS are suitable for university research and teaching programs. The TRIS offers a unique compilation of resources of major importance to individuals involved in toxicology teaching and public education. The TRIS will be demonstrated on computer, and displayed in the printed format in which it is available for distribution.

HAZARDOUS SUBSTANCES DATA BANK. G J Cosmides. National Library of Medicine, Bethesda, MD.

The Hazardous Substances Data Bank (HSDB) is a factual data bank focusing on the toxicology of chemicals. It is enhanced with data from such related areas as emergency handling procedures, environmental fate, human exposure, detection methods, and regulatory requirements. Data are derived from a core set of standard texts and monographs, government documents, technical reports and the primary scientific literature. HSDB contains complete references for all sources utilized. This data bank is peer-reviewed by experts drawn from the major subject disciplines within the scope of HSDB. The Data Bank is being built, maintained, reviewed and updated on the National Library of Medicine’s Toxicology Data Network (TOXNET). HSDB is organized by chemical record and contains over 4200 records. There are 150 data fields arranged in eleven subject categories. It utilizes free text search capability, Boolean logic, a powerful and flexible command language, and a variety of online user assistance features. Online and offline printing of entire or specified parts of records is available. HSDB is available 24 hours/day, 7 days/week. Registered users can access all files on the TOXNET system by direct dial or through the TELENET or TMMNET telecommunication networks.

The National Library of Medicine (NLM) developed and maintains TOXLINE, an extensive collection of online bibliographic information covering the pharmacological, biochemical, physiological, and toxicological effects of drugs and other chemicals. TOXLEARN is an interactive, microcomputer-based training package designed to teach toxicologists and environmental scientists, as well as librarians and information specialists, how to search TOXLINE effectively. TOXLEARN is organized into seven chapters. Chapters two, four and six present information on the content and use of TOXLINE, followed by multiple choice and/or completion problems. Chapter two covers the basic information used in searching TOXLINE. A detailed discussion on the structure and use of MeSH is presented in chapter four and chapter six covers selected search limiters. Chapters three, five, and seven provide interactive searches which simulate actual online sessions. TOXLEARN may be used in place of formal training, or as a precursor to, or a refresher following formal training, or for review of a particular concept. TOXLEARN provides inexpensive and easily accessible instruction for searching TOXLINE.


The EXICHEM database was introduced by the Organisation for Economic Cooperation and Development (OECD) in 1987, as a mechanism to exchange information among member countries on planned and ongoing activities on existing chemicals. The database served as the backbone for the establishment of 12 chemical-specific information clearinghouses which share available toxicology data and other related information. It is designed to provide information about planned and ongoing activities on existing chemicals. The database presently contains information on over 3500 chemical-related activities reported by 35 different member country government and industrial organizations, and the International Programme on Chemical Safety of the World Health Organization. This will be useful to the international community in the regulatory and hazard evaluation arena. It enables member countries to share results of toxicity testing and hazard/risk evaluation and avoid duplication of effort. Present plans are for the data base to be updated twice annually.


The Report of the Task Group on Reference Man or International Commission on Radiological Protection No. 23, first published in 1975, is now being revised. The present Task Group, with chairman Dr. Chester R. Richmond of ORNL, Oak Ridge, TN, is comprised of 21 members from Japan, People's Republic of China, England, and the United States and there are several corresponding members from various countries. Dr. Henri Jammet of SCPRF, France, is the critical reviewer. The new publication will focus on revising the concentrations of trace elements in various organs, employing recent sophisticated measurement techniques; revising measurements for the composition of the skeleton, for the layers of skin, and for the mass of the thyroid; adding the latest research on the cells of the islets of Langerhans; and possibly introducing a new weight and height for the standard "70 kg man". A new section on embryo and fetus will be added, and the inclusion of more graphics will be a highlight of the revised version. The Task Group does not plan to develop an Asian Reference Man, but will cover data of interest in the text and appendix.


EFFECT OF 2,3,7,8-TETRACHLORIDIBENZO-P-DIOXIN (TCDD) ON HEPATIC PROTEIN BOUND (PB) AND NON-PROTEIN (NPB) SULPHHYDRYL (-SH) GROUPS IN FEMALE RATS. M. A. Shara, W. J. Murray, and S. J. Stohe. University of Nebraska Medical Center, Omaha, NE.

TCDD induces oxidative stress in rats. Sulphydryl groups are important in maintaining structural integrity of proteins. Therefore, the effect of TCDD on PB-SH and NPB-SH groups was examined. TCDD (100 μg/kg) was administered P.O. to female Sprague-Dawley rats. Control animals received the vehicle. Animals were killed 3, 5, 7 or 10 days post-treatment and liver homogenates were fractionated by differential centrifugation. PB- and NPB-SH groups were determined by the method of Sedlak and Lindsay. Decreases in mitochondrial (MT) NPB-SH of 50, 61, 68 and 76% occurred 3, 5, 7 and 10 days post-treatment, respectively. Similar changes occurred in microsomal (MC) NPB-SH. PB-SH of MT increased 12, 40 and 25% 3, 5 and 7 days after treatment, respectively, with similar increases occurring in MC PB-SH groups. Cytosolic NPB-SH decreased 34 and 32% on days 3 and 10 after treatment, respectively, with no change in PB-SH. NPB-SH groups of nuclei fraction decreased 10, 20 and 13% on days 3, 5 and 7, respectively, with no change in PB-SH. Thus, TCDD decreased NPB-SH while increases were observed in PB-SH of MT and MC. These sulphhydryl changes may contribute to the membrane alterations produced by TCDD.
TCDD-INDUCED DECREASES IN RAT LIVER MEMBRANE FLUIDITY. N Alsharif, G J Grandjean, and S J Stoks. University of Nebraska Medical Center, Omaha, NE.

TCDD-induced lipid peroxidation (LP) has previously been demonstrated by assessing the hepatotoxic content of thiobarbituric acid reactive substances (TBARS) as well as the NADPH-dependent, microsomal formation of TBARS using malondialdehyde as the standard. Changes in membrane fluidity as a result of LP may occur. Therefore, the dose and time dependent effects of TCDD on LP in mitochondrial and plasma membranes and changes in membrane fluidity in the above tissues and microsomes were examined. Animals were treated with either 50 or 100 μg TCDD/kg p.o., and killed 3, 6 or 9 days post-treatment. A 2-fold increase in LP was observed in mitochondria 6 days post-treatment. A significant increase in LP was also observed in plasma membranes. Following TCDD administration, fluorescence polarization measurements as determined by the fluorescence polarization (r) and anisotropy parameter (a.) values demonstrated a significant decrease in membrane fluidity in the above membrane fractions, indicative of membrane structural alterations. An inverse correlation between lipid peroxidation and membrane fluidity was observed. Thus, decreased membrane fluidity and increased membrane damage may contribute to the toxic manifestations of TCDD.

THE MODULATION OF THE PHOSPHATIDYLINOSITOL SIGNAL TRANSDUCTION PATHWAY BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD). T Rosenbach and W F Greenlee, Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

The phosphatidylinositol (PI) signal transduction pathway is a major regulatory component of cell growth and differentiation. The receptors for growth factors, hormones, and neurotransmitters are coupled via G proteins to a specific phospholipase C (PLC) which in turn cleaves phosphatidylinositol-biphosphate (PIP2) into the second messengers inositol (1,4,5)-triphosphate (Ins(1,4,5)P3) and diacylglycerol. The modulation of this pathway by TCDD was examined in the human squamous cell carcinoma line SCC-12F. Inositol phosphate metabolism of cells pretreated with 1 μM TCDD for 48 h and of 0.1% DMSO controls was measured by HPLC. A survey of several of the reported growth factor and hormone stimulators of the PI pathway was carried out and only bradykinin was found to stimulate the PI pathway in these cells. TCDD pretreatment of SCC-12F cells resulted in a 50% decrease in the bradykinin stimulated generation of Ins(1,4,5)P3. In contrast, TCDD pretreatment enhanced the direct activation of G proteins by the GTPmimicking agent, ALF2, and of PLC by the Ca2+-ionophore A23187, as judged by the increased production of inositol mono- and inositol -biphosphates. These observations suggest that TCDD-dependent modulation of the PI-signal transduction pathway in SCC-12F cells can occur at two sites: (1) receptor recognition of a stimulator; and (2) altered activity of the G protein-PLC complex. These actions may take place secondarily to TCDD-induced terminal differentiation.

TCDD AND RETINOIC ACID (RA) INTERACT SYNERGISTI-
CALLY IN CLEFT PALATE (CP) INDUCTION IN MICE. M W Harris, L M Stockling, R E Morrisey, A M Clark and L S Birnbaum, NIH/NS, RTP, NC, and UNC, Chapel Hill, NC.

TCDD and RA are potent teratogens in mice. Both produce CP in susceptible strains of mice. TCDD produces hydronaphrosis while RA produces limb defects. The incidence of CP following RA exposure on gestation day (gd) 10 is greater than that observed after gd12 exposure while the reverse is true for TCDD. Enhancement of CP formation in C57BL/6N mice has been reported after coadministration of TCDD and RA on gd10. To examine the effects of these compounds following the formation of the palatal shelf bud, timed-mated mice were treated by gavage on gd12 with 10 μl corn oil/kg body wt containing either TCDD (0 to 15 mg/kg), all trans RA (0 to 200 mg/kg) or combinations of the two compounds. Dams were sacrificed on gd18 and maternal and fetal toxicity assessed. Fetuses were examined for hydronaphrosis and limb anomalies. Other than the expected increase in relative liver weights in TCDD exposed mice, no significant effects were observed in the dams. The incidence and severity of TCDD-induced hydronaphrosis was not affected by coadministration of RA. In contrast, the CP incidence was increased by coadministration of TCDD and RA above that expected of either compound alone. Based on these observations after administration on gd12, the interaction of TCDD and RA in the induction of CP appears to be synergistic.

ROLE OF THE Ah LOCUS IN THE REGULATION OF THE EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON EPIDERMAL GROWTH FACTOR (EGF), GLUCOCORTICOID (GCR), AND ESTROGEN (ER) RECEPTORS IN MOUSE LIVER. F H Lin, S Stoks, L S Birnbaum, G Clark, G Lucier, and J A Goldstein, NIH/NC, Research Triangle Park, NC.

The purpose of this study was to determine whether the effects of TCDD on binding of EGF, GCR and ER to their receptors are mediated by the Ah receptor. Mouse response curves for the effects of TCDD on EGF, GCR and ER receptors were examined in female strains of C57BL/6N mice congenic at the Ah locus: responsive, Ahb/b; nonresponsive, Ahb/d. As expected, Ahb/b mice were 10 times more sensitive to the induction of ethoxyresorufin O-deethylase than Ahb/d mice (ED50 1.8 vs 18 μg/kg). TCDD decreases the maximum binding capacity of both the high and low affinity EGR and ER autophosphorylation by >90% in hepatic plasma membranes of Ahb/b mice. These effects are regulated by the Ah locus, since the ED50 for the high affinity receptor is shifted from 1 μg/kg in Ahb/b mice to 10 μg/kg in Ahb/b mice, and that of the low affinity EGR from 0.7 to 7 μg/kg. TCDD also decreases the maximum GCR and ER binding capacity of hepatic cytosol of Ahb/b and Ahb/d mice by 30-35%. In contrast to the effects on the EGR, dose response curves for the effects of TCDD on the GCR (ED50 2.5 μg/kg) and ER (ED50 0.6 μg/kg) were identical in Ahb/b and Ahb/d mice. These data suggest that not all TCDD effects are mediated by the Ah locus.
TCDD produces a chloracne-like response in the skin of hairless HRS/J mice but not of their hairless littermates. The effect of dermal TCDD treatment (12 μg/kg in 100 μl acetone) was examined on liver and skin ethoxyresorufin-O-de-ethylase (EROD) activity, glucocorticoid receptor (GCR) binding, and epidermal growth factor (EGF) receptors in hairless mice. No difference was seen in the induction of hepatic EROD, but maximal induction in the skin of hairless mice was only 60% of that observed in hairless mice. No strain difference existed in the number (Rmax) or affinity (Rk) of GCR in the liver and skin of control hairless and hairless mice. TCDD resulted in a 30% decrease in the hepatic GCR Rmax. However, in skin, TCDD decreased GCR Rmax by 40% in the hairless mice but only 18% in the haired. No differences could be seen by sucrose density gradient centrifugation in the properties of the dexamethasone-GCR complex from the skin and liver of control and treated mice. The moderate decrease in the numbers of GCRs occurred later than the marked increase in EROD. No change was seen in the dermal EGF receptors following TCDD treatment in either strain as determined immunohistochemically. TCDD treatment resulted in epidermal hyperplasia and hyperkeratosis in the hairless mice. These changes were detectable 1 day after treatment. Thus, the genetic difference in TCDD sensitivity appears restricted to the target tissue.

In nature animals the acute toxicity of TCDD exhibits marked interspecies variability, with the LD50 in the hamster being approximately 10-fold greater than that in the rat. The fetal toxicity of TCDD was evaluated in these species to assess whether there is a similar interspecies variability in the toxicity of TCDD in the developing fetus. Pregnant Holtzman rats (22 day gestation period) were treated with TCDD in corn oil (0, 1.5, 3, 6, or 18 μg/kg, po) on gestation day (gd) 10 and were sacrificed on gd 20. Pregnant Golden Syrian hamsters (16 day gestation period) received TCDD in corn oil (0, 1.5, 3, 6, or 18 μg/kg, po) on gd 9 and were sacrificed on gd 15. In both species, there was a dose-related increase in the incidence of fetal resorptions and mortality. At 18 μg/kg, TCDD produced 72% fetal mortality in rats and 60% fetal mortality in hamsters, with no overt maternal toxicity in either species. Most fetal deaths occurred late in gestation and in the rat were associated with extensive hemorrhaging within the gastrointestinal tract. Fetal deaths in the hamster were associated with extensive edema and renal congestion. Although a few cleft palates and hydromidrotic were observed in viable rat fetuses, gastrointestinal hemorrhaging was the most sensitive indicator of in utero exposure to TCDD, with an incidence of 41% at a dose of 1.5 μg/kg. Kidney abnormalities were the most sensitive indicator of exposure in hamster fetuses, with an incidence of 42% at a dose of 3.0 μg/kg. Although there are species differences in the expression of fetal toxicity, the results suggest that the toxic potency of TCDD is similar in the developing rat and hamster. (Supported by March of Dimes Grant No. 15-12.)

Cleft palate (CP) and hydrophobia (HN) are sensitive indicators of TCDD teratogenicity, with CN occurring at doses below those inducing CP. The stage in organogenesis when the kidney is most sensitive to insult with TCDD has yet to be determined. The extreme persistence of TCDD makes identification of a critical period difficult. To characterize the window of sensitivity for the induction of CN, as well as to attempt to dissociate the induction of CN from CP, dams were treated with 0.12, or 24 μg TCDD/kg on gestation day (gd) 8 or 10, or 0.3, 6, 9, 12, or 24 μg TCDD/kg on gd 6. Fetuses were examined on gd 18 for the presence of CN and CP. Maternal weight gain and liver weight, as well as the CP/litter increased in a dose-related fashion for all days, except for the absence of CP at 3, 6, and 9 μg/kg on gd 6. A dose-related increase in maternal liver/body weight and mean fetal weight was observed for gd 6 and 0, respectively. Palatal sensitivity to TCDD increased with gestational age. The incidence for the renal lesion was close to 100% for all doses regardless of the day of exposure, but severity varied with dose. In conclusion, while sensitivity to CP was greater on gd 10, a critical window was not identified for the kidney. Dosing earlier in gestation allowed for dissociation of CN from the induction of CP, which suggests differences in organ sensitivity and/or compound distribution rather than just TCDD persistence.
AGE-RELATED CHANGES IN DERMAL ABSORPTION OF TCDD AND 2,3,4,7,8-PENTACHLORODIBENZOFURAN (4PeCDF).
Y B Banks, D W Brewster, and L S Birnbaum, NTENS, RTP, NC.

Physiologic changes in aging skin may alter dermal absorption of xenobiotics, including halogenated aromatics. These toxic and persistent compounds accumulate in human tissues. In order to examine changes in potential for systemic exposure to TCDD and 4PeCDF, the absorption, distribution, and elimination of these compounds were examined following dermal exposure in male Fischer 344 rats of various ages (TCDD - 3, 9, and 24 mo; 4PeCDF - 3, 9, 16, 24, and 30 mo). The compounds were applied to the back as a dose of 0.1µmol/kg in 60% acetone and covered with a perforated cap; animals were held in individual metabolism cages for 3 days. Dermal absorption of TCDD was significantly greater in the youngest age group: 17% of the administered dose at 3 mo vs 5% at 9 and 24 mo. Absorption of 4PeCDF was also significantly decreased in older age groups compared to the 3 mo rats. Major tissue depots for both compounds were liver, fat, muscle, and skin in 3 month rats. Liver was a major depot in the older age groups and fat was a secondary depot. Due to decreased absorption, liver and fat concentrations were significantly decreased in older TCDD-treated rats. Elimination of the absorbed dose of both compounds was limited at all age groups. Results indicate that these compounds are poorly absorbed through aging skin and that the potential for systemic exposure is decreased in older age groups.

KEY ENZYMES OF GLUCONEOGENESIS IN LIVERS OF TCDD-TREATED RATS. M Lebofsky, LWD Weber, H Greim and K Rozman. University of Kansas Medical Center, Kansas City, KS (USA), and Institut für Toxikologie, GSF München, Neuherberg (FRG).

Male Sprague-Dawley rats (350-360g) were injected ip with a lethal dose of TCDD (125 µg/kg in corn oil). An equal number of rats, matched by body weight, received vehicle only and was pair-fed to the TCDD-treated animals. 4, 8, and 16 days after treatment, 5 animals per group were sacrificed and activities of gluconeogenic enzymes were determined in liver. The activity of phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32) decreased slightly with time after treatment in the livers of pair-fed rats. The activity of microsomal glucose-6-phosphatase (G-6-Pase, EC 3.1.3.9), on the other hand, increased about 10%; neither change was statistically significant. The activity of PEPCK in the livers of TCDD-treated rats decreased with time, losing 32% by day 4 after treatment (p=0.05), 44% by day 8 (p=0.025), and 45% by day 16 (p=0.001), as compared to pair-fed controls. The activity of G-6-Pase decreased to a level 21% below that of the pair-fed controls by day 4 (p=0.01), 27% by day 8 (p=0.1), and 39% by day 16 (p=0.01). These findings, together with the reduced turnover of gluconeogenic precursors in TCDD-treated rats previously shown in our laboratory, suggest that TCDD affects gluconeogenic enzymes in a way which prevents synthesis of sufficient amounts of glucose. This may explain the low levels of blood glucose consistently observed in TCDD-treated rats. Together with the as yet unexplained reduction in feed intake this leads to a progressively increasing hypoglycemia, the apparent cause of TCDD-induced death in rats.

472 PENETRATION OF TCDD INTO HUMAN SKIN in vitro. LWD Weber, A Zesch and K Rozman. University of Kansas Medical Center, Kansas City, KS (USA); Institut für Arzneimittel, Bundesgesundheitsamt, Berlin (FRG); and Institut für Toxikologie, GSF München, Neuherberg (FRG).

Human post mortem skin was exposed to 650, 65, or 6.5 ng/cm² of 3H- or 14C-labeled TCDD, in Franz cells at 32°C for up to 1,000 min. Vehicles were acetone ("dry exposure") or mineral oil ("oily exposure"). Damage to skin was simulated by removal of the horny layer in one-half of all skin samples. Circular punches were taken from frozen skin samples and sectioned along their natural layers. Radioactivity in skin layers was determined by scintillation counting. Dermal absorption of TCDD increased with time of exposure. The absorbed fraction did not change much with dose under conditions of dry exposure, but increased with oily exposure. After dry exposure to TCDD maximum concentrations in epidermis were in the 50 nM range, with the horny layer removed. Intact horny layer decreased this concentration by one order of magnitude, and mineral oil as the vehicle decreased it by another order of magnitude. Epidermal concentrations directly reflected the amount of TCDD administered per unit area of skin. It is generally assumed that only that portion of a topically administered substance is available for systemic absorption that passes the epidermis and reaches the dermal compartment. Under such premises, after 1,000 min dry exposure of skin with intact horny layer, only 2% of the dose would be absorbed systematically, and about 5% when the horny layer had been destroyed. After oily exposure about 7 times lower values of absorption were recorded. Rates of absorption were 0.1-0.3% of dose per hour with acetone, and 0.02-0.05% of dose per hour with mineral oil as the vehicle.

Exposure to TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) reduces food intake. Disregulation of amino acid metabolism by TCDD might result in increased plasma tryptophan (TRYP) levels thereby enhancing serotonin (5-HT) neurotransmission in the hypothalamus to reduce feeding. To test this hypothesis, male Sprague-Dawley rats were injected with TCDD (125 µg/kg, ip) or vehicle and sacrificed 4, 8 or 16 days later. TRYP, 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were measured in the hypothalamus and striatum. TRYP was also measured in the plasma. Compared to pair fed controls, plasma TRYP levels were increased in TCDD-treated rats on all days; brain TRYP tended to increase similarly. TCDD treatment also increased hypothalamic 5-HT levels (4, 8 and 16 days), striatal 5-HT levels (8 and 16 days), and hypothalamic 5-HT (16 days) concentrations. No change in melatonin, dopamine, or DOPAC concentrations were observed in either brain area. Thus, TCDD may decrease food intake in part through increased hypothalamic serotoninergic mechanisms secondarily to elevated plasma TRYP levels. Furthermore, TCDD also increases striatal 5-HT but not dopamine turnover.

(Supported in part by NIH HL-38072 to RHA)


Despite much research, the mechanism of action of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is still unclear but appears to involve interactions with hormonal systems, especially estrogens. Due to the extreme complexity of the underlying processes, mathematical modeling and computer simulations can provide useful information. A multi-compartmental model was constructed that includes a number of components or variables such as estrogen levels, steroid serum binding proteins, estrogen-receptor complexes, E2 receptors, and DNA. Each compartment was modeled as an ordinary differential equation describing the time-dependent behavior of the variable. The synthesis of a variable and its interactions such as binding, dissociation and the metabolism of other variables were considered. The dynamic behavior of the estrogen system modeled by differential equations was further examined under the perturbation of TCDD through numerical solutions of the equations and computer simulations. (Supported by Environ. Occupat. Health Sci. Institute).

MOLECULAR PROPERTIES OF THE RAT AND MOUSE CYTOSOLIC Ah RECEPTOR COMPLEX: RADIOLIGNAND-DEPENDENT EFFECTS. J Fiskorska-Pilszczynska and S Safe. Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX.

Using rat and mouse hepatic cytosol prepared in low salt buffers, it was demonstrated that for [3H]-2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and [3H]-2,3,7,8-tetrachlorodibenzo-furan (TCDF), the following molecular properties were radioligand-independent: sedimentation coefficients, Stokes radii, relative molecular masses, frictional and axial ratios. In contrast, after incubation in high salt buffer (0.4 M NaCl), the molecular properties of the complexes were highly variable and depended on the source of the cytosol and the structure of the radioligand. Moreover, using a series of unlabeled competitive ligands, it was evident that the displacement of the radioligands was dependent on the incubation conditions (high or low salt), the source of cytosol (rat or mouse) and radioligand structure (TCDD or TCDF). For example, in high salt buffers, unlabeled 3-methylcholanthrene competitively displaced [3H]-TCDD but not [3H]-TCDF from the rat hepatic cytosolic receptor (ES-0354).


Female SD rats (ca. 100g) were treated by gavage with decontaminated soil or soil to which TCDD was added immediately before use (final dose 10 µg TCDD/kg). Four rats per group were sacrificed 1 or 4 days after dosing and liver microsomes were prepared. Aryl hydrocarbon hydroxylase and cytochrome P-450 activities were induced by TCDD treatment at both time points. Membrane fluidity was determined by the fluorescence depolarization technique using all trans-1,6-diphenyl-1,3,5-hexatriene as the fluorescent lipid probe. One and four day controls had polarization (p) values of 0.164±0.004 and 0.167±0.003 respectively while rats treated with TCDD on soil had p values of 0.160±0.005 (1 day) or 0.160±0.003 (4 day), at 37°C. These values are not statistically different, suggesting that a TCDD dose sufficient to induce microsomal enzymes was not sufficient to cause alterations in the lipid fluidity of microsomal membranes. (Supported in part by USEPA CRAB11A-01-1).
HEPATIC DNA SYNTHESIS IN C57BL/6J AND DBA/2J MICE TREATED WITH 2,3,7,8-
TETRACHLORODIBENZO-P-DIOXIN (TCDD). P L Scala, T H Umbreit, W Lutz, and M A.
Gallo. Dept. Environ. Comm. Medicine., UMDNJ/AbbV. W. Johnson Medical School,

DNA synthesis was studied in young female C57Bl/6J and DBA/2J mice. Hepatocyte
division was synchronized by limiting food availability to 4 hours per day for 14 days.
Mice were given ip doses of 0 to 50 ug TCDD/kg 24 hours before receiving tritiated
thymidine (TDR) by gavage. Mice were sacrificed after three hours, and thymidine
incorporation into hepatic DNA measured. Food consumption and body weight gain
returned to normal levels by 7 days on restricted availability and was unaffected by
TCDD. TDR incorporation index did not differ from control mice in either strain, nor
were differences between strains detected. Thus stimulation of DNA synthesis
by TCDD does not appear to be an early effect of TCDD on the liver of female mice, nor
is there a difference in DNA synthesis between sensitive and non-sensitive mouse
strains.

THE EFFECTS OF TCDD ON ESTRADIOL
LEVELS AND ESTROGEN RECEPTOR LEVELS
IN LIVER AND UTERI OF CD-1 MICE. M DeVito, S
Mackenzie, E Martin, T Umbreit and M Gallo. Graduate
Program in Public Health and Dept. of Pathology, UMDNJ-RW Johnson Medical School Piscataway NJ.

Ovariectomized (OVX) and intact female 40 day old CD-1 mice received corn oil or 2,3,7,8-tetrachloro-
dibenzo-dioxin (TCDD) (1, 3, 10, 30 or 100 ug/kg) and were sacrificed two days later. Estrogen receptor (ER) levels were determined using an ER-EIA kit (Abbott). Treatment of intact animals with TCDD resulted in a dose-dependent decrease in cytosolic ER levels in liver and uterus and a dose-dependent increase in AHH activity and P-450 levels. TCDD did not alter serum estradiol levels. OVX animals showed a decrease in uterine ER levels and a decrease in liver ER levels when compared to the intact control group. Treatment of OVX animals with TCDD resulted in a dose-dependent decrease in liver ER levels but did not alter uterine ER levels. However, OVX animals were less sensitive to the ER-decreasing effects of TCDD when compared to control animals. In OVX animals, treatment with TCDD also resulted in a dose-dependent increase in AHH activity and P-450 levels, but TCDD did not alter serum estradiol levels in OVX animals. These results suggest that the ER-decreasing effects of TCDD are not directly related to alteration in serum estradiol levels. In addition, OVX, which lowers serum estradiol levels by two orders of magnitude, markedly affects the ER-decreasing ability of TCDD possibly through a centrally mediated mechanism. (Supported in part by NJDOJ Grant # 45127-3503.)

HISTOPATHOLOGICAL OBSERVATIONS OF TCDD-
ESTROGEN INTERACTION IN MOUSE LIVER. MA Gallo
and TH Umbreit. Dept Environ Comm Med, JGPT,
UMDNJ-RW Johnson Med Sch, Piscataway, NJ.

Immature female CD-1 mice were dosed with
TCDD (200ug/kg), estradiol (E2, 40ug/mouse/day) or tamoxifen (Tmx, 5mg/mouse/day) in a Latin Square design, and
estrogen receptor (ER) levels determined by
EIA. Upon autopsy, mice receiving TCDD/E2 or
TCDD/E2/Tmx had severely mottled livers (with
visible nodules in some), and altered uteri and
vomaries. In these livers there was marked
extramedullary hematopoeisis and a remarkable
dysplastic adenomatous proliferation of bile
duct epithelium which had taken on the
appearance of extrahepatic ductal tissue. The
cuboidal epithelium was transformed to a
highly dysplastic columnar epithelium. A novel
lesion appears to be a well circumscribed
proliferation of basophilic cells manifested
as a packet of small cells with dense nuclei
and scant cytoplasm. These lesions appear
to be an extension of bile ducts, possibly from
basement membranes. Interestingly, Tmx, a
mixed antagonist of ER, exacerbated this liver
lesion while antagonizing the E2
uterotrophic. (Supported in part by NJDOJ
Grant #45127-3503).

INDUCTION OF CYTOCHROME P-450 AS A MARKER OF
THE TRANSPULMONARY ABSORPTION OF TCDD. C S
Nessel, M A Amoruso, T H Umbreit, R J Meeker, M A
Gallo. Graduate Program in Public Health, Dept. of
Environ. and Comm. Medicine, UMDNJ-R W Johnson
Medical School, Piscataway, NJ.

Intratracheal instillation was utilized as a surrogate for
inhalation to examine the transpulmonary absorption of
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Female
Sprague-Dawley rats (200-250 g) were
intragatthally administered 0, 3, 10, or 30 ug
TCDD/kg in 0.25 ml corn oil and sacrificed 4 days later.
There was a significant increase in relative liver weight in
to TCDD-treated groups compared to controls. Total
cytochrome P-450 and aryl hydrocarbon hydroxylase
(AHH) were determined in hepatic microsomal fractions
and increased in a dose-responsive manner. Total
P-450 increased two-fold at the high dose (1.27 ± 0.16
vs. 0.65 ± 0.06 nmols P-450/mg prot), while low and
mid dose groups increased 55% and 68%, respectively.
AHH increased eleven-fold at the high dose (1.94 ± 0.18
vs. 0.18 ± 0.10 pmols AHH/mg prot/min), while low
and mid dose groups increased nine- and ten-fold,
respectively. This was comparable to induction following
ip administration of TCDD, illustrating similar
absorption across the lung and peritoneum. These
results indicate that TCDD is absorbed across the lung
following intratracheal instillation and that inhalation
may be an important route of exposure for TCDD risk
assessment. (Supported by NJ DEP Grant C29510).
POTENTIATION OF TCDD TOXICITY IN CD1 MICE BY TAMOXIFEN. S A Mackenzie, M J Devito, T H Umbreit, M A Gallo. JGPT, UMDNJ/RW Johnson Medical School and Rutgers U., Piscataway, NJ.

Previous studies in our laboratory showed increased TCDD toxicity in CD1 mice dosed concurrently with tamoxifen (TMX). Female CD1 mice received a total i.p. dose of 0, 50, 100 or 200 ug/kg TCDD and 0 or 1 mg/kg/day s.c. TMX, both in corn oil, and were observed for 60 days. TCDD produced ascites and lethality and these effects were potentiated by TMX. LD50 values were 250 ug/kg for TCDD alone and 170 ug/kg for TCDD-TMX. Other female CD1 mice received 0 or 200 ug/kg i.p. TCDD and 0 or 1 mg/kg/day s.c. TMX and were sacrificed on day 11. TCDD and/or TMX altered specific organ/body weight ratios (liver, thymus) but produced only minor changes in selected clinical chemistry parameters. TMX potentiated the TCDD induction of total P450 (p < 0.05) but decreased AHH activity/mg P450 (p < 0.05). Thus TMX is able to potentiate TCDD by a mechanism not involving AHH induction. Supported in part by NJDOJ Grant #45127-3503.

INTERCELLULAR COMMUNICATION AND RODENT HEPATOCYTE HETEROGENEITY. J A Hampton, C M Weghorst, R J Ruch, and J E Kleinin. Department of Pathology, Medical College of Ohio, Toledo, OH.

Using cell culture techniques, recent emphasis of this laboratory has assessed rodent hepatocyte intercellular communication (IC) following exposure to a variety of compounds. In 24 hr mouse hepatocyte cultures, previous experiments have shown that phenobarbital treatment (4 hrs) inhibits IC. However, when hepatocytes are isolated by collagenase perfusion, the structural heterogeneity of the lobule is destroyed. We have designed experiments to investigate the effects of phenobarbital on IC in enriched populations of periportal and pericentral cells. In the male strain A mouse, hepatocytes were isolated into periportal (high density) and pericentral (low density) enriched fractions using Percoll discontinuous density gradients (1.06-1.12 g/ml). IC was assessed through dye transfer. In 24 hr cultures of pericentral enriched cells, phenobarbital treatment (4 hrs) resulted in 8% of the cells communicating, while 80% of DMSO treated cells exhibited dye transfer. In 24 hr cultures of periportal enriched cells, phenobarbital treatment (4 hrs) resulted in 62% of cells communicating, while 95% of DMSO treated cells exhibited dye transfer. These results indicate methods have been developed to study in culture, functional hepatocyte heterogeneity to phenobarbital.


Structurally similar cyclic hydrocarbons were evaluated in the V79 metabolic cooperation assay and their response correlated to their known carcinogenicity. The V79 metabolic cooperation assay is based on the recovery of 6TG resistant mutants from mixed cultures of wild type and mutant cells. Inhibition of cell-cell communication results in the enhanced recovery of mutant cells. For a compound to be considered as positive, it must significantly enhance the recovery of mutant cells (p<0.005). At least for two concentrations in two separate experiments or at least two consecutive concentrations in one experiment. The sensitivity of the V79 metabolic cooperation assay to detect known carcinogens was 92% (the suspect carcinogens were included in this calculation). The specificity of the assay was 75% and the accuracy, the ability to correctly identify both carcinogens and non carcinogens was 80%. Supported in part by US EPA contracts 63-02-4032 and 68-02-4450.


In vitro morphological transformation of primary Syrian hamster embryo cells is one of the most frequently used transformation systems for identification of carcinogenic compounds. Tumor promoting phorbol esters potentiates the induction of transformed morphology and do also inhibit intercellular communication in these cells as well as in transformation sensitive cell lines. Phorbol esters have however, no effect on cell-cell communication between cells that are resistant towards transformation by phorbol esters. Indicating a relation between morphological transformation and inhibition of communication. Vanadium compounds are on the other hand potent inducers of morphological transformation without affecting intercellular communication. This show that morphological transformation is not necessarily linked to inhibition of cell-cell communication. Supported by The Norwegian Cancer Society.
Our group has been studying the effects of carcinogens and other agents on primary cultured rodent hepatocyte intercellular communication (IC). Since culture conditions may modify hepatocyte IC, we examined the effects of three media (RPMI 1640, William's E, and Leibovitz's L-15) on mouse and rat hepatocyte IC. Each medium was supplemented with 10% fetal bovine serum, 100 IU penicillin/ml, and 100 μg streptomycin/ml. IC was evaluated by Lucifer Yellow CH dye-coupling. In each of the media, mouse and rat hepatocyte IC increased rapidly to >85% coupled cells after 12 h in culture. In RPMI 1640, hepatocyte IC decreased over the next 12 h to <10% coupling and no communication could be detected after 48-120 h culture. Hepatocyte IC in William's E and L-15 media was maintained at >80% coupling after 24 h culture then gradually decreased to 10-20% coupled cells over the next 96 h. The rapid loss of hepatocyte IC in RPMI 1640 medium was not due to decreased cell viability. Addition of 1 μM dexamethasone, 5 mM sodium pyruvate, and/or 5 mM L-alanine to RPMI 1640 enhanced hepatocyte IC. Thus, hepatocyte IC and gap junction expression is dependent on the composition of the culture medium.

Peroxisome proliferators have been shown to induce rodent hepatocarcinogenesis, possibly through nongenotoxic mechanisms. Inhibition of intercellular communication (IC) by nongenotoxic carcinogens may serve to isolate preneoplastic initiated hepatocytes from the growth control of surrounding normal cells. This may allow the altered hepatocytes to progress into neoplasia. We have examined the effects of the hypolipidemic agent, nafenopin (NAF), and other peroxisome proliferators (clofibrate, M2H, and TCA) on IC in cultured male F-344 rat hepatocytes that were chronically treated with NAF (50 μM). IC was evaluated by dye-coupling. Peroxisome proliferation was evaluated by measuring palmitoyl-CoA oxidase. Inhibition of IC occurred after 48, 72 and 96 h in cells treated chronically with Naf. Enhanced peroxisome proliferation and DNA synthesis were also detected after 48 h exposure to Naf. Inhibition of IC by peroxisome proliferating compounds may require prior induction of peroxisome enzymes.

Gap junctional intercellular communication (GJIC) has been postulated to play an important role in tumor promotion. The tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) has been reported to inhibit GJIC in several mammalian cell types in culture. In order to further investigate the effect of tumor promoters on GJIC, we measured the kinetics of GJIC inhibition in rat liver WB cells exposed to varying concentrations of TPA. This information may be useful in understanding the mechanism(s) associated with alteration of gap junctional communication by TPA. GJIC was measured using fluorescent recovery after photobleaching (FRAP) analysis following a 1 hour TPA exposure in synchronized cultures of rat liver WB cells. TPA inhibited GJIC in WB cells in a dose-dependent manner at all concentrations tested (0.01, 0.1, 1, 10 and 100 ng/ml). GJIC was completely inhibited following incubation with 100 ng/ml TPA. At the remaining concentrations of TPA tested (0.01, 0.1, 1 and 10 ng/ml), the kinetics of fluorescent dye (5,6-dicarboxyfluorescein diacetate) recovery following photobleaching was affected in a concentration-dependent manner. These findings indicate that the rate of transfer of material between communicating cells is concentration dependent, suggesting that GJIC in this model may involve a mechanism that is associated with a graded response rather than an all-or-nothing effect.

Inhibition of gap junction mediated intercellular communication (IC) by nongenotoxic carcinogens may upset normal growth processes and permit the proliferation of preneoplastic cells. In this study, we evaluated the effects of several nongenotoxic carcinogens on primary cultured rodent hepatocyte IC paying particular attention to the correlation between the inhibition of IC and the in vivo carcinogenicity of these chemicals. IC was detected by dye coupling using lucifer yellow. Carcinogens were examined at sublethal concentrations. Pb, sodium barbital, amobarbital, barbituric acid, DDT, lindane, dieldrin, TCE, TCA, and Arco 1254 were examined for their effects on rat and mouse hepatocyte IC. A direct correlation was observed between the ability of a compound to inhibit IC and the hepatocarcinogenicity of the compound. The observed inhibition of IC also correlated with the strain and species susceptibility to hepatocarcinogenesis seen in vivo.
ROLE OF INHIBITION OF HEPATOCYTE INTERCELLULAR COMMUNICATION IN TUMOR PROMOTION. J. E. Klaunig, R. J. Ruch, S. G. Lilly and C. M. Weghorst, Department of Pathology, Medical College of Ohio, Toledo, Ohio.

Intercellular communication through gap junctions (IC) may serve to regulate normal cellular growth and differentiation. The inhibition of IC by carcinogens may upset normal growth and differentiation processes and permit the proliferation of preneoplastic cells, thus enhancing tumor formation. In this study we examined the effect of 2 nongenotoxic carcinogens (PB and lindane) on IC in preneoplastic hepatocytes (PNH) and normal hepatocytes (NH). IC was detected as the passage of fluorescent Lucifer Yellow CH dye from microinjected, dye-loaded hepatocytes to neighboring hepatocytes. PNH and NH exhibited IC at 80-90% coupling when cultured separately. PB and lindane inhibited IC in a dose-responsive manner. When co-cultured, the PNH and NH cells displayed a dramatic decrease in coupling between PNH and NH cells. IC was completely absent when the cocultured cells were exposed to PB or lindane.


Various environmental chemicals were tested for carcinogenic potential using a 6-week bioassay system based on the two-stage concept of hepatocarcinogenesis. Rats were initially given a single ip dose (200 mg/kg) of diethylnitrosamine (DEN) and starting 2 weeks later were treated with test compounds for 6 weeks and then killed. All rats were subjected to partial hepatectomy at week 3. Carcinogenic potential was scored by comparing the number and/or area per cm² of glutathione S-transferase placental form-positive (GST-P⁺) foci in the test group livers with those of the control group given DEN alone. Test compounds used were as follows: hepatocarcinogens: N-ethyl-N-hydroxyethylnitrosamine (SEHEN, 0.05% in water), dibutylaminonitrosamine (DBAN, 0.1% in water), o-toluidine (0.1% in diet), quinoline (0.25% in diet), auramine (0.05% in diet), aldrin (0.005% in diet), HC-blue No. 1 (0.3% in diet), nonhepatocarcinogens: N-methyl-N-nitrosourea (MNNU, 10 mg/kg, 2 weeks, ip) and noncarcinogens; HC-blue No. 2 (0.3% in diet). In this assay, AHC, DBN, quinoline, auramine, aldrin and HC-blue No. 1 increased either the numbers or areas of GST-P⁺ foci, but o-toluidine showed no significant effects. MNNU and HC-blue No. 2 were negative. The system therefore appears useful for rapid detection of carcinogenic agents.


WB rat liver epithelial cells were infected with a retrovirus containing the v-H-ras gene associated with the neomycin drug resistance marker gene (WB-Hras). Intercellular communication (IC) was compared in WB-Hras, non-infected WB cells, and WB cells infected with only the neomycin resistance gene (WB-neo). IC was studied using FRAP, dye injection and scrape-loading/dye transfer assays. Only the WB-Hras cells had significantly reduced IC. When the WBHrass cells were injected subcutaneously or into the portal vein of F344 rats, tumors developed rapidly, whereas those rats injected with the WB-neo cells did not develop tumors. Further study of the relationship between the expression of the H-ras oncogene, cell communication and tumorigenicity in these cells may aid in the elucidation of the relationship between IC and tumor development.

INITIATION-PROMOTION BIOASSAY IN MOUSE LIVER. M. W. Khoury, P. L. Barnwell, R. K. Wassmund, J. E. Klaunig and M. A. Pereira. Environmental Health Research and Testing Inc., Cincinnati, OH; Medical Center of Ohio, Toledo, OH.

A short-term/limited liver initiation-promotion bioassay in 15 day old mice is being developed for determination of carcinogenic, tumor initiating and tumour promoting activity. The bioassay consists of administering the test substance for initiation on day 15 of age to male mice followed by promotion with 500 ppm sodium phenobarbital administered in the drinking water starting on day 28 of age and continuing until sacrificed on day 180 of age. Liver tumor incidence is determined. C3H mice were sensitive at day 15 of age to seven of the eight chemical carcinogens tested and phenobarbital promotion was required for activity by 5 of 7 carcinogens ( aflatoxin B1, 2-acetylaminofluorene, benzidine, DMBA, and benzenepthalamine). Diethylnitrosamine (DEN) and dimethylnitrosamine were active in C3H mice with/without phenobarbital promotion. B6C3F1 mice were sensitive to only DENA, dimethylnitrosamine and DMBA and phenobarbital inhibited the occurrence of DENA-initiated tumors. Thus, C3H mice were more sensitive than B6C3F1 mice. Once developed and validated this liver initiation-promotion bioassay in 15 day old mouse liver would be very useful for the evaluation of the carcinogenic hazard of substances.
The efficacy of the short-term assay for predicting the outcome of 2-year studies for positive or negative trends for leukemia was evaluated with 13 chemicals. Male Fischer rats were injected with leukemic mononuclear cells from spleens of syngeneic donors. The effects of chemical dosage on tumor progression were evaluated 60 days later. In all cases the short-term assay accurately predicted the results for leukemia in 2-year tests. In transplant recipients dosed with chemicals positive for leukemia there were dose-related increases in spleen weights and WBC counts, and decreases in RBC indices and platelet counts by comparison with responses in rats with transplants but no chemical treatment. The reverse was true for chemicals that caused negative trends for leukemia in 2-year tests. These data were confirmed by histopathological examination of spleen and liver. There were examples of structure-activity relationships for chemicals that were both positive and negative for leukemia. Dichlorovos and other chemicals containing the dimethyl ester of phosphoric acid enhanced tumor progression, while short-chain glycol ethers inhibited and/or arrested tumor progression.

A comparison of the polyaromatic aromatic compound (PAC) content and skin carcinogenicity was made for a series of complex oil mixtures. Results showed a significant correlation (r=0.84) between the 3-7 ring PAC content and the carcinogenic potency, as determined in a mouse skin-painting bioassay, for oil samples ranging from those with median (50% recovered) boiling points above 500°F to those with initial boiling points of 107°F. Two variables not considered in the original correlation study, oil viscosity and oil dermal penetration rate, were subsequently measured and found to correlate with the carcinogenic potency of the oils determined using simple and multiple regression techniques. Although a weak correlation was observed between oil viscosity and the dermal penetration rate of 14C-benz[a]pyrene from the oils (co-linear variables), no statistically significant correlation was observed between viscosity or penetration rate and carcinogenic potency or between transformations or combinations of viscosity and penetration rate data and carcinogenic potency. It is suggested that the repeated dosing of mice in a two-year skin-painting bioassay results in the attainment of a steady-state concentration of the oils in the skin compartments such that the concentration of 3-7 ring PAC at the sites of cutaneous metabolism, and the concentration of carcinogenic metabolites is proportional to the 3-7 ring PAC content of the applied oil.
Male and female B6C3F1 mice were given 1 or 2 g/L of dichloroacetate (DCA) or trichloroacetate (TCA) for 5 days. To provide an intermediate total dose of each compound, other groups of mice were given 2 g/L of DCA or TCA for 9 mo and removed from treatment for the remaining 3 mo. At termination, 18, 73, and 96% of the male mice treated with DCA at the low, intermediate, and high total doses had multiple hepatic tumors, respectively. The same doses of TCA produced 45, 36, and 79% incidence. There were no treatment-related liver tumors in female mice. DCA produces a marked hypertrophic effect on the liver that is associated with focal necrotic lesions, effects not observed with TCA. DCA also induces low frequency basophilic and glycogen-poor foci in the liver at 6 mo. Thus, DCA and TCA induce hepatic tumors in B6C3F1 mice in accordance with the sex-linked spontaneous liver tumor rates. Unlike TCA, DCA accelerates tumor progression to an irreversible stage by 9 mo. This is signalled by an early appearance of foci which apparently have a high probability of progressing to tumors. (Funded by U.S. Air Force Grant #AFSOR-86-0284).

Various non-genotoxic chlorinated hydrocarbons many of which are known hepatic tumor promoters, were examined for their ability to stimulate protein kinase C (PKC) activity in mouse brain 10pg kg utilizing lysine-rich histone as a phosphate acceptor. Chlorodane, kepone, heptachlor, DDD, DDT, aldrin, DFO, and lindane were the most potent stimulators of PKC activity. Chlorodane (100 μM) stimulated PKC to a maximum velocity similar to that obtained when the enzyme was maximally stimulated with 12-0-tetradecanoylphorbol-13-acetate (TPA), a potent skin tumor promoter. Chlorodane-stimulated PKC was calcium-dependent, and in the presence of calcium, chlorodane-stimulated PKC activity was five fold greater than in the absence of added calcium. In contrast, the addition of calcium only minimally affected the TPA-stimulated PKC activity (1.3%). Concentrations of chlorodane and TPA which maximally stimulate PKC did not produce an additive effect on PKC activity. Chlorodane-stimulated PKC was phospholipid-dependent and could be inhibited by quercetin, an inhibitor of PKC. Chlorodane also stimulated mouse epidermal and hepatic PKC and purified rat brain PKC in the presence of calcium. Therefore, a wide variety of chlorinated hydrocarbons which are known rodent hepatic tumor promoters stimulate hepatic, epidermal and brain PKC.

Both cigarette smoke and nitrosamines have been suggested as contributors to human risk of perinatal cancer initiation. The transplacental (TP) carcinogenicity of NNK was tested in A/J, (C3H/He x 57BL/6) F1, and Swiss (Nijm) mice. Three doses of 100 mg/kg were given ip during the last week of gestation. In A/J offspring killed at 24 wks, 12/66 (18%) had a lung tumor, vs 4/87 (5%) of controls (p<0.05). In C3H/He male offspring at 72 weeks, TP NNK resulted in 12/30 (40%) with liver tumor vs 8/46 controls (17%) (p<0.05). Postnatal treatment of these mice with Na barbital (0.5% in the drinking water) or a single 500 mg/kg dose of a mixture of polychlorinated biphenyls (PCB) did not alter liver tumor numbers. NNK-exposed Swiss male offspring also developed liver tumors, in significantly greater numbers after PCBs given on day 56 (5/26, 19%) vs 3/57 (5%) for NNK only (p<0.05). Total liver tumor incidence for both NNK groups, 8/83, was significantly greater than for controls, 0/66. The NNK mothers all developed lung tumors in high incidence; the C3H mothers presented liver tumors also. Thus NNK is a weak transplacental carcinogen for sensitive fetal target organs in the mouse.

Pentachlorophenol (penta) is a broad spectrum biocide which has been used for many years for the preservation of wood. Groups of 35-50 male or female 6 week old B6C3F1 mice were exposed via feed to 2 commercial grades of penta; a technical grade (composite from 3 manufacturers) and Dowicide EC-7 (a grade containing much lower amounts of dibenz-0-dioxins and dibenzofuran) for a period of two years. Based on the results of 6-month prechronic studies the doses were used were 100 and 200 ppm for technical penta and 100, 200, and 600 ppm for EC-7. Survival was not affected by either grade of penta. Treatment related neoplasms were observed in the liver (hepatocellular adenomas and carcinomas), adrenal medulla (pheochromocytomas) and vascular system (hemangiosarcomas of the liver and spleen) with both grades of penta, although the carcinogenic response was less marked at a given dose of EC-7. The results of these studies show that pentachlorophenol is carcinogenic in the mouse and that the carcinogenic response is primarily due to penta itself rather than its contaminants.
CARCINOGENIC EVALUATION OF 3,3'-DIMETHYLBENZIDINE (DMB) IN BALB/C MICE. G J Schieferstein, Y Shimohara, R R Allen, D L Greenman, and W T Allaben, National Center for Toxicological Research, Jefferson, AR

DMB, a chemical congener of the known human bladder carcinogen benzidine, is used to manufacture colorfast dyes. Dyes derived from DMB are known to be metabolized to parent DMB by laboratory animals. This study investigated possible DMB-induced carcinogenicity in mice because DMB, a known carcinogen in the rat, was non-carcinogenic in the hamster. For an appropriate carcinogenic risk assessment, another species was required. Male and female BALB/C mice were exposed to 0, 0.9, 1.8, 3.5, 7.0 or 140 ppm of DMB in their drinking water and were sacrificed at 13, 26, 39, 52, 78, and 116 weeks on dose. A complete necropsy and histological analysis was performed at all sacrifices and on all dead and moribund animals. There were no treatment-related effects on body weight, water or food consumption. In male mice only, treatment-related increases in the incidence of fatal lung alveolar cell bronchial adenomas, bronchial carcinomas, or fatal neoplasms combined were observed. Non-fatal lung tumors did not have a statistically significant dose-related trend. The fatal lung tumors appeared around 78 weeks on the 140 ppm DMB dose. A treatment-related decrease in the time-to-death from fatal lung neoplasms was also observed. This study is the first to report DMB-induced neoplasia in the mouse.

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ACTIVATED K-ras IN 1,3-BUTADINE-INDUCED B6C3F1 MOUSE LUNG TUMORS. T Goodrow, S Reynolds, R Marnoport, and M Anderson. NIH, RTP and *University of North Carolina, Chapel Hill, NC.

1,3-Butadiene is used extensively in the production of polymers and synthetic rubber. Male and female B6C3F1 mice have been shown to develop lung tumors following chronic exposure to butadiene (NTP, 1984). We obtained lung tumors from male and female B6C3F1 mice treated chronically with 20 ppm to 625 ppm butadiene by inhalation. High molecular weight DNA was isolated from 9 lung tumors from 4 female and 5 male mice. 7 of 9 lung tumor DNA samples induced morphological transformation of NIH 3T3 fibroblasts in the NIH 3T3 DNA transfection assay. Transforming efficiency was relatively high for 6/7 of the lung tumor samples (0.25 - 0.75 foci/μg DNA) and subsequent Southern blot analysis of representative NIH 3T3 foci DNA indicated that the transforming gene present in these samples was an activated K-ras oncogene. Both rearranged bands and amplified signals were detected in the foci DNA. The identity of the putative activated gene in the 7th lung tumor sample has not been determined. The specific activating lesions in the K-ras containing transforming DNA will be characterized by amplification of the K-ras genes by the polymerase chain reaction followed by direct sequencing. Identification of the lesion and comparison with lesions found in B6C3F1 mouse spontaneous lung tumors may aid in the risk assessment of this chemical.

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ALtered METHYLATION OF RAS Oncogenes IN BenZIDINE-INDUCED B6C3F1 Mouse Liver Tumors (MLT). R L Vorce and J L Goodman, Michigan State University, East Lansing, MI.

The B6C3F1 mouse is a hybrid strain exhibiting sensitivity to chemical induction of MLT and a spontaneous hepatoma incidence (30%) intermediate between that of its paternal C3H/He (60%) and maternal C57BL/6 (1%) strains. Activated oncogenes, Ha-ras and, to a lesser extent, Ki-ras have been reported in B6C3F1 MLT. Because alterations in capacity for expression, as well as mutation, may be involved, this study examined a putative control point for transcription, i.e., the methylation state of a gene. Hypomethylation is necessary, but not sufficient, for transcription. Restriction enzyme analysis was used to assess DNA methylation. Msp I digestion of B6C3F1 and C3H/He DNA revealed the absence of a 15 kb Ha-ras band present in Msp I-digested C57BL/6 DNA, suggesting that Ha-ras of B6C3F1 and C3H/He mouse liver lacks a methylated site. In other respects, the Ha-ras and Ki-ras oncogenes are hypermethylated. Oncogene methylation was also assessed in benzidine-induced MLT and adjacent non-tumor tissue from B6C3F1 mice. In 4/4 cases, Ha-ras was hypomethylated in MLT as compared to non-tumor tissue; Ki-ras was hypomethylated in 2/4 cases. These results suggest that hypomethylation of oncogenes may provide an epigenetic mechanism for facilitating their aberrant expression. Both the methylated site in Ha-ras in B6C3F1 and C3H/He mouse may indicate an increased potential for its expression which could, in part, account for the high propensity for hepatoma development in these two strains. (Supp. by ILSI Risk Sci. Inst.)

Our objective is to test the hypothesis that alterations in DNA methylation may be an epigenetic mechanism by which non-mutagenic compounds that are positive in carcinogenicity bioassays could facilitate the aberrant expression of oncogenes. In these studies, the effect of TCE treatment on the methylation status of the Ha-ras and Ki-ras oncogenes in the liver of the male B6C3F1 mouse was assessed using the restriction enzyme isoschizomers Msp I/Hpa II. Activation of both of these oncogenes (primarily Ha-ras) has been implicated in hepatocarcinogenesis in the B6C3F1 mouse. Hypomethylation is necessary, but not sufficient, for transcription. TCE was administered by gavage, in corn oil, at a dose of 0.25 g or 1.0 g/kg/day, the maximum tolerated dose (MTD) to mimic bioassay conditions, for 3 days prior to and 3 days following partial hepatectomy (PH). Samples were obtained at 84 hours post-PH. Following treatment with 1.0 g/kg/day there was a trend toward hypomethylation of the Ha-ras, but not the Ki-ras oncogene. This indicates that the MID of TCE, under conditions of hepatocyte proliferation, might have the potential to facilitate expression of Ha-ras in the B6C3F1 mouse which could contribute to hepatoma development. Supp. by ILSI Risk Sci. Inst.

Lipoxygenase catalyzed peroxyl radical formation and epoxidation of 7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene (BP-7,8-diol). M F Hughes, W Cham- ultrat, R P Mason, and T E Elzing. Laboratory of Molecular Biophysics, NIH, Research Triangle Park, NC. Sponsor: G L Foureman.

A lipoxygenase catalyzed peroxyl radical formation and epoxidation of BP-7,8-diol was examined. Epoxidation of BP-7,8-diol was catalyzed by 8- and 15-lipoxygenase in the presence of either arachidonic acid (AA), y-linolenic acid, or 15-hydroperoxyeicosatetraenoic acid (15-HPETE). The anti-BP-7,8-diol-9,10-epoxide isomer was formed in greater quantities than the syn isomer, indicative of peroxyl radical mediated epoxidation. Epoxidation was dependent on time, enzyme and arachidonic acid concentration. The lipoxygenase inhibitor nordihydroguaiaretic acid inhibited epoxidation in a dose-dependent manner in incubations initiated with either AA or 15-HPETE. The anti-oxidant butylated hydroxyanisole also inhibited the epoxidation. Incubations conducted under an argon atmosphere significantly decreased epoxidation. This suggests that the oxygen inserted into BP-7,8-diol is derived from the atmosphere. A peroxyl radical was detected by electron spin resonance in incubations of 15-lipoxygenase and AA. Since lipoxygenases are widely distributed in vivo, peroxyl radical formation by these enzymes may have an important role in chemical carcinogenesis.

PATTERNS OF ONCOGENE EXPRESSION IN RETINOID-PROMOTED PAPILLOMAS AND PHENOTYPICALLY NORMAL MOUSE SKIN. D L McCormick and B J Bagg. IIT Research Institute, Chicago, IL.

Although cancer chemoprevention by retinoids has been demonstrated in a number of animal models for human cancer, several retinoids can promote, as well as inhibit, tumorigenesis in mouse skin. In order to determine if the mechanism of retinoic acid action in skin tumor induction may involve differential expression of oncogenes, levels of oncogene transcripts in papillomas and phenotypically normal skin from mice fed retinoids were compared with levels in grossly normal skin from mice fed a control diet. RNAs isolated from normal and prneoplastic mouse skin were blotted onto nitrocellulose membranes and hybridized with 32P-labelled DNA probes for H-ras, K-ras, N-ras, p53, N-myc, neos, fos, ras, and MAP-1. Dot blots were visualized by autoradiography and quantitated by scintillation counting. Levels of H-ras and p53 transcripts were increased in papillomas promoted by 13-cis-retinoic acid or N-(4-hydroxynpehyl)retinamide (4-NPR) in comparison to normal skin from control mice. In phenotypically normal skin, levels of all oncogene transcripts were decreased from control in mice fed 13-cis-retinoic acid, while K-ras, N-ras, and p53 were decreased in mice fed 4-NPR. These data demonstrate that retinoids can modulate oncogene expression in mouse skin, and suggest possible mechanisms for their promoting and antipromoting activities. (NCI-NO1-CP-41063)

REDUCTION BY DIETARY RESTRICTION OF IN VIVO BINDING OF AFLATOXIN B2 TO HEPATIC NUCLEAR DNA IN RATS. R A Pegram, U T Allaben, and W W Chou. National Center for Toxicological Research, Jefferson, AR.

To gain additional mechanistic insight into the anti-tumorigenic effect of dietary or caloric restriction, the initiation phase of aflatoxin B1 (AFB1) hepatocarcinogenesis was studied in male Fischer 344 rats fed ad libitum (AL) or restricted to 60% of AL intake (DR). Three hours after receiving a single oral dose of [14C]AFB1 (0.1 mg/kg body wt), hepatic nuclear AFB1 binding (pmoles/mg DNA) in AL rats was 2.1 times greater than in rats restricted from 10-16 weeks of age. Moreover, this 2-fold accumulation difference between AL and DR rats was maintained at both 1 and 7 days after dosing. These data appear more significant in light of the fact that liver DNA content in DR rats averaged 30% greater than in AL rats, thus providing more target DNA per unit volume for activated AFB1. Compared with the AL group, the DR plasma concentration of [14C] after 3 hr was significantly lower (42%) and urinary excretion of [14C] was greater (40%), suggesting an enhancement of second metabolism. Furthermore, hepatic DNA synthesis was markedly decreased (65%) in rats restricted for 4 weeks. These results indicate that dietary restriction can beneficially modulate chemical carcinogenesis at the initiation level in rats, and that alterations in both metabolism and DNA synthetic rates may be mechanistically involved.

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HEMOGLOBIN ADDUCTS FORMED ON ADMINISTRATION OF ACRYLONITRILE (AN) TO RATS. T R Fennell, J P MacNeela, M J Turner, and J A Swenberg. Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

AN, which is widely used in the manufacture of plastics and fibers, is carcinogenic in the rat, producing tumors in the brain, stomach, and Zymbal's gland. AN is electrophilic and can react with proteins, or it can undergo metabolism to cyanoethylen oxide (CEO), which is also capable of reacting with proteins. Measurement of specific adducts formed by reaction of either AN or CEO with hemoglobin could be used as a monitor of exposure and metabolism. After administration of [2,3-14C]AN p.o. to male Fischer 344 rats, blood was removed at 6h (4 mg/kg) or 24h (10 or 28 mg/kg). The isolated globin contained 96, 1180, and 3670 nmol equivalents/g at doses of 4, 10 and 28 mg/kg, respectively. Chromatography on Dowex 50 of acid-hydrolysed globin revealed 7 radioactive peaks. The major radioactive peak co-eluted with S-(2-carboxyethyl)cysteine (CEC). This is produced on hydrolysis of S-(2-cyanoethyl)cysteine formed by Michael addition of cysteine residues to AN. A GC/MS assay is being developed for CEC, which may be useful as an indicator of exposure to AN. The remaining adducts are being characterized.

LACK OF IN VIVO DNA BINDING OF MEKAPTOBENZOThIOAZOLE (MBT) TO SELECTED TISSUES OF THE RAT. D W Brester, K J Iroby, A G Wilson, J W Barnett. Monsanto Company, St. Louis, MO.

The objective of this study was to determine the extent of covalent binding of MBT to DNA from selected tissues of the rat. Male and female SD rats were gavaged with 375 mg 14C-MBT/kg body weight and killed 8 hours later. DNA was extracted from the liver, adrenal glands, pituitary gland, pancreas, and bone marrow using standard exhaustive solvent extraction techniques and the amount of radioactivity associated with the DNA was determined. Hepatic DNA was further purified with CsCl ultracentrifugation or hydroxyapatite chromatography. Binding results were expressed in terms of pmol MBT bound/mg DNA and in terms of a covalent binding index (CBI) which normalizes DNA binding with respect to dose and allows a comparison to be made with other chemicals in terms of their binding and carcinogenic potency. Results from this study suggest very little or no in vivo MBT binding with DNA from any of the tissues examined. Hepatic CBI values for the 3 methods of purification were all similar. Other chemicals with similar CBI values include 3-methylcholanthrene, estrone and diethylsterbesterol. The CBI values for strong hepatocarcinogens such as dimethyl-nitrosamine and aflatoxin range from 6000 to greater than 20000. Results from this study agree with other short term bioassays in which MBT was found to be nongenotoxic.


Although in situ metabolic activation of B(a)P results in DNA adduct formation, uptake of B(a)P metabolites from blood may contribute to the DNA adduct levels in tissues. We measured electrophilic metabolites in mouse serum after B(a)P dosing (200 mg/kg p.o.) by trapping metabolites with salmon sperm DNA (ssDNA), followed by 32P-postlabeling analysis for adducts in ssDNA. In vitro studies demonstrated that mouse serum sequesters B(a)P-7,8-diol-9,10-epoxide (BPDE), protects it from hydrolysis, and transfers it to ssDNA or splenocytes. After B(a)P dosing, mouse serum contained 2 reactive metabolites; the major metabolite formed a DNA adduct spot which co-migrated with a BPDE adduct standard. Serum BPDE levels reached a peak of 12.5 nM by 2.5 hours after B(a)P, and then remained constant through 24 hours. Serum B(a)P levels (HPLC analysis) peaked at 1 hour and declined thereafter. BPDE-DNA adduct levels in liver, lung, kidney, spleen and stomach increased rapidly through 5 hours and then less steeply through 24 hours. These results indicate that serum can stabilize and transport BPDE, and that the levels of BPDE in serum may be sufficient to account for a substantial portion of the tissue load of BPDE-DNA adducts after B(a)P administration.

3-METHYLCOLANTHRENE (MC)-INITIATED LUNG TUMORS CORRELATE WITH CYTOCHROME P-450IA1 INDUCTION IN FETAL BUT NOT ADULT MICE. M S Miller, A B Jones, and L M Anderson. Laboratory of Comparative Carcinogenesis, NCI, Frederick, MD.

Inducibility (I) of polycyclic aromatic hydrocarbon metabolism, conferred by the Ah1 gene, has been shown to increase susceptibility of mouse fetuses to lung tumorigenesis by transplacental MC exposure. In I ([C57 x DBA] F1) fetal lung, aryl hydrocarbon hydroxylase (AHH) activity, a measure of levels of cytochrome P-450IA1, increased 50-fold after treatment with MC, with a maximal induction by 8 hr that persisted for at least 48 hr. Northern blot analysis showed a rapid and early induction of P-450IA1 RNA by MC with detectable signal at 2 hrs and a maximum at 4 hrs. For comparison, adult (F1 x DBA) F2 mice received 3 weekly treatments with 100 mg MC/kg. No differences in the incidence of lung tumor bearing mice of either sex or in tumor multiplicity in males was observed between I and non I mice after 16 wk. However, female non I mice had a higher lung tumor multiplicity (p<0.05) than did I mice. Adults had much higher levels of constitutive lung AHH than did fetuses; maximally induced fetal levels were similar to basal adult levels. These results suggest that the correlation between susceptibility to MC-initiated lung tumors and P-450 inducibility may be a unique property of the fetus, in part due to the low basal levels of activating enzymes and their high induction ratio in the fetus.
The present study evaluated the ability of phenobarbital (PB) to promote liver tumorigenesis in the 15-day-old initiated mouse model. At 15 days of age, male (m) C3H, C57/Bl, B6C3F1, and female (f) B6C3F1 mice received a single injection (i.p.) of either diethylaminoethylnitrosamine (DEA; 50 mg/kg) or saline. At 4 weeks of age, mice were given either PB (500 ppm) in their drinking water or deionized water. Mice were killed at 28 weeks of age, livers removed and lesions quantitated. PB promoted liver tumor formation in the C3H(m) and B6C3F1(f). PB had no effect in the C57/Bl(m) and inhibited hepatic tumorigenesis in B6C3F1(m). In a separate experiment, individual preneoplastic foci induced at 15 days of age with DENA in mice from the 3 strains were evaluated for their responsiveness to the mitogenic effects of PB. At 28 weeks of age, mice containing foci were exposed to either PB water or deionized water for 7 days. DNA synthetic rates were increased in foci from C3H(m) and B6C3F1(f) exposed to PB. DNA synthetic rates in foci from C57/Bl(m) and B6C3F1(m) treated with PB were not significantly increased.

While examining the ability of various sedative/anticonvulsant drugs (oxybutynin, oxazepam, diazepam) and alkylating agents (diethylnitrosamine) to promote diethylnitrosamine-initiated liver tumorigenesis, we observed that a number of these compounds (phenobarbital, barbital, pentobarbital and ethylphenylhydantoin) promoted the expression of follicular cell tumors of the thyroid. These compounds were also potent inducers of a pleiotropic "phenobarbital-type" response in the liver, causing increases in activities mediated by cytochrome P-450, epoxide hydrolase and UDP-glucuronoyl transferase. In contrast, a variety of related compounds which failed to induce hepatic pleiotropic response were inactive as thyroid tumor promoters. In addition, barbital and diethylnitrosurea, a ring-opened and decarboxylated analog, were effective promoters of DEN-initiated renal tumors. This promoting activity for the kidney bore no relationship to induction of the hepatic pleiotropic PB-type response, since barbital is a potent hepatic enzyme inducer while diethylnitrosurea is totally inactive in this regard. These results imply that the structural and biologic requirements of thyroid tumor promoters are distinct from those necessary for kidney tumor promotion.

The urinary bladder tumor-promoting potential of the phenolic antioxidants, catechol (CC), 3-t-butyl-4-methylphenol (TBMP), propylparaben (PP), roxorinol (RC) and hydroquinone (HQ) which are structurally similar to BHA, was examined. Male F344 rats were initially given 0.05% BBN as an initiator in their drinking water for 4 weeks, and then groups of 20 rats received daily containing 0.8% CC, 1.0% TBMP, 3% PP, 0.5% RC, 0.5% HQ or basal diet alone for 32 weeks. All survivors were sacrificed for histopathological analysis at the end of week 36. Of the compounds tested, only TBMP significantly increased the incidence and average numbers per 10 cm of blood vessel of papillary or nodular hyperplasias and papillomas of the bladder over those in animals treated with BBN alone. Treatment with test chemical alone did not result in any bladder lesions. The results clearly showed that TBMP, whose chemical formula most closely resembles BHA, promoted bladder carcinogenesis. The similar effects of TBMP and BHA on bladder and forestomach carcinogenesis suggest a direct link between chemical structure and biological potency.

The process of neoplasia is a process shared by both man and animals. In only a few cases are the exact mechanisms of induction and promotion understood.

Those cases where there appears to be no fundamental difference between the cancer development that occurs in experimental animals and that which occurs in man provides the best basis for scaling these results to the human situation and performing realistic risk assessments. In such cases the results transcend species. These cases should be identified and utilized to the fullest.

Certain neoplastic transformations are known to be completely unique to the species tested. In such cases a risk in one species does not predict a risk in another species.

A study of species comparisons has been completed examining various model assays and various site-specific carcinogenesis. It was concluded that results in the A strain mouse and the Scn strain mouse can not be used quantitatively or qualitatively to predict carcinogenicity in man. Other site-specific lesions having defined mechanisms are also examined and compared regarding their relative value for risk assessment.
CADMIUM AND BIORESPONSES TO 1,25 (OH)₂ VITAMIN D₃. S A Swanson and C R Angle. Toxicology Program and Department of Pediatrics, University of Nebraska Medical Center, Omaha, NE.

Studies in the chick intestine model define the binding of 1,25 (OH)₂ vitamin D₃ to DNA as Zn dependent with equivalent inhibition by Cd. A series of experiments in human fibroblasts, osteosarcoma-derived osteoblasts (HOS TE 85) and rat osteosarcoma osteoblasts (ROS 17/2.8) test the hypothesis that the bioterror responses to 1,25 (OH)₂ D₃ are similarly inhibited by Cd. In human fibroblasts, cultured after confluence in serum-free α MEM, we found the alkaline phosphatase (AP) response to 1,25 (OH)₂ D₃ 10⁻⁸ to be inhibited approximately 20% by Cd 10⁻⁸ with a comparable modulation of the AP response to estradiol. In HOS TE 85 cells, also cultured in serum free α MEM, the time and dose related responses to 1,25 (OH)₂ D₃ 10⁻¹¹ to 10⁻⁷, assessed by AP synthesis and 3H-proline incorporation in collagen and non-collagen protein, were comparably modulated by Cd. The effect was also defined in the third bioterror response to 1,25 (OH)₂ D₃, synthesis of osteocalcin by ROS 17/2.8 cells. The observations indicate one of the mechanisms of cadmium induced skeletal toxicity. The data are also compatible with the critical nature of the zinc domains in the transcription regulatory proteins of the glucocorticoid-metallothionein-vitamin D family of receptors.

IN VIVO EFFECTS OF CADMIUM ON CALMODULIN AND CALMODULIN REGULATED ENZYMES P J S VIG and R NATH, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

There was significant accumulation of cadmium in cerebral cortices of young male adult Wistar rats after 4 weeks of cadmium (6 mg/kg body weight) exposure (through gastric intubation). Biological activity of calmodulin decreased significantly (p<0.001) in the cerebral cortices of these animals in comparison to the control group. Phosphodiesterase and synaptic membrane Ca²⁺-Mg²⁺ ATPase were also significantly affected (p<0.01 and p<0.001 respectively). However, cadmium treatment did not alter synaptic membrane adenylylcyclase activity. Alcohol (2% via gastric intubation) and diethylthioucarbamate (DDC) (9.2 mg/kg body weight, IP) treatment along with cadmium (6 mg/kg body weight) enhanced cadmium accumulation in cerebral cortices of treated animals resulting in an increased inhibition of calmodulin and calmodulin dependent enzymes. It is suggested that cadmium may act via binding to calmodulin and uncoupling it from its normal cellular control by calcium.

CADMIUM DEPENDENT BINDING OF CALMODULIN (CaM) TO MICROTUBULE-ASSOCIATED PROTEINS (MAPS) AND TUBULIN. B A Ferrone and L N Chou, Dept. of Microbiology, Boston Univ. Sch. of Medicine, Boston, MA

Although Cd has been shown to inhibit microtubule (MT) assembly in vitro by binding to tubulin sulfhydrils, Cd also binds to the Ca-binding domains of CaM, apparently because the ionic radius of Cd (0.997nm) is almost identical to that of Ca (0.999nm). We have shown that Cd can induce disassembly and inhibit assembly of MT in vitro by binding to and activating CaM in a manner similar to Ca. Previous studies showing the Ca-dependent binding of CaM to MAPs and tubulin suggest that the inhibitory effect was due to the formation of a Ca/CaM complex with MAPs or tubulin. To determine whether CaM binds to MAPs and tubulin in the presence of Cd, we examined the binding of a biotinylated CaM probe to Western blots of bovine brain MT protein in the presence of Ca or Cd. Specific biotinylated CaM binding was detected using avidin-horseradish peroxidase, and chloronaphthol as the chromogenic substrate. CaM bound to MAP2 and tubulin in the presence of Ca or Cd. In addition, this binding was prevented by the cationic chelator ECTA. These results demonstrate the Cd-dependent binding of CaM to MAP2 and tubulin, and support our conclusion that Cd can substitute for Ca in activating CaM, resulting in Cd/CaM complex formation with MAP2 and tubulin and inhibition of MT assembly in vitro.

LOCALIZATION OF METALLOTHIONEIN IN PLACENTA. R A Goyer, M.D. Haust and M G Cherian. Univ. of Western Ontario, London, Canada.

Cellular localization of MT in placenta may provide information on its function as a metal binding protein. Rabbit antibodies to rat liver MT cross-reacted with human MT (Am. J. Pathol. 129: 177-182, 1987) and were used to localize MT in human term placenta by avidin-biotin peroxidase technique. Serial sections (5 microns) were cut from paraffin embedded placenta obtained at term from five normal women and incubated with rabbit anti-bodies to MT. Normal rabbit serum was used as a negative control. The slides were incubated first with biotinylated swine anti-rabbit IgG (linking antibody) then with avidin-biotin horseradish peroxidase complex and developed with diaminobenzidine in hydrogen peroxide (0.03%) substrate. The optimum staining of MT was obtained at a 1:800 antibody dilution. MT was identified in fetal amniotic cells, syncytial trophoblasts and villus interstitial cells, and in maternal decidual cells. The presence of MT at specific cellular sites suggests that it may regulate the transplacental transport of metals such as zinc, copper and cadmium because of the difference in its affinity to these metals and subsequent susceptibility to proteases. Since cadmium is less in fetal blood and zinc and copper are more in fetus than mother, MT may restrict cadmium while enhancing zinc and copper transport.
LOW MOLECULAR WEIGHT (MW) Zn AND Cd BINDING PROTEINS IN MOUSE PLACENTA. D J Thomas, R K Johnson, and T S O’Gara. Depts Peds and Pharm Sci, Univ Neb Med Ctr, Omaha, NE.

Pregnant C57BL/6 or NAW mice received ip injections of 1 mg of Cd or of 5 mg of Zn per kg on gestational day 14. Mice were killed 24 hours later and maternal liver and placenta used to prepare 104,000 xg supernate (cytosol). Supernates were chromatographed on a Sephadex G-75 gel and eluate fractions analyzed for Cd and Zn. Maternal liver cytosols from Cd-treated C57BL/6 and NAW mice contained two Cd peaks in elution profiles. The second and larger Cd-containing peak had an apparent MW of 10 to 12 kDa. Placental cytosol Cd concentration was lower than maternal liver and the elution profile for Cd in placental cytosol contained two peaks. The second peak had an apparent MW of 10 to 12 kDa. In maternal liver cytosol from Zn-treated pregnant C57BL/6 or NAW mice, Zn eluted in two peaks. The second peak had an apparent MW of 10 to 12 kDa. In C57BL/6 placental cytosol the 10 to 12 kDa Zn-binding peak was absent. Thus, Zn or Cd treatment of pregnant C57BL/6 and NAW mice results in the appearance of a low MW protein in maternal liver and placenta. Differences in the kinetics of metal distribution and binding may underlie differences in the amount of protein in this form of placenta from the two strains. (Supported by State of Nebraska LB506 Smoking and Health Grant).

HEPATIC ENDOTHELIAL CELLS APPEAR TO BE AN IMPORTANT CELL TYPE IN PRODUCING CADMIUM HEPATOTOXICITY. Y Liu, Y P Liu, W C Kershaw and C D Klassen. Univ of Kansas Medical Center, Kansas City, KS.

Liver is a target organ for Cd toxicity. Histopathological changes have been observed both in parenchymal and endothelial cells after Cd exposure. However, the relative importance of injury to each cell type in Cd hepatotoxicity is not known. In an attempt to obtain information on this topic, experiments were designed to determine whether liver endothelial cells (EC) or parenchymal cells (PC) of Cd-sensitive mice (C3H/He) were more susceptible to the toxic effects of Cd than those of Cd-resistant mice (DBA/2J). EC were isolated by enzymatic perfusion and incubation followed by differential centrifugation and percoll separation. PC and EC were grown in monolayer cultures for 22 hr and subsequently treated with Cd for 4-24 hr. Cytotoxicity was assayed by enzyme leakage and intracellular K+ loss for PC, or by trypan blue exclusion and protein synthesis for EC. The toxicity of Cd towards PC was observed at the same concentration of Cd in both strains but was different towards EC. Specifically, when EC were exposed to 2.5-100.0 μM Cd for 8 hr, cell viability and protein synthesis in C3H mice were much less than in DBA mice. Metallothionein (MT) concentration and Cd uptake (1-24 hr) in PC and EC of the two strains was similar. In summary, EC but not PC, from Cd-sensitive C3H mice, were more sensitive to Cd toxicity than the corresponding cells of Cd-resistant DBA mice. This suggests that EC may be important target cells in the production of hepatotoxicity by Cd. (Supported by USPHS Grant ES-01142 and ES-07079).

LACK OF COORDINATION OF METALLOTHIONEIN AND HEAT-SHOCK PROTEIN PRODUCTION IN RESPONSE TO METALS. J W Bauman, J Liu and C D Klassen. Univ of Kansas Med Ctr, Kansas City, KS.

Acute stress, such as heat, and some metals, such as arsenite, will induce a specific group of stress proteins referred to as heat-shock proteins. Similarly, metals such as cadmium and zinc, will increase the synthesis of metallothionein (MT). The purpose of the present study was to determine if there is a coordinate production of these proteins in response to these stresses. Rat hepatocytes were grown in monolayer culture for 22 hr and subsequently treated with various concentrations of metals for 4 hr or incubated at 43.5°C for 13-45 min. Following two washes with fresh media, the cells were labeled with 35S-methionine (25 μCi/ml) in methionine-free media for 4 hr for determination of heat-shock protein production or reincubated in fresh media for 20 hr for MT determination. Heat-shock protein production was determined by SDS polyacrylamide gel electrophoresis followed by autoradiography. MT was determined by the Cd/hemoglobin affinity assay. Heat-shock treatment was found to cause a major elevation in heat-shock protein levels while actually decreasing the levels of MT. All three metals (arsenite, cadmium and zinc) increased heat-shock proteins. Whereas arsenite was a much less effective inducer of metallothionein than was cadmium or zinc, arsenite was not less effective than the other metals in inducing heat-shock proteins. In conclusion, there is not a good correlation between the various stresses that induce heat-shock proteins and those that induce MT, indicating there is not a coordinate production of heat-shock proteins and MT. (Supported by USPHS Grant ES-01142 and ES-07079).

EFFECT OF IN VIVO LOW-DOSE CADMIUM PRETREATMENT ON THE IN VITRO INTERACTIONS OF CADMIUM WITH ISOLATED INTERSTITIAL CELLS OF THE RAT TESTES. Z Z Wahba and M P Waalkes. National Cancer Institute-FCRF, Frederick, MD.

Recent studies have shown that Cd-induced testicular interstitial cell (IC) tumors can be prevented by low-dose Cd pretreatment. However, the mechanism by which low-dose Cd induces such tolerance is unclear. Thus, in this study we assessed the effects of in vivo Cd pretreatment (3 μmol/kg) on Cd uptake, cytotoxicity and metal content (Zn, K and Ca) of isolated ICs exposed to Cd in vitro. ICs were isolated by collagenase dispersion of Wistar (WF/NCr) rat testes and incubated with Cd (1.0 mM) up to 1 h. In vivo Cd pretreatment increased in vitro Cd uptake by 17% after 1 hr of incubation with Cd. In vivo Cd pretreatment also resulted in a marked reduction of in vitro Cd-induced cytotoxicity, as reflected by reduced loss of cellular K and glutamic-oxaloacetic transferase as well as reduced lipid peroxidation. Although Cd pretreatment did not alter basal levels of Zn, Ca or K, Ca uptake from the media was increased by Cd pretreatment. In vivo Zn pretreatment, which is also effective in inhibition of Cd-induced testicular tumors, results in a similar reduction in Cd-induced cytotoxicity in ICs. Thus, it appears that treatments that result in reduced Cd-induced IC tumors are consistently able to reduce in vitro Cd-induced cytotoxicity in isolated ICs.
SPECIES SPECIFICITY OF ACUTE CADMIUM-INDUCED RENAL TOXICITY: SUSCEPTIBILITY OF THE SYRIAN HAMSTER. S Rehm and M P Waalkes, National Cancer Institute-FCRC, Frederick, MD.

It has previously been reported that cadmium (Cd) induces renal lesions only after chronic exposure. However, in this study we reevaluate the findings of acute Cd-induced renal lesions in the Syrian hamster, which appear to be species specific as neither rats nor mice showed such lesions. Adult rats (WF/NCr), mice (BALB/cAnNcr) and hamsters (Cr:GH) were given Cd doses ranging from 30 to 50 μmol/kg, sc, and assessed for renal histopathology by standard techniques between 1 and 7 days later. Hamsters developed mild to moderate cell necrosis of the proximal renal tubules 24 h after Cd in 20 to 60% incidence in both sexes. Within one week, however, essentially complete tissue restitution had occurred. Rats and mice showed no such lesions even at lethal doses of Cd (> 35 mg/kg). At maximum tolerated doses (approx. LD₅₀) of Cd (rats and mice, 35 μmol/kg; hamsters, 50 μmol/kg) renal Cd content was not higher in hamsters than in the other species 24 h after injection. In fact, the highest Cd contents were found in mice (6.48 ± 0.75 μg/mg) followed by rats (2.55 ± 0.12 μg/mg) and hamsters (2.28 ± 0.10 μg/mg). These results indicate the hamster is uniquely susceptible to the acute effects of Cd on the kidney and that this effect is not related to an unusually high concentration of Cd in the kidney.

SELENIUM AND CADMIUM INTERACTIONS ON HEPATIC GLYCOGEN CONTENT. M B Izard, V Nonavinakere, J L Early, and R R Bell. Florida A&M University, College of Pharmacy, Tallahassee, FL. Sponsor: R C Schnell

Previous studies have shown that selenium (Se) administration to rats results in hyperglycemia, and an increase in liver glycogen at 1 h after 1.6 mg/kg Cd in both fed and fasted rats. In this study, male Sprague-Dawley derived rats (151-175g) were fed ad libitum and treated with sodium acetate (1.23 mg/kg), selenium (1.65 mg/kg cadmium (Cd) (0.84 mg/kg) or Se and Cd, intraperitoneally. Liver samples were collected by freeze-clamp technique at 15, 30, and 60 min following treatment. Liver glycogen was determined with amyloglucosidase. Present results indicate that Se, Cd or Se and Cd treatment did not significantly alter liver glycogen, during the first 30 min of treatment. However, Se and Cd treatment resulted in a significant decrease (p<0.05) in liver glycogen at 60 min as compared to 30 min. Supported by NIH/DRR/MBRS grant (RR08111) and NIH/DRR/RCMI grant (RR03620).

THE PROTECTIVE EFFECTS OF ZINC ON DIETHYL-DITHIOCARBAMATE (DCC) CYTOTOXICITY ON ASTROCYTES IN CULTURE. M F McManus, M Toulon, L D 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% fetal bovine serum. Cells, in the log phase of growth, were treated for 1 h with 35 μg of DCC/ml (1.5 x 10⁻⁴ M) (Aldrich) of medium or pretreated with 50 μM ZnCl₂ in medium for 15 hours and washed in buffer prior to the 1 h exposure to DCC. After treatment the cells were fed complete medium. Within 1 hour cells treated with DCC alone retracted their processes, became highly vacuolated and detached from the tissue culture flasks. By 24 hours only 3.3% of these cells remained adherent. DCC-treated cells were unable to survive further passages. Cells pretreated with ZnCl₂ prior to DCC exposure became vacuolated and contained numerous inclusion bodies. Many of these cells retained their processes and 23.5% of these cells remained adherent. A significant difference in adherence was seen between groups (p < 0.03). Results indicate that ZnCl₂ reduces injury caused by DCC.

EFFECTS OF INTRATHRACHEAL V O₂ ADMINISTRATION ON RAT LIVER MITOCHONDRIA. L Zychlinski and J Z Byczkowski. Toxlab Research Institute and Florida Toxicology Research Center, College of Public Health, University of South Florida, Tampa, FL.

The bioenergetic functions of liver mitochondria were studied following the acute and chronic exposure of rats to V O₂ via respiratory tract to determine whether vanadium, absorbed from the lung, can affect subcellular organelle in liver. The mitochondrial respiration with glutamate or succinate as substrate was inhibited significantly when compared to the control animals. No inhibition was found with ascorbate. The same effects were observed when mitochondria from control animals were treated in vitro. Pentavalent vanadium was responsible for these inhibitory effects. It is postulated that significant amount of vanadate is accumulated in the intermembrane space of liver mitochondria of the exposed rats. The process of "detoxification" by reduction of vanadate in the tissue may be insufficient to prevent the deleterious action of this compound on liver mitochondria.

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EFFECT OF GALLIUM (Ga) ON INHIBITION OF ANGIOLYSINIC ACID DEHYDRATASE (ALAD) IN RATS. P.E. Geering and S. Rehmi*. Food and Drug Administration, Rockville, MD and National Cancer Institute*, Frederick, MD.

Selective inhibition of enzymes in the human biosynthesis pathway with concurrent urinary excretion of some precursors serve as potentially important biomarkers of chemical exposure and cell injury. This study was undertaken to evaluate ALAD inhibition and concurrent urinary excretion of ALA as biomarkers of chemical exposure using Ga as a model compound. Male C57BL/6 mice received a single ip injection of GaCl3(SO4)2 at doses of 12.5, 25, 50, 100, and 200 mg Ga/kg. A dose-dependent inhibition of ALAD was observed 24 hr after injection in blood, kidney, and liver. After injection of 25 mg Ga/kg, maximal inhibition of ALAD occurred between 12 and 24 hr in liver and kidney with full recovery of activity at 96 hr. In blood, maximal inhibition of activity occurred between 12 and 24 hr with partial recovery of activity at 96 hr. Mild to moderate renal proximal tubular cell degeneration was observed at higher doses. No consistent changes in urinary excretion of ALA were observed. Whole blood analyses of renal and hepatic ALAD activities in the absence and presence of Ga indicated that the inhibition of ALAD by Ga is noncompetitive (same Km decreased Vmax). The data suggest that determination of ALAD activity in various tissues may be of potential value as a biomarker of exposure/toxicity to metals such as Ga.

Inhibition of Superoxide (O2-*) and Hydrogen Peroxide (H2O2) Production by Organotin Compounds in Stimulated Phagocytic Cells. C. Whiteside, M. A. Bagnasco, N. Sadikah and B. D. Goldstein. Graduate Program in Toxicology. Rutgers University, UMRI/Robert Wood Johnson Medical School, Piscataway, N.J.

The effects of the organotin compounds, tri-butyltin bromide (TBB), diethyltin dichloride (DEE), and tri-ethyltin bromide (TEB), on stimulated phagocytic cells were studies to investigate mechanisms by which these compounds act as membrane toxicants. Incubation of PMN-stimulated human polymorphonuclear leukocytes and rat alveolar macrophages with 50 μM TBB resulted in a 95% inhibition of O2- production. Much higher concentrations of DET and TEB (400 μM or greater) were required to achieve comparable effects. Similar decreases were observed for H2O2 production. Results from experiments investigating the capability of dithiothreitol and cysteine to protect against the organotin-induced inhibition of O2- showed a complete reversal of this inhibition by micromolar concentrations. Membrane fluidity studies were performed to determine if 1,6-diphenyl-1,3,5-hexatriene showed significant increases in polarization values for DET-but not TBB-treated neutrophils compared with controls. These studies suggest that organotin toxicity may involve interaction with critical sulfhydryl groups. Supported by NIH grants #ES02910.

GLUTATHIONE ATTENUATION OF PORPHYRIN SYNTHESIS IN VITRO. ROLE IN TRACE METAL-INDUCED PORPHYRIA. C. C. Calas and J. S. Woods. University of Washington, Seattle, WA.

Chemically-induced porphyria may involve direct oxidation of porphyrins to porphyrins reactive with reactive oxygen species (ROS) produced during chemical exposure. We have used a model in vitro ROS generating system to investigate the antioxidant potential of glutathione (GSH) to attenuate uroporphyrinogen (uro) oxidation, and the effects of trace metals which complex with GSH on this action. ROS were generated in a stoichiometric system containing 10 μM FeCl3, 40 μM EDTA, and 2.5 mM H2O2 in 3 μM Hepes buffer, pH 7.45. Uroporphyrinogen oxidation was followed at 370 μm by measuring the increase in UV absorption at 405 nm. Reactions were initiated by addition of uroporphyrinogen to the sample cuvette. In the absence of GSH, the uroporphyrinogen oxidation rate was ω 100 pmol/min. GSH at 5 or 10 μM in the reaction mixture reduced the oxidation rate to 52 and 19% of control, respectively. When metals which complex with GSH were added, the effect of GSH to attenuate uroporphyrinogen oxidation was significantly reduced. Thus, oxidation rates in the presence of Hg-GSH (5 mM eq) or Sn(II)-GSH (5 mM eq) were 1.5 and 1.5 times that seen with 5 mM GSH alone, respectively. These results show that GSH significantly attenuates porphyrinogen oxidation at biologically relevant concentrations. Trace metals which deplete GSH may promote porphyria by compromising the action of GSH to attenuate oxidation of porphyrinogens by endogenous reactive oxidants. (Supported by ES03628)
TET produces potent behavioral toxicity when given to adult or developing animals. The present study used TET to examine the effect of neonatal neurotoxicant administration on associative learning during early development. Long-Evans rat pups received an i.p. injection of either TET (5 mg/kg in 10 ul/g saline) or saline vehicle on postnatal day 5 (PND 5), or PND 10. These pups were then trained and tested on an aversive olfactory discrimination task (Kucharski and Spear, Develop Psychobiol, 1984, 17, 465-479). Training and testing took place on either PND 12 or PND 18. Pups injected with TET on PND 5 showed learning impairments, relative to vehicle injected controls, at both 12 and 18 days of age. TET administration on PND 10 impaired learning only on PND 18. An additional experiment showed that TET administration on PND 16 impaired learning on PND 18. Further experiments established the dose-dependency of this TET induced impairment in early learning. These findings indicate that neonatal exposure to organotin compounds impairs cognitive development in a manner that interacts with age of exposure and age of testing. They also illustrate the value of olfactory conditioning as an animal model for assessing neurotoxic effects on the ontogeny of learning and memory.

Ni(II) compounds are toxic and carcinogenic to humans and animals. One possible mode by which these compounds act may involve active oxygen species, e.g., \( \text{H}_2\text{O}_2 \). The present study was undertaken to determine the effects of Ni(II) in vitro on enzymes detoxifying \( \text{H}_2\text{O}_2 \), i.e., catalase (CAT) and the glutathione peroxidase (GSH-Px)/glutathione reductase (GSSG-R) system. Two essential metals, Mg(II) and Fe(III), served as controls and were also tested for their influence on Ni(II) effects on the enzymes. The activity of CAT decreased proportionally to increasing concentration of Ni(II) to 61% of its original value at 24 mM Ni(II). Mg(II) up to 24 mM and Fe(III) up to 0.2 mM had no significant influence on the CAT activity or its inhibition by Ni(II). Catalytic activity of the GSH-Px/GSSG-R system decreased also with increasing concentration of Ni(II) to 12% of its original value at 4.5 mM Ni(II). Mg(II) had no effect on the GSH-Px/GSSG-R activity and its inhibition by Ni(II). The catalytic activity of GSSG-R alone was reduced by Ni(II) to 90% of its original value at 0.9 mM Ni(II). Mg(II) up to 0.9 mM had no effect on this enzyme. Hence, Ni(II) inhibits enzymes decomposing \( \text{H}_2\text{O}_2 \) that may contribute to its toxicity. This effect is not prevented by Mg(II) or Fe(III).

Thallium intoxication produces several neurological symptoms including tremors, convulsion, and neuropathy. Several neurochemical biomarkers were measured in adult male Sprague-Dawley rat brains after thallium exposure. Acute injection of thallium (20 mg/kg, ip) produced significant increases in frontal cortex concentrations of glutamine after 6 hr and taurine after 24 hr. In hippocampus, a significant decrease of aspartic acid and taurine concentration was found after 6 hr. Subacute exposure to thallium (5 mg/kg, ip daily for 10 days) produced significant increases of dopamine (DA), DOPAC and serotonin in amygdala and also increased serotonin in the hypothalamus. DA or muscarinic cholinergic (MCh) receptor binding did not show any significant alteration in caudate nucleus or frontal cortex after acute or subacute exposure to thallium. However, when membranes prepared from the control caudate nucleus were incubated with thallium (1-100 uM), we observed a dose dependent decrease in DA and MCh receptor binding. These data suggest that neurotoxicity produced by thallium exposure may be associated with changes in amino acids and neurotransmitter levels in various regions of the brain.
538 MACROMOLECULAR BINDING OF NICKEL IN RAT LUNG. J D Benson, C B Mitchell, J D Young, and A Waddoups. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

The objectives of this study were to characterize nickel binding proteins in rat lung, to quantitate nickel binding to DNA, and to determine whether previous exposure to Ni affects binding of a subsequent dose of Ni to either protein or DNA. Two groups of 54 male rats were exposed by inhalation 6 hr/day for 21 days to either 0 or 2.5 mg Ni/22. On day 22, both groups were administered 63mg/kg (3 uCi) by intratracheal instillation. Rats were sacrificed 24 hours later and lungs were removed for analysis. Trace metals, protein, and electrophoresis was used to determine the molecular weights of the Ni-binding proteins. For quantitation of 63Ni binding to DNA, lung homogenates were treated with trichloroacetic and RNAase and DNA was isolated by phenol extraction and ethanol precipitation. Proteins having molecular weights of 57,000, 48,000 and 17,000 daltons bound 10 – 20% of the 63Ni in the post mitochondrial supernatant of the lung homogenates. Approximately 80 – 90% of the 63Ni in this fraction was bound to small peptides or amino acids. The pattern of 63Ni binding to proteins was not altered by previous exposure to Ni135. The extent of Ni binding to DNA was 28 ± 4 pg Ni/mg DNA. Previous exposure to Ni135 did not alter the amount of 63Ni bound. Results indicate that several Ni binding proteins exist in lung, with some binding proteins being Ni administered. Results also suggest that previous exposure to Ni does not quantitatively affect the extent of binding of protein or DNA of a subsequent dose of Ni. (Research performed under Funds-in-Agreement No. DE-F104-87AL44742 between the U.S. DOE/ONR and the Nickel Producers Environmental Research Association, Inc.).

540 FEASIBILITY OF AI, SI AND TI AS TRACERS FOR SOIL INGESTION MEASUREMENTS BY XRF AND ICP SPECTROMETRY: A SURROGATE STUDY IN MINIPIGS. J E Ballou and L B Sasser. Pacific Northwest Laboratory, Richland, WA.

The possibility that soil may become contaminated with unacceptable levels of hazardous chemicals is of great concern and could represent a potential health problem, especially to children. A surrogate study in pigs was conducted to test the feasibility of using Al, Si, and Ti as tracers for soil ingestion to evaluate analytical procedures, and to investigate metabolic effects as a function of age. Eight 20-week-old Hanford minipigs (8 of each age) were maintained in metabolism cages for sample collection. Soil was administered daily (3-7 g/day) in gelatin capsules for 11 days, these samples and soil and feed samples were analyzed for Al, Si, and Ti content using XRF and ICP-emission spectrometry. Al appeared to be the best indicator of soil ingestion although Ti and Si were also useful indicators. Both XRF and ICP methods were acceptable for tracer analysis. No clear-cut differences in the metabolism of or rate of excretory clearance of soil tracer elements were observed between the 8- and 20-week-old pigs. Supported by US EPA Contract 10183 under a Related Services Agreement with the US DOE under Contract DE-AC06-76RL01830.

539 TOXICITY AND ALUMINUM CONCENTRATION IN BONE AND BRAIN FOLLOWING DIETARY ADMINISTRATION OF BASIC SODIUM ALUMINUM PHOSPHATE (KASAL®) IN BEAGLE DOGS. J C Pettersen, D S Hackett, G M Zwicker, and G L Sprague. CIBA-GEIGY, Environmental Health Center, Farmington, CT.

This study was conducted to determine the chronic dietary toxicity of KASAL and aluminum deposition in bone and brain in Beagle dogs. Groups of 4 dogs each of sex were fed constant dietary concentrations of 0, 3000, 10000, or 30000 ppm KASAL for 26 weeks. Minimal toxicity characterized by a transient decrease in body weight and food consumption was observed in high-dose (30000 ppm) males. At study termination, body weights in males fed 3000, 10000 and 30000 ppm KASAL were 85.89 and 81 percent of control, respectively. Corresponding weights in females were 93.48 and 83 percent of control. Biologically significant histopathological findings were mild, only apparent at the highest dose level, and did not suggest obvious tissue toxicity. aluminum concentrations in trabecular bone were similar in KASAL-treated and control dogs. Aluminum concentrations in brain tissue were similar in treated and control males. However, aluminum levels in brain tissue were significantly increased (p<0.05) in high-dose females. This increase was relatively small (1.6 fold over control) and of no biological significance. In summary, KASAL administration did not cause significant toxicity.

541 COMPARISON OF THE EFFICACY OF SEVERAL CHELATING AGENTS IN ACUTE URANIUM INTOXICATION. J M Lloubet, A Ortega, J L Domingo, M Gomez, and J Lorbella. Laboratory of Toxicology & Biochemistry, School of Medicine, E-4300, Reus, Spain.

Sixteen chelating agents were examined to determine their relative efficacy as antidotes in acute uranyl nitrate intoxication in mice after subcutaneous administration. Chelators were administered intraperitoneally to male Swiss mice at a dose equal to one-fourth of their respective LD50 and the therapeutic effectiveness was calculated. Eight compounds resulted in a significant enhancement of the survival rate: Tiron, gallic acid, DTPA, p-amino salicylic acid, sodium citrate, EDTA, and 5-amino salicylic acid and ETA. Therapeutic indices (TI) were then determined for these chelating agents. Tiron (TI:158), gallic acid (TI:116) and DTPA (TI:44.9) were the most effective antidotes. In subsequent experiments, uranyl acetate dihydrate was administered subcutaneously (10 mg/kg) in a 24-hrs excretion and distribution study. The most effective light chelators were given intraperitoneally ten minutes after the uranium administration. 5-amino salicylic acid and Tiron were consistently the most effective in increasing the urinary and fecal excretion of uranium respectively. A decrease in the uranium concentration in kidneys and bone was also noted with Tiron. Tiron appears to be the most effective agent of those tested in the prevention of acute uranium poisoning.
PRODUCTION OF AN IN VIVO REDUCING ENVIRONMENT FOR GSH BY THE CHELATING AGENT DMPS. W. Zeng*, R. Maiorino and H. V. Aposhian. Dept Pharm & Toxicol & Dep't Mol & Cell Biology, Univ of Arizona, Tucson, AZ.

N-(2,3-dimercaptopropyl)phthalamic acid (DMPS) increases the biliary excretion of GSH by at least two fold, DMSA and DMPS do not. The mechanism by which DMPS increases biliary GSH has been investigated. When GSH was incubated in vitro with normal or heated bile, GSH was oxidized to GSSG, but the total GSH (unaltered + altered) was unchanged. The incubation of DMPS with GSH in normal bile resulted in significant inhibition of both the oxidation of GSH and the formation of GSSG. Incubation of heated bile with DMPS and GSH did not affect the protective effect of DMPS in the oxidation of GSH. On the other hand, DMPS enhanced the formation of GSH when DMPS was incubated with GSSG in 0.05 M phosphate buffer. The disappearance of DMPS was equal to the formation of GSH. DMPS (100 dithiol : 1 substrate) inhibited γ-glutamyltranspeptidase in vitro 42%. DMPS increases the biliary content of GSH by producing a reducing environment, in vivo. The reduction of GSSG and protection against the autoxidation and enzyme-mediated degradation of GSH by DMPS appears to account for its activity in maintaining the biliary concentration of GSH. (Supported in part by NIDH Grant ES 03356)

DECORPORATION OF POLONIUM-210 BY DMPS. G. M. Boyd and H. V. Aposhian. Dep'ts of Pharmacology & Toxicology and Molecular & Cellular Biology, University of Arizona, Tucson, AZ.

Polonium-210, an alpha emitter, has a number of uses including the reduction of static electricity in packaging processes. An estimated 20,000 polonium-210 ionizing units are in use in the U.S. for this purpose. The decorporation of this radioactive metal was studied by giving rats 3.33 x 10^7 cpm Po-210/kg ip. One hour later they were given 0.20 mmol DMPS or DMSA/kg s.c. Treatment was repeated each day for 12 days. DMPS and DMSA increased the urinary excretion of Po-210, as compared to control animals, 8-fold and 5-fold, respectively. DMPS also increased the fecal excretion of Po-210. The results indicate that DMPS has greater specificity in chelating and decorporating Po-210 than DMSA. A more frequent and long treatment schedule showed that DMPS was three times more potent than DMSA in reducing kidney levels of Po-210. (Supported in part by NCI Grant CA49252)


Sprague-Dawley rats (20/sx/group) were treated by gavage with an aqueous solution of thallium sulfate at concentrations of 0, 0.01, 0.05, or 0.25 mg/kg/day for 90 days. The data generated included body and organ weights, food consumption, hematological and clinical chemistry parameters, neurotoxicity findings, opthalmologic observations and histopathology findings including neuropathy. No differences were observed in body weight, body weight gain, food consumption or absolute and relative organ weights at any of the doses tested. Increased mortality was not seen in the treated rats. A dose-related trend in the incidence of alopecia, lacrimation and exophthalmos was seen throughout the study. Moderate dose-related changes were observed in some blood chemistry parameters such as increased SGOT, LDH, and sodium levels and decreased blood sugar levels. The only grossly observed finding at necropsy was treatment-related alopecia, especially in female rats; however, microscopic evaluations did not reveal any histopathologic alterations.


We have investigated the prenatal effects of trimethylarsine and its oxidation product trimethylarsine oxide upon exposure to a postimplantation rat embryo culture system. Sprague-Dawley rat embryos, explanted on day 11 of gestation, were cocultured with trimethylarsine for 24 hours in a defined cell culture medium consisting of 50% Waymouth's 752/1 medium and 50% fresh rat serum supplemented with antibiotics. At higher dose levels (1 to 2 μg/ml), embryo lethality monitored as loss of yolk sac circulation and embryonic heart beat was observed. At lower dose levels (0.5 μg/ml), embryotoxic effects were observed including decreased growth, limb bud and somite development, and embryonic levels of DNA and protein. Culture supplementation with hepatic S-9 fractions isolated from Aroclor 1254-pretreated rats significantly decreased trimethylarsine prenatal toxicity; trimethylarsine oxide was observed to exhibit minimal embryotoxicity, with or without S-9 culture supplementation, over this same dose range. These findings suggest trimethylarsine possess direct prenatal toxicity and that prenatal toxic effects observed can be altered by enzyme-mediated biotransformation.
SIMULTANEOUS EXPOSURE TO METALS SYNERGISTICALLY INHIBITS Na⁺/K⁺-ATPase ACTIVITY IN SYNAPTIC PLASMA MEMBRANES. MA Carfagna,† GD Ponsler‡ and BB Muhoberac§. Department of Pharmacology and Toxicology, † Indiana University School of Medicine, and Department of Chemistry, ‡ Purdue University School of Science, Indiana University-Purdue University, Indianapolis, IN. Sponsor: BB Forney, Sr.†

Cadmium (Cd²⁺), lead (Pb²⁺) and manganese (Mn²⁺) have separately been shown to exert toxicologically dangerous effects. In this study, the inhibition of Na⁺/K⁺-ATPase and Mg²⁺-ATPase activities by in vitro exposure to Cd²⁺, Pb²⁺ and Mn²⁺ was investigated in rat synaptic plasma membranes. Metal concentrations causing 50% inhibition of Na⁺/K⁺-ATPase activity were: Cd²⁺ (0.6 μM) < Pb²⁺ (2.1 μM) < Mn²⁺ (3 mM). Separately, Cd²⁺ and Pb²⁺ were more potent inhibitors of Na⁺/K⁺-ATPase than Mg²⁺-ATPase activity. Simultaneous exposure to the combinations Cd²⁺/Mn²⁺ or Pb²⁺/Mn²⁺ inhibited Na⁺/K⁺-ATPase activity synergistically (i.e. greater than the sum of the metal-induced inhibition assayed separately), while Cd²⁺/Pb²⁺ caused additive inhibition. In contrast, simultaneous exposure to Cd²⁺/Pb²⁺ antagonistically inhibited Mg²⁺-ATPase activity while Cd²⁺/Mn²⁺ or Pb²⁺/Mn²⁺ additively inhibited Mg²⁺-ATPase activity at low Mn²⁺ concentrations, but inhibited antagonistically at higher concentrations. These data reveal a synergistic inhibition of Na⁺/K⁺-ATPase activity by certain metal combinations which may be physiologically important. [Supported by NIAAA predoctoral fellowship AA05285 (MAC) and NIAAA grant AA06935 (BBM)]


SFP-VAF, F-344 rats were exposed 6 hr/day 5 da/wk for up to 24-mo to a special test toner at 0, 1, 4, or 16 mg/m³ or TiO₂ at 5 mg/m³ by the inhalation route. Animals were allowed extra 6 weeks in filtered air. The test toner material was enriched in fine particles; thus the resp. aerosol conc. (ACGIH) were 0, 0.35, 1.4 and 5.6 mg/m³. Surviving animals were sacrificed at 25.5 months after start of exposure. The mean retained mass of toner and TiO₂ at terminal sacrifice were 0, 0.21, 1.77, 14.98 and 3.13 mg/ lung, respectively. No evidence for systemic toxicity or upper-resp. system effects were found. The incidence of lung tumors was comparable in all groups and in accordance with historical background values. A slight-moderate degree of fibrosis was observed at the high (16 mg/m³) exposure level in all animals, while a very slight degree of fibrosis was noted in 25% of the animals at the middle (4 mg/m³) exposure level. The fibrogenic potency of the test toner was comparable to TiO₂. No pulmonary changes were seen in the toner low (1 mg/m³) and environmentally most relevant exposure level.


This is the first report of selenite induced peroxidation. Forty male Sprague-Dawley rats were housed four to a cage and given food and water ad libitum. Twenty were implanted subcutaneously with osmotic minipumps containing 20 mg of sodium selenite, the remaining twenty were sham operated. After two weeks of treatment animals were necropsied and livers excised. There was a significant (p < 0.01) decrease in reduced glutathione and a significant (p < 0.01) increase in oxidized glutathione in both cytosolic and mitochondrial fractions. There was no alteration in glutathione reductase. Associated with these changes there was a significant (p < 0.01) 42% increase in peroxidation in whole homogenate and a significant (p < 0.01) 38% increase in mitochondrial peroxidation.

COMPARISON OF THREE METHODS OF EXPOSING RATS TO CIGARETTE SMOKE. J.L. Mauderly, W E Bechtold, J.A. Long, A L Brooks, B T Chen, J R Hackena, R F Hambrecht, W J Johnson, M R Riddick, D G Thomas, and R G Cuddihy. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

Studies of rats using repeated nose-only exposures to puffs of smoke have not duplicated the carcinogenicity of human smokers. The dose delivered by this method is limited by reduced breathing during puffs, stress to the animals, and labor requirements. Whole-body exposures might overcome these problems. We examined the smoke characteristics and short-term health effects of nose-only intermittent (ROI), nose-only continuous (ROC), and whole-body continuous (WBC) exposures of rats to diluted mainstream smoke. All groups received 1200 mg particulate/hr-m⁻³ daily 5 days/wk for 4 wk (preceeded by one-half concentration for 1 wk). ROI received 10, 10-min, exposures at 720 mg/m³ during 6 hrs/day. ROC and WBC received 200 mg/m³ continuously for 6 hrs/day. We compared the physical, chemical, and mutagenic characteristics of smoke, and health effects, including carboxyhemoglobin, urine nicotine/cotinine, body weight, bronchoalveolar lavage, chromosomal damage in alveolar macrophages, respiratory function, particle clearance, nasal epithelial proliferation, tracheal epithelial transformation, lung DNA adducts and histopathology. There were few differences in smoke characteristics. Health effects were apparent, but differed little among groups. These results suggest that whole-body exposures produce effects similar to those of nose-only exposures, and might be useful for chronic studies. [Supported by U.S. DOE/DOEER under Contract No. DE-AC04-76EV01011]
SYNERGISTIC EFFECTS OF NITROGEN DIOXIDE AND CARBON DIOXIDE FOLLOWING ACUTE INHALATION EXPOSURES IN RATS. B G Levin, M Faabo, L Highbarger, and N Eller. National Institute of Standards and Technology, Gaithersburg, MD.

The National Institute of Standards and Technology (NIST) is developing a model to predict the toxic interactions of the binary gases generated in fires. The model now consists of four gases: carbon monoxide, carbon dioxide ($CO_2$), hydrogen cyanide, and low oxygen. Previous work at NIST has shown the synergistic effects of $CO_2$ when combined with the other three gases.

Nitrogen dioxide ($NO_2$), a pulmonary irritant, has been detected in levels as high as 150 ppm in fires involving nitrogen-containing products. In Fischer 344 male rats, the 30 min $LC_{50}$ for $NO_2$ was 200 ppm (190-210 ppm, 95% C.L.), whereas that for $NO_2$ in the presence of 5% $CO_2$ was 90 ppm (67-118 ppm, 95% C.L.). $NO_2$ deaths (all post-exposure) occurred earlier in the presence of 5% $CO_2$, i.e., 13% deaths were noted in the first 1.5 hrs, whereas with only $NO_2$ (200 ppm), all deaths ($n=15$) occurred between 3 and 24 hrs. $CO_2$, a respiratory stimulant, is probably acting to increase the body burden of $NO_2$. At death, lung edema was evident. The mean lung wet weight/body weight ratio from rats exposed to 200 ppm $NO_2$ plus 5% $CO_2$ was 3.5 times that of the non-exposed rats. At the end of the 30 min exposures, the methemoglobin levels were 3 times higher in the animals exposed to the combination of $NO_2$ and $CO_2$ than those exposed to $NO_2$ only.

553 THE USE OF A LIPID PROFILE IN A 90-DAY INHALATION EXPERIMENT IN RATS, COMPARING SMOKE FROM CIGARETTES WHICH BURNED OR ONLY HEATED TOBACCO. C R E Coggins, P H Ayres, A T Mosberg and C H Chen. R.J. Reynolds Tobacco Co, Winston-Salem, NC and Alpha Biomedical Labs, Seattle, WA.

Sprague-Dawley rats were exposed nose-only to three concentrations of smoke from each burner or only heated tobacco, in a 90-day inhalation experiment. Further groups of animals were sham-exposed (machine control) or left in their cages (room control). Blood samples were taken at necropsy, and plasma analyzed for total and free cholesterol (CHOL), high-density lipoprotein CHOL, total and net triglycerides (TRIG), and endogenous lecithin: CHOL acyltransferase (LCAT). Other plasma lipids (% free CHOL, low and very low density lipoprotein CHOL, glycerol) were obtained by calculation. Despite the very high exposure concentrations used (blood carboxyhemoglobin concentrations of over 55% and plasma nicotine concentrations of up to 250 ng/ml), there were no differences between the smoke-exposed and control groups for any of the lipid profile parameters. Plasma glycerol concentrations were unaffected by the very high concentrations of glycerol in the smoke produced by the test cigarette.
INDUCTION AND REGRESSION OF HISTOPATHOLOGY IN THE RAT LARYNX AFTER ACUTE EXPOSURE TO SMOKE FROM IR4F CIGARETTES. P H Ayres, C R E Coggins, J W Sagartz*, and G T Burger. R.J. Reynolds Tobacco Company, Winston-Salem, NC and *Veritas Laboratories, Burlington, NC.

Sprague-Dawley rats were exposed nose-only to high concentrations of mainstream smoke from IR4F cigarettes at a wet total particulate material concentration of 0.2 mg/l for 1-6 hrs. Rats were killed after exposure or 3, 7 or 14 days later. Exposure to smoke for 1, 2, or 3 hrs resulted in inflammatory changes and minimal necrosis and erosion of the larynx were examined after exposure. Changes in the larynx in rats killed 3 days after exposure were compatible with minimal squamous metaplasia. These changes had regressed to normal in rats killed 7 or 14 days after exposure. Rats exposed for 4, 5, or 6 hrs and killed, showed ulceration of the ventral epitheum of the larynx. Ulcerated areas had regressed in rats killed 3, 7 or 14 days after exposure. The changes in laryngeal epithelium had regressed to normal in rats killed 14 days after exposure, due to repeated exposures for 1 to 5 hours. A minimal degree of squamous metaplasia remained. The nature and time course of regression of the laryngal changes were related to the duration of inhalation exposure. Considering the sequence of tissue changes and the regenerative nature of the changes, the term squamous re-epithelialization may be a more appropriate term than squamous metaplasia for describing this change.

RELATIONSHIPS BETWEEN AMOUNTS OF NICOTINE PRESENTED AND INHALED, AND RESULTING PLASMA CONCENTRATIONS, IN 90-DAY INHALATION STUDIES IN RATS. A T Moseberg, C R E Ayres, and A P Wohner. R J Reynolds Tobacco Co, Winston-Salem, NC and Battelle, Richland, WA.

Sprague-Dawley rats were exposed nose-only to three concentrations of smoke from cigarettes which burned or only heated tobacco, in two 90-day inhalation experiments. Blood samples were taken at the end of 60-minute daily inhalation exposures, from animals whose minute ventilation was monitored during the exposure. The cigarette content of the smoke for both experiments was in the range 2-25 ug/cigarette for the test cigarette (tobacco only heated) and 6-50 ug/liter for the reference (tobacco burned). Plasma nicotine concentrations in the different groups at the end of the exposures were 30-215 ng/ml for the test cigarette, and 50-195 ng/ml for the reference. Plasma nicotine was corrected for the amounts of nicotine inhaled by the animals. Unlike an earlier study, and in agreement with subsequent work, there were no differences between the corrected plasma nicotine concentrations produced by inhalation exposure to smoke from cigarettes which burned or which only heated tobacco.

SUBCHRONIC INHALATION TOXICITY STUDY OF ISOBULYL NITRITE (IBN) IN F344/N RATS AND B6C3F1 MICE. C Aranyi, C L Gavorski, A Hall, III, K M Abdo* and D Jackson. IITRI, Chicago, IL, PAI, Chicago, IL, and NIEHS/NTP, RTP, NC, and NCTR, Jefferson, AR.

IBN, a volatile nitrite inhaled as a recreational drug by a population with a high incidence in AIDS, may have potential immunotoxic and carcinogenic effects. This report summarizes results of the completed subchronic exposures that will be followed by 2-year chronic carcinogenicity studies. F344/N rats and B6C3F1 mice were exposed to IBN vapors 6 hrs/day, 5 days/wk for 13-weeks at concentrations of 0, 10, 25, 75, 150 and 300 ppm. Mortalities occurred only in male mice at 150 or 300 ppm. Rats at 300 ppm had reduced body weight gains, with no adverse body weight effect in mice. Reductions in RBC counts were seen in rats at 275 ppm, and mice at 300 ppm. Methemoglobin was increased in male rats exposed to >150 ppm, female rats at >150 ppm, and male mice at 300 ppm. Bone marrow hyperplasia was noted in both sexes of rats at 300 ppm and female mice at >150 ppm, and excessive splenic pulp hematopoiesis occurred in mice at all exposure levels. Relative lung weights were increased in rats at 300 ppm and in female mice at 150 ppm. Hyperplasia occurred in the nasal mucosa of male rats at 275 ppm and female rats at 2150 ppm, and in the epithelium of the lungs of male mice at >150 ppm and female mice at 275 ppm. (Supported by NIEHS/NTP Contract No. N01-ES-65143)

SUBCHRONIC INHALATION TOXICITY STUDY OF 2-MERCAPTOBENZMIDAZOLE, (2-MBI) AEROSOL. C L Gavorski, C Aranyi, S Vana, N Rajendran, A Hall, III, and K M Abdo. IITRI, Chicago, IL, PAI, Chicago, IL, and NIEHS/NTP, RTP, NC.

2-MBI, a rubber processing chemical additive, is a suspect carcinogen. F344/N rats were exposed 6 hrs/day, 5 days/wk for 13-weeks to 2-MBI at aerosol mass concentrations of 0, 3.1, 6.2, 12.5, and 50 mg/m³. Aerosol particle sizes ranged from 2.0 to 2.5 µm MMAD, with ages of 2.4 to 2.9. Mortalities included 10/19 males and 10/10 females at 50.0 mg/g, and 1/10 control females. Rats at 225 mg/g displayed emaciation, abnormal posture, hypoactivity, and reduced body weight gain. Clinical pathology changes at the two highest exposure levels included; anemia; increased SGPT, SODT, SDH, DUN and cholesterol; and increased free fatty acid. Endocrine system toxicity was indicated by increased thyroid weight and thyroid follicular cell hyperplasia in both sexes at >6.2 mg/m³, and reduced T₃ and T₄ levels in both sexes at >12.5 mg/m³. Thyroid hyperplasia also appeared in 80% of the males and 30% of the females at 3.1 mg/m³. Thyroid weights were significantly reduced in both sexes at >3.1 mg/m³, with liver weight increases at >6.2 mg/m³. Exposure-related histopathologic changes were also seen in the pituitary, thymus, mesenteric lymph nodes, respiratory epithelium of the nose, kidney, adrenal cortex, pancreas, bone marrow, liver, and skin on the feet and tail. (Supported by NIEHS/NTP Contract No. N01-ES-65135)

An inhalation exposure of F-344 rats to the spontaneous oxidation products of TEB, a fuel additive, at concentrations below autoignition point (~1000 ppm) resulted in immediate lethality attributed to severe, diffuse pulmonary edema accompanied by hepatocytic degeneration. Incidence of lethality as well as incidence and severity of the pulmonary and hepatic lesions were a function of the time delay between initiation of the oxidation, in air, of pure TEB vapor and exposure. All rats exposed to TEB vapor 37 ms after the initiation of oxidation (fresh TEB) expired within 50 minutes and all of these animals exhibited severe congestive perivascular pulmonary edema accompanied by tracheal necrosis. Fifty percent of the animals exposed to an equivalent concentration of TEB vapor 4.6 min. post initiation of oxidation (aged TEB) expired within 24 hr. Nearly all of these animals exhibited non-congestive, pulmonary edema. No other deaths occurred in 14 days post exposure. None of the surviving animals demonstrated pulmonary lesions of any type, however the majority of the survivors showed hepatocytic degeneration.

(Contract No. F33615-85-C-0532).

HISTOPATHOLOGIC ANALYSIS OF SEQUENTIAL LESIONS IN THE RESPIRATORY SYSTEM OF F344 RATS RESULTING FROM A SINGLE 2 HOUR INHALATION EXPOSURE TO METHYL ISOCYANATE (MIC), C A Boorman, H R Brown and J R Bucher. Experimental Pathology Laboratories, Inc. and National Toxicology Program, N.I.E.H.S., Research Triangle Park, NC.

Histopathologic evaluations of sequential lesions resulting from MIC exposure in laboratory animals are important not only as predicitors of long term consequences of the disaster in Bhopal, India but also to provide insight into the pathogenesis of lesions encountered in any subacute and chronic studies where an agent or its metabolite has necrotizing potential. F344 rats were exposed to concentrations of MIC ranging from 0 to 30 ppm for 2 hours and then sacrificed at the following intervals post exposure: 3 hours (Day 0), Days 1, 3, 7, 14, 49 and 91. Acute epithelial necrosis was followed by epithelial denudation, fibrinocellular exudation, regeneration, respiratory epithelial hyperplasia, mucus pooling and intraluminal fibrosis with partial airway obstruction. Intragranulam fibrosis appeared to result from fibrous organization of trapped fibrinocellular debris overlying deep epithelial defects which failed to reepithelialize within 24 to 48 hours following injury. Several structural alterations did not resolve during the course of the study, although remarkable healing and resolution of lesions did occur in animals surviving the initial two week post exposure period.
SUBCHRONIC INHALATION TOXICITY OF METHYLTHIOACETATE (MTA) in F-344 RATS. LC Griffith, ED Bruce and WR Richter. Chevron Environmental Health Center, Inc., Richmond, CA.

MTA is a volatile by-product produced in the manufacture of the insecticide acephate.

Male and female rats were exposed to mean vapor concentrations of 0, 3, 15, 60 and 115 ppm, 6 hrs/day, 5 days/wk for 13 wks. The highest concentration was reduced from 150 to 100 ppm after 4 wks due to toxicity. Exposure resulted in the death of one male at 115 ppm. At 60 and 115 ppm, toxic effects included abnormal respiratory sounds (ARS), decreased body wt and food consumption and increased lung wt. Histopathologic lung changes were observed at 60 and 115 ppm but not at 3 or 15 ppm. Effects in the lung included mucus accumulation in the respiratory lobule, bronchiolar hyperplasia, increased alveolar macrophages and interstitial pneumonia with slight collagen formation. Signs of toxicity and lung changes were still present, but less severe in additional 115 ppm rats at the end of an 8 wk. recovery period. MTA produces optic neuropathy in rabbits which has not been seen in other species; no effects on the visual system were observed in the rats in this inhalation study. With the exception of a slight increase in the incidence of ARS at 15 ppm, this concentration was considered to be the no-observed-effect level.

PULMONARY PATHOLOGY IN RATS EXPOSED TO FLUOROPOLYMER/WOOD SMOKE FROM A FULL-SCALE FIRE. R Valentine, G T Makovec, B B Baker, D J Kasprzak; P B Clarke; and C H Herpol and M Jannsens. E.I. du Pont de Nemours & Co., Wilmington, DE; Benjamin/Clarke Associates, Kensington, MD; and State University of Ghent, Belgium.

Respiratory tract pathology was evaluated in rats exposed to the smoke produced from burning 20–30 kg Teflon® FRP cable and 110 kg wood. Groups of 10 Crl:CD rats were exposed nose-only to the cooled smoke for 30 minutes and killed either immediately or 14-days after exposure for gross and microscopic examination. Animals exposed to fluoropolymer/wood smoke (FFW) exhibited pulmonary edema whose incidence and severity was related to the amount of cable burned. Edema, ranging from minimal to mild severity, tended to be more severe in rats that died 1 day after exposure. Although pulmonary hemorrhage was also found, neither edema nor hemorrhage was considered to be the primary cause of death in FFW-exposed rats. Two weeks after exposure, some FFW-exposed rats exhibited interstitial pulmonary fibrosis. In contrast, neither edema, hemorrhage nor fibrosis was found in control groups. Tissue injury was not observed in the liver, kidneys, brain, heart or adrenal glands of any rat. Despite the apparent involvement of fluoropolymer decomposition products in producing lung injury, deaths could be attributed to the high levels of carbon monoxide present in the smoke.


The effects of inhalation of 0, 100, 2000 and 4500 ppm acetaldehyde (6 hr/day, 5 days/wk for 2 or 4 wks) upon the nasal cavity, trachea and lungs were examined in adult female Wag/Rij rats. The predominant treatment-related changes were observed in the high dose group at 2 and 4 wks and were characterized as marked degeneration, hyperplasia and metaplasia of the nasal and tracheal epithelium. Pulmonary morphologic changes in this group included multifocal interstitial thickening accompanied by hypertrophied type II alveolar epithelial cells and numerous alveolar macrophages. Significant increases in lung wet and dry weights occurred in the high dose group as compared to controls. There were no differences in lung hydroxyproline content. Minimal to moderate changes occurred in the nasal epithelium of the 1000 or 2000 ppm groups, and no differences were observed in lung weight or composition. The results of this study demonstrate that the greatest morphologic change occurred in the rostral respiratory system and was associated with increasing acetaldehyde concentrations. High concentrations of acetaldehyde can also affect the lung of a species that is an obligate nasal breather.

VINYLTRIMETHOXYSILANE (VMS) NINE-DAY VAPOR INHALATION STUDY WITH RATS. D Ballantyne, F F Lodge, G M Troup, and B Dodg. Union Carbide Corporation, Danbury, CT; “Bushy Run Research Center, Export, PA.

Male and female Fischer 344 rats were exposed 6 hours per day for 9 days to a vapor of Vinyltrimethoxysilane (VMS) at concentrations of 148, 746, 153 or 0 ppm. Parameters evaluated for toxicity included clinical observations, body and organ weights, food and water consumption, hematologic analyses, urinalyses, ophthalmic, gross pathologic, and microscopic evaluations. All rats in the 1484 ppm group died within the first 5 days of exposure. Clinical signs in the 1484 and 746 ppm groups included nasal and ocular discharge, dyspnea, hyperventilation, corneal opacities, urinary incontinence, and weight loss. The 153 ppm group appeared normal but had reduced weight gain. The 746 ppm group rats had polyuria, polydipsia, hematuria, decreased urine specific gravity, and mild anemia. Urinalyses and hematologic parameters were normal for the 153 ppm group. Absolute kidney weights were increased 7% in 746 ppm group rats. Gross necropsy lesions included bloody urine, renal papillary necrosis, corneal opacities, and subdural hemorrhage. Major histologic lesions in the 1484 and 746 ppm group rats included necrotizing rhinitis, keratitis, and necrosis of the renal papilla, pelvis and tubules. VMS vapor produces local irritant effects on the nasal and ocular mucosa and in the airways, producing toxicity in the urine. Additional studies are in progress to further characterize the toxicity of VMS vapor.
ACUTE AND 9-DAY VAPOR INHALATION STUDIES WITH E8 (2 - DIMETHYLAMINOETHYL) ETHER (DMEA).

DMEA (CAS #3303-62-3) is an amine catalyst used in the urethane foam industry. SD rats were exposed for 6 hr to DMEA by different generation methods and then observed for 14 days. Under static conditions, a vapor conc of 24 ppm produced no deaths, BW gains, and clinical signs of periocular/perinasal wetness. An inverse relationship between chamber relative humidity and DMEA vapor conc was observed. Under dynamic conditions, higher vapor conc were produced and the 6-hr LC50 (95% C.I.) was 166 (155-178) ppm. Most deaths occurred within 48 hr. Clinical signs included cloudy corneas, mouth breathing, and hypoactivity. A transient loss of BW occurred in survivors. Subsequently, SD rats were exposed 6 hr/day for 9 days to 90, 40, 20, or 0 ppm. All rats exposed to 90 ppm died on days 3-4; at 40 ppm all died on days 6-11. Corneal opacities, lacrimation, gasping, bright red ears and paws, and BW loss were observed in all DMEA groups. Alterations in urinalysis and serum chemistry results were attributed to the debilitated condition of the animals. Noteworthy microscopic lesions were vascular cytoplasmic swelling of epithelial cells of the respiratory tract as well as squamous metaplasia of the epidermis, trlobular hepatocytes, and walls of the coronary arteries. Additional studies are being conducted to define a 9-day NOEL.


Terephthalic Acid (TPA) is a component in several smoke grenades and is a candidate material for safe training smoke. Since exposure of troops could occur from pyrotechnic dissemination of TPA, an acute inhalation study of TPA from thermally disseminated devices was conducted to mimic field exposure. Groups of male, Fischer 344 rats were exposed by nose-only inhalation to 100, 200, and 400 mg/m³ of TPA for 30 min. Exposed rats and respective groups of air and fuse/fuel exposed controls were evaluated for physiological, bronchoalveolar lavage (BAL), and histopathological changes at 24-hr and 14-day post-exposures (PEs). There were no adverse changes in pulmonary function, BAL parameters, or histopathology. Inhalation of TPA resulted in a reversible, dose-related rhinorrhea; TPA is a mild irritant to mucous membranes. Gas-phase byproducts (CO, CO2, NO2, and SO2) were below the threshold limit values for short-term exposures as established by the American Conference of Governmental Industrial Hygienists. The particulate products were nonmutagenic in the Ames mutagenicity assay. TPA appears to be an excellent candidate for a "safe" training smoke.

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COMBUSTION TOXICITY OF AN AIRCRAFT LUBRICANT
V J Forrest, J F Wyman, *C J Hixon, "J R Cooper, L H Lee, "J A Rivera, D A Macyo, Naval Medical Research Institute, Toxicology Detachment, WPAB OH and *AMRL, Wright Patterson Air Force Base OH Sponsor: R Garder, Wright State Univ., Dayton OH

Fire-resistant lubricants of military specification L-23699 are widely used in military and civilian jet propulsion systems. Some formulations consist of a trimethylolpropane base with triaryl phosphate additives. These constituents when thermally degraded produce a neurotoxic bicine phosphate ester, trimethylolpropane phosphate, which is potentially hazardous for personnel involved in aircraft fires. The inhalation hazard of one such lubricant was investigated. Male Fischer 344 rats were placed in a slowly rotating drum inside a chamber and exposed for 30 min to combustion products produced in an adjoining furnace. Mineral oil and another lubricant of the same specification (MIL-L-17331) were tested in the same manner. Three dose levels of each lubricant and appropriate controls were included. Combustion of MIL-L-23699 did not result in greater incapacitation or deaths than the other lubricants. All rats exposed to smoke demonstrated altered behavior as indicated by auditory startling response and open field activity. Rats exposed to combustion products of the different lubricants exhibited similar pathology. The data do not suggest an increased inhalation hazard from the combustion of MIL-L-23699 compared to other lubricants.

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SUBCHRONIC INHALATION TOXICITY STUDY WITH FORMAMIDE IN RATS. PE Ross, LA Kinney, MC Carakostas, and DB Warheit. Du Pont-Haskell Lab., Newark, DE.

Formamide is used as a solvent in the manufacture of plastics. The current TLV® is based primarily on the toxicity of an analog, i.e., dimethylformamide (DMF). Therefore, a study was carried out to determine the toxic effects of repeated inhalation of sublethal concs. of formamide. Four groups of 10 male CD rats were exposed 6 hrs/day, 5 days/week for 2 weeks to design concs. of 0, 100, 500 and 1500 ppm vapor in air. At the end of the exposure period, clinical and pathologic evaluations were carried out. Male rats exposed to 1500 ppm had depressed body weights. Clinical pathologic examinations revealed that rats exposed to 500 and 1500 ppm of formamide were thrombocytopenic after 10 days of exposure and following 14 days of recovery. Pathologic examinations revealed compound-related microscopic changes in the kidneys of rats exposed to 1500 ppm formamide. Nephrosis was characterized by necrosis and regeneration of renal tubular cells in the deep cortical nephrons. These alterations were prominent after the 10th exposure, however, only regeneration of the tubular cells was evident after 14 days of recovery. Based upon the hematologic and clinical chemical parameters measured, the no-effect exposure conc. for repeated inhalation of formamide was considered to be 100 ppm. The findings of kidney lesions concomitant with increases in kidney-to-body weight ratios reflect the target organ toxicity.
570 ASSESSMENT OF LUNG TOXICITY TO ACRAWAX® FOLLOWING ACUTE EXPOSURE. C.F. Reinhardt, M.C. Carakostas, M.A. Hartsky, and D.E. Warheit. Du Pont–Haskell Lab., Newark, DE.

Acrawax is a trademark for a series of synthetic waxes which are used as flatteners in paint, and lubricants in plastics, and is routinely regarded as a nuisance dust. Due to a paucity of toxicologic data, we investigated the effects of acute inhalation of Acrawax® in rats. CD® rats were exposed to aerosols of Acrawax®C for 6 hrs at 100 mg/m³. Fluids and cells from sham and exposed rats were recovered by lavage (BAL) and measured for cellular and biochemical parameters at 0, 24, 48, 172 hrs, and 1 month postexposure. Pulmonary macrophages (PM) were cultured and studied for morphology, chemotaxis, as well as in vitro and in vivo phagocytosis. The lungs of additional animals were processed for histopathology, and scanning and transmission electron microscopy. Our results showed that Acrawax®C exposure produced a small inflammatory response at 24 hrs postexposure, but cell differentials were nearly indistinguishable from controls at 48 hrs after exposure. BAL levels of LDH, alkaline phosphatase and proteins were not different from controls at any time postexposure. Based on acute studies, our results suggest that the lung response to inhaled Acrawax®C is not substantially different from other nuisance dusts such as carbonyl iron and titanium dioxide particles.


In order to assess the potential health effects resulting from exposure to paraffinic lube oil mists, both acute and subacute (9-day) inhalation studies were conducted on rats. The test materials comprised of highly refined, solvent-extracted paraffinic base stocks which varied in their molecular weight/viscosity (51-158 @ SUS 100F). Acute 4-hr. exposures to the highest attainable atmospheric concentrations (4 g/cm³) did not produce any mortalities and/or adverse effects. Subacute exposures to concentrations of 50, 500, or 1500 mg/m³ did not produce any mortalities and the no-adverse effect level (NAEL) was > 50 mg/m³. Treatment-related clinical signs indicative of CNS and dermal effects and alterations in feed consumption, organ and body weight parameters were observed at ≥ 500 mg/m³. Microscopic examinations revealed evidence of irritation and inflammation in the pulmonary tissue only at the highest concentration. These findings are consistent with those observed when evaluating materials of this type. The NAEL of > 50 mg/m³ supports the current ACGIH TLV of 5mg/m³ for oil mist exposure.


Previous 2-wk aerosol inhalation studies on a 4000 mw EO/PO polymer indicated that this member of this series produced unexpected pulmonary toxicity in rats. Since the 4-hr LC50 for this 1700 mw polymer was 4670 mg/m³, 44x higher than for the 4000 mw polymer, mean exposure conc. of 504, 982, and 2460 mg/m³ were evaluated for this 2-wk (6 h/d, 5 d/wk; 9 exposures in 11 days) study. All rats survived at 2460 mg/m³. Signs of ocular and nasal irritation and respiratory difficulties and reduced BW and/or BW gain occurred in all aerosol-exposed groups. Many of the hematologic, serum chemistry, and urinalysis parameters were abnormal for the 504 and 982 mg/m³ groups when compared to controls. Pulmonary congestion was observed at necropsy in all aerosol-exposed groups; absolute and relative lung weights were increased for the 504 and 982 mg/m³ groups. Of the organs evaluated, only the lung had histopathologic lesions; these included interstitial pneumonitis, bronchioalveolar cell hyperplasia, and intraalveolar macrophage infiltrates. The severity of the lesions was concentration related. In conclusion, despite wide differences in the LC50 of these compounds, data for this 1700 mw polymer indicate that the toxicity is cumulative and aerosol of this polymer should be regarded as a potential health hazard.


Cobalt hydrocarbonyl (CHC) is a gas used as a catalyst in certain chemical operations. The limited inhalation toxicity data which exist indicate that CHC and its spontaneous decomposition products are irritating to the mucous membranes of the eye and respiratory tract. The current experiment was undertaken as part of an effort to develop an Emergency Planning Guideline for CHC. Each of three exposure groups consisted of 10 rats (5/sex). All groups were exposed once for 30-minutes to either air, or an "aged" atmosphere of CHC at 110 mg (Co)/m³ or 210 mg (Co)/m³. At the end of the 16-day observation period, 0 of 10 rats in the 110 mg (Co)/m³ group and 7 of 10 rats in the 210 mg (Co)/m³ had died. At the 110 mg (Co)/m³ exposure concentration, 0 of 10 rats showed respiratory distress, 3 of 10 rats showed slight respiratory effects, and 7 of 10 showed grossly observable lung effects at necropsy. At the 210 mg (Co)/m³ exposure concentration, all the surviving rats showed significant weight loss during the first 7 days of postexposure observation. Significant clinical signs seen in most of these animals were labored breathing, dried nasal discharge, and decreased activity. All rats in this group were observed to have discolored lungs at necropsy. The LC50 from this study was estimated to be 180 mg (Co)/m³, confirming the accuracy of a previously reported value.
Inhalation exposure to Skydrol® 500 B-4 fire resistant hydraulic fluid was evaluated using Sprague-Dawley rats (25/group) at average atmospheric concentrations of 5, 50, 100 or 300 mg/m³ for 5 hr/day, 5 days/wk for 13 weeks. Parameters evaluated included clinical signs, body weights, ophthalmic exams, hematology and clinical chemistry, organ weights and gross- and histopathology. All animals survived until scheduled necropsy. Eyes and gross condition were not affected. Signs of irritation, e.g., reddish nasal discharge and salivation were observed at the mid-conc. and, more extensively, in high-conc. group. Body weights were decreased and liver weights were increased in high-conc. animals. Plasma cholinesterase activity was decreased in high-conc. females at the interim and final bleeding periods. Slightly decreased RBC, HGB and HCT were observed in high-conc. group at 6-week bleeding. Mild hepatocellular vacuolization was considered treatment-related in high-conc. group. Urinary bladder transitional epithelium hyperplasia reported previously after ingestion of the major components (tributylphosphate and dibutylphthalate), was not observed. Other than irritation, the NOAEL is considered to be at least 100 mg/m³.

Chlorotrifluoroethylene (CTFE) oligomer is an inert, nonflammable, saturated and hydrogen-free chlorofluorocarbon oil of interest to the Air Force. It is noncorrosive, has high thermal stability, good lubricity, and high dielectric strength. Past acute inhalation studies indicated CTFE had a low degree of toxicity. To determine the potential subchronic inhalation toxicity of CTFE, male and female F-344 rats were exposed to air only, 0.25mg/L, 0.50 mg/L, and 1.00 mg/L of CTFE in 65 6-h inhalation exposures over a 90-day period. A dose dependent depression in body weight gains was noted in male rats only. Alkaline phosphatase, AST and ALT values examined at the conclusion of the study indicated a treatment-related effect in the male test rats, but not the female test rats. Notable, concentration-related, increases (p < 0.01) in relative kidney and liver weights occurred in both sexes of rats at all test concentration levels. The male rats showed slight to minimal hyaline droplet formation in the kidney proximal tubule epithelium. However, pronounced cyto-megaly of hepatocytes was the predominant lesion recognized. (Supported by US Air Force Contract # F33615-85-C-0532.)

Formic acid (HCOOH) is a natural component of plant and animal tissue and an air pollutant. Thirty-one million kg are produced annually in the U.S., resulting in the exposure of over 500,000 workers. Rats and mice were exposed by inhalation to 0, 31, 62.5, 125, 250 or 500 ppm of HCOOH for 12 exposures to establish concentrations for a subchronic study. Mortality was 70% in the 500 ppm group and 5% in the 250 ppm group. Lesions consisted of necrosis, regeneration, and squamous metaplasia of the nasal respiratory epithelium, and suppurative rhinitis and laryngitis. Lungs were largely unaffected except for some congestion in the highest dose group. Thymus and kidney weights were reduced. Based on the upper respiratory tract lesions, concentrations of 0, 8, 16, 32, 64 or 128 ppm were utilized in the 13-week study. One male mouse in the 128 ppm group died but all other animals survived. Mice in the 128 ppm group had decreased body weight gain beginning after 6 weeks of exposure, but body weights of the rats were not affected. There were no exposure-related adverse clinical signs or gross necropsy observations. Lung weights were generally decreased. Lesions occurred in the respiratory and olfactory epithelium of these groups. Degeneration of the olfactory epithelium and squamous metaplasia of respiratory epithelium in the two highest dose groups. There were no exposure-related lesions present in the lungs of either species. Thus inhalation exposure to HCOOH resulted in nasal lesions with little apparent effect on the lungs or other tissues.

Uniform distribution of test compound is required in inhalation exposure chambers to assure that all animals are exposed to the same concentration. Uniformity tests must be done on a regular basis at numerous specified sample locations throughout the chamber, as distribution can be affected by small leaks in the chamber door seals or by a nonuniform distribution at the chamber inlet. Changes in average chamber concentration during the measurement routine (e.g., generator variation) introduce a time-varying element to the spatial measurements. The method described was derived for uniformity determinations in the Hazleton 2000 exposure chamber, but is applicable to most chambers. Samples are taken at the chamber inlet and exhaust and at 12 locations within the chamber, one of the locations serving as a reference for temporal variation. The time varying element is statistically eliminated from the spatial concentration variation. The method requires a minimum number of samples to arrive at a value for spatial variability and provides a simple uniformity acceptance criteria. In the case of materials which are reactive with the chamber or the animals (specifically glutaraldehyde and ozone), the method will reveal discrepancies between materials supplied to the chamber and the concentration measured in the breathing zones of the animals.

Forest firefighters rely on field deployed heat reflective personnel shelters to escape backfires. Since shelters of old construction had the potential for generating toxic internal atmospheres due to thermodegradation of the laminate used, a combustion toxicity test (USDA Method TM 5100-1) was previously developed. Problems with TM 5100-1 lead to a project to revise the test. The optimization of thermodegradation and combustion gas evolution from materials which had been, by design, high heat performance and low combustibles was the study objective. Features of the revised test method include: (1) a new inhalation chamber design; (2) validation steps to check for chamber integrity; (3) a dose-response scheme instead of "limit testing". New chamber design lead to larger specimen acceptance and elimination of heat stress in test animals. Using a known toxic laminate, data were developed showing consistent thermodegradation yields (expressed as mass/mass area) for shorter experiment time was feasible thus better discriminating HCN versus CO toxicity: a 7 day pre-exposure period was possible shortening unnecessary post-exposure study time. A screening test without animals and combustion gas measurement was also introduced. Work supported by Forest Service, USDA, contract 53-0343-00917.

A FLUIDIZED-BED DUST GENERATOR FOR USE IN INHALATION TOXICITY STUDIES. B R Dudek, T A Kaepple, C L Bechtel, S R Becktame and W V Roloff. Monsanto Company, Environmental Health Laboratory, St. Louis, MO.

Solid aerosol atmospheres in inhalation toxicity studies require that test material concentrations be controllable and that the atmospheres have a relevant particle-size distribution. A design for a simple, inexpensive generator is available that can be used for these types of studies. Construction is from readily-available materials and requires the services of a glass-blower. Total cost for one unit is approximately $200. Aerosol data from a 1-month study with solid cadmium chloride at target concentrations of 0.2 (low) and 2 mg/m³ (high) generated with this system resulted in mean analytical concentrations of 0.20±0.03 and 2.0±0.3 mg/m³ in air. Corresponding mean nominal concentrations were 14.99 mg/m³. Mass median aerodynamic diameter (MMAD) for the low and high levels were 4.55 and 5.12 micrometers, respectively. Corresponding geometric standard deviations were 1.56 and 1.56 microimeters, respectively. The percentages of particles less than or equal to 10 micrometers were 94.3 (low) and 93.1 (high). This system has also been effective in generating solid aerosol atmospheres at concentrations greater than 1 mg/l during acute inhalation studies.


Inhalation toxicity studies with drug aerosols in dogs or primates presents difficulties related to the numbers of animals involved, the achievement of adequate dose levels and limited availability of research drugs. A system for mask exposure of large numbers of primates for extended periods has been developed. Aerosols of saline, metaproterenol sulfate 1% (MPS), MPS in liposomes or empty liposomes were generated using two Resa® nebulizers and delivered into six pediatric anesthetic breathing loops. Seven groups of six cynomolgus monkeys were restrained in a neck yoke apparatus, fitted with Cushion flex® pediatric endonasal masks and exposed to one of various aerosols during two daily sessions, each up to 2 hours, for 28 days. The mean aerosol presented to the animals was 31.5 ± 6.1 mg/mL with a mass median diameter of 3.7 µm ± 2.0. MPS in the aerosol averaged 0.35 ± 0.041 mg/mL (1% MPS solution) and 0.31 ± 0.056 mg/mL (MPS-liposomes). The achieved drug dose (based on aerosol presented, duration of exposure, estimated minute volume and deposition fraction) ranged from 2.7 to 3.1 mg/kg/day and represented 7 to 8 fold the anticipated human dose. This apparatus permits the extended studies in large numbers of animals with limited amount of drug and at reasonable multiples of human dose.

PULMONARY, RENAL AND HEPATIC DISTRIBUTION OF CADMIUM IN MICE AND RATS CHRONICALLY EXPOSED TO CIGARETTE SMOKE. C Gariola*, and G J Wagner**, Tobacco & Health Research Institute*, Graduate Center for Toxicology*, and Department of Agronomy**, University of Kentucky, Lexington, KY.

Cadmium (Cd) is implicated in various organ toxicities in humans and animals. Cigarette smoking constitutes a major source of Cd exposure via inhalation in man. To determine how smoke exposure affects the organ distribution of Cd, male C57Bl mice and Sprague Dawley rats were exposed daily to mainstream smoke from University of Kentucky reference cigarettes (2Rl) in a nose-only exposure system for 52-60 consecutive weeks. Exposed mice and rats averaged blood carboxyhemoglobin values of 17.7 and 7.2%, and a daily TPM dose of 7.2 and 3.2 mg/kg body wt/exposure, respectively. These markers suggested effective inhalation of smoke by the animals. The tissues were acid oxidised and analysed for Cd by flame atomic absorption spectrometry. Five to 6 and 2-3 fold greater than control levels of Cd were detected in the lungs and kidneys, respectively, of exposed animals of both species. Liver did not show increased Cd levels in exposed mice or rats. It is, therefore, concluded that low-dose chronic inhalation exposure leads to the highest Cd accumulation in the lung, followed by the kidney, with insignificant amounts in the liver (supported by KTRB 5-41031).
Evidence for a Lack of Selective Vulnerability of Large Diameter Afferents to 2,5-Hexanediode (HD). T. C. deRojas and R. D. Goldstein. Dept. of Pharmacol & Toxicol, Medical College of Ga., Augusta, GA.

A selective vulnerability of large diameter axons in central-peripheral distal axonopathy (DA) has been suggested. This study was conducted to determine if this vulnerability exists in HD toxicity; a model of DA. Cats were given either 40, 120, or 240 mg/kg HD (sc), twice weekly for six weeks. Four days after the last injection soleus muscle spindle afferents were isolated using standard techniques. The position sensitivity of primary (group Ia) and secondary (group II) muscle spindle afferents and velocity sensitivity of only Ia afferents was determined. Behaviorally, the animals exhibited a dose response relationship with an endpoint of severe splaying being reached. The position sensitivity (area under curve; AUC) of Group II afferents was significantly decreased 33%, 62%, and 66% from control at 40, 120, and 240 mg/kg HD respectively. The position and velocity sensitivity of Group I afferents was unaffected until 240 mg/kg HD; at which point a 43% and 25% decrease in AUC from control was observed, respectively. These data suggest that the large diameter primary muscle spindle afferents are more resistant to HD than are their smaller diameter counterparts; the secondary muscle spindle afferents. Supported by NS-18664.

Non-selective Effects of Acrylamide (AC) on Retrograde HRP Transport in Slow and Fast Motoneurons. D. W. Sickles, and R. D. Goldstein. Medical College of Georgia, Augusta, GA.

Recent evidence suggests that non-adaptable neurons are most susceptible to ACR neurotoxicity and not the largest and longest fibers, as classically believed. To test this hypothesis, we have compared, using quantitative histochemical procedures, the effects of a single 50 mg/kg injection of ACR on the rate and quantity of retrogradely transported horseradish peroxidase (HRP) in the non-adaptable slow soleus motoneurons (MN), with the effect on transport in adaptable fast sensor fascia latae MN in the rat. The rate of transport, measured as first appearance of HRP and as time to peak MN accumulation was not significantly affected in either MN type. However, the total quantity of HRP transported was reduced 56% in the TFL MN and 46% in the SOL MN. Therefore, the quantity of material transported but not the rate of retrograde transport was significantly reduced, as previously found with fast anterograde transport (Toxicologist 8:43, 1988). In addition, retrograde transport was simultaneously and significantly decreased in both MN types demonstrating a non-selective effect of the toxicant upon the two neuron types. (Supported by NS18664, EHO2020 & MCGR)

Extensive Proximal Degenerative Changes in Sensory Neurons Produced by Acrylamide Administration to Neonatal Rats. S. F. Matheson and R. G. Gold. Joint Graduate Program in Toxicology, Rutgers Univ./UMDNJ-R W Johnson Medical School, Piscatawy, NJ.

Acrylamide (AC) produces a dying back neuropathy upon repeated exposure which is associated with an early defect in retrograde axonal transport. We postulated that sensory neurons in young neonatal rats may demonstrate enhanced vulnerability to AC due to their dependence upon retrogradely transported trophic factors. Newborn rats received daily (5 days/week) i.p. injections of AC (30 mg/kg) for 2 weeks and were perfused, along with saline-treated siblings, with 5% glutaraldehyde. Extensive axonal degeneration was observed in sensory fibers of the L4 and L5 dorsal root ganglia (DRG) and in distal portions of the sciatic nerve. Remaining dorsal root fibers were markedly atrophic. Neuronal cell bodies in DRG exhibited degenerative (vacuolation and shrunken perikarya) and reactive (chromatolysis) changes. In contrast, motor neurons showed relatively few alterations at this time. These results suggest that the pattern of AC-induced alterations is age-dependent, the more proximal changes observed in neonatal rats perhaps being due to an interference with trophic interactions. Supported by NIEHS.


Nine male ND-4 mice were dosed with 25 mcg/kg of 14C-acrylamide (200 mg/kg). The animals were killed 1, 3, and 7 days after dosing. The labeling of a number of different proteins in mouse brain and spinal cord was assessed by polyacrylamide gel electrophoresis and autoradiography. On a molar basis, the proteins which showed the highest degree of labeling were the medium (130 kDa) and high (180 kDa) molecular weight neurofilament proteins and unknown proteins with molecular weights of 31, 33, and 57 kDa. About 0.05% of the neurofilament proteins were labeled with the dose employed. It is estimated that over 0.1% of the 31, 33, and 57 kDa proteins bound acrylamide. With a neurotoxic dose of 100 mg/kg, the stoichiometry for acrylamide binding to all five of these proteins could be expected to approach unity. Although some of the label was found to decrease with time for all the proteins, there were significant amounts of acrylamide bound seven days after exposure, with labelling ranging from 15 to 60% of the levels observed one day after exposure. These results indicate that a covalent binding of acrylamide to several major proteins present in the mouse nervous system may play a role in the neurotoxicity of acrylamide.
COMPARISON OF SUBCHRONIC NEUROTOXICITY OF 2-HYDROXYETHYL ACRYLATE (2HEA) AND ACRYLAMIDE (ACR) IN RATS. W.C. Hoser, 1 P.N. Phillips, 1 D.C. Anthony, 2 V.P. Sette, 2 P. M. Nashall, 4 JST Technology Services, RTP, NC; 2DPM, Durham, NC; 3US EPA, Washington, DC & 4US EPA, RTP, NC.

The comparative neurotoxicity of subchronic exposures to 2HEA and ACR was evaluated using a functional observational battery (FOB) followed by neuropathological examination of compound (2HEA: 3, 20, 60 mg/kg; ACR: 1, 4, 12 mg/kg) were administered ip to male and female Long-Evans rats (N=10/dose/sex) 5 days/wk for 13 wks; 2 vehicle control groups were also included. The FOB, which includes home-cage and open-field observations and interactive tests of sensory, neuromuscular, and autonomic function, was administered before dosing, at monthly intervals, and on the day after the last dose. Rats (N=6/dose/sex) were then perfused and tissues from brain, spinal cord, and peripheral nerve were prepared for light microscopic evaluation.

There were clear differences between the effects of 2HEA and ACR. ACR produced time- and dose-related changes in measures of muscle tone and equilibrium and characteristic signs of degeneration in peripheral nerves and spinal cord. 2HEA also produced neuromuscular changes, but the magnitude was not as great as for ACR and often not dose-related; in addition, no neuropathological changes were detected. Thus, 2HEA had effects that did not resemble those produced by ACR.

SENSITIVITY OF RAT BRAIN CALCIUM ATPase TO SCORPION AND SNAKE VENOMS. B. Venkaiah, C.H. Trottman, M. Veerepalli and D. Desai. Dept Neurol, Univ Miss Med Ctr, and Dept Chen, Jackson State Univ, Jackson, MS.

Scorpion (Heterometrus fulvipes) and snakes (Russel, vipers, Naja naja) possess highly potent neurotoxic venoms. The neurotoxic action of these venoms is the subject of this study. Scorpions were collected in Tirupati, India, and the venom was extracted and partially purified. Viper and cobra venoms were purchased from commercial source. Rat brain P, fraction was prepared by differential centrifugation method. Calcium ATPase was assayed by determining inorganic phosphate. Test solutions of venoms were prepared by dissolving 6 mg protein in 1 ml distilled water. Different amounts of venoms (6-18 mg/1.5 ml reaction mixture) were incubated with rat brain P, fraction. The calcium ATPase was inhibited by all three venoms in a concentration dependent manner. However, the sensitivity of the enyme to venoms differed considerably. The IC50 values were 12.15 and 18 mg for scorpion, cobra and viper respectively suggesting the scorpion venom is the most effective inhibitor of the mammalian brain calcium ATPase activity. These results also suggest that these venoms may produce neurotoxicity by altering calcium regulated events in the neurons. (Supported by NIH/MBRS Grant #RR 08047).

HIPPOCAMPAL NECROSIS RESULTING FROM SUBCHRONIC ADMINISTRATION OF TRIS(2-CHLOROETHYL)PHOSPHATE (TECP) IN RATS. D. Dillingham, S. G. Mathews, D. W. Herr and H. Tolson. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

TECP, a flame-retardant plasticizer, was evaluated as part of the National Toxicology Program's chemical study of flame-retardant phosphates. Subchronic administration (16 wks) of 350, 175, 88, 44, 22 or 0 mg of TECP/kg in corn oil to male and female F344 rats by oral gavage resulted in bilateral hippocampal necrosis in 8/10 females receiving 175 mg and 10/10 females receiving 350 mg of TECP/kg. Similar hippocampal lesions were observed in only 2/10 male rats given 350 mg TECP/kg. Hippocampal lesions were characterized by necrosis of neurons, predominantly involving the CA1 pyramidal neurons. In severe cases, there was tissue mineralization, microgliosis and more extensive involvement of the CA1 region with extension to adjacent hippocampal regions. Hippocampal damage increased with dose in both male and female rats, but was consistently greater in females. Hippocampal lesions were not observed in B6C3Fl mice receiving 700, 350, 175, 88 or 44 mg of TECP/kg.

Toxicity observed in mice was limited to increased liver weights in both sexes and decreased kidney weights in males. Other toxicity observed in rats was limited to increased liver and kidney weights in both sexes. Thus, a unique lesion in the hippocampus appears to be the most obvious effect of TECP toxicity and sensitivity to this lesion appears to be both species and sex dependent.

COCAINE-INDUCED ALTERATIONS OF ELECTRO-MECHANICAL ACTIVITY IN HUMAN FETAL HEART IN VITRO. I. S. Richards and A. P. Kulkarni. Florida Toxicology Research Center, College of Public Health, University of South Florida, Tampa, FL.

Although the mechanisms are not fully established evidence suggests that fetal death in utero may occur following acute exposure to moderate doses of cocaine. This study examines the acute in vitro effects of cocaine on cell membrane potentials and contractility of 12-18 week old human fetal left ventricle (n=6) as determined using intracellular microelectrodes and microforce transducers. Cocaine (600 ng/ml) produced significant decreases in action potential amplitude and upstroke velocity within several minutes of exposure accompanied by alterations in developed force. Within an hour preparations became electrically-quiet and did not respond to supramaximal electrical field stimulation. Our study suggests that moderate concentrations of cocaine alter cellular membrane potentials and may precipitate serious cardiac arrhythmias. Whether this contributes to the development of fetal cardiac abnormalities during pregnancy is presently unknown.
Acetylcholine (ACh) stimulation of M1 receptors after DFP inhibition of cholinesterase (ChE) increases intracellular cGMP, a proposed second messenger. With continued DFP animals develop tolerance to its toxicity despite elevated ACh. Uncoupling of the muscarinic receptor-cGMP complex has been demonstrated after repeated exposure of cell cultures to cholinolametics. To study the role of cGMP in DFP tolerance rats were given 2 mg/kg DFP, sc, on Day 1 then 1 mg/kg on alternate days from Days 4-14. On Day 1 ChE was inhibited 60% prior to a 2-fold elevation of cGMP in brainstem (BS) and cerebellum (CE), which preceded toxicity by 30 min. Atropine pretreatment (10 mg/kg, ip) prevented cGMP elevations and signs of toxicity. On Day 14 tolerance to DFP was evident. A significant decrease in muscarinic receptor Bmax, but not Kd, was observed in striatum and cortex (53% and 64% control) and not BS or CE. As a result, cGMP measurement in BS and CE was uncomplicated by possible receptor loss. However, cGMP levels were also elevated 2-fold after DFP challenge on Day 15. These data suggest that uncoupling of the M1 receptor-cGMP complex is not a mechanistic factor in DFP tolerance. (NIM ES07163 and the Center for Biochemical Toxicology)

The role of acetylcholinesterase (AChE) in the central actions of organophosphates (OP) and carbamates was examined, in vitro, on spinal cords from 6-9 day old rats. Stimulation a dorsal root (L3-L5) evoke a monosynaptic reflex (MSR) in a corresponding ventral root while stimulating an adjacent dorsal root evoked inhibition. Sarin potentiated the MSR at 2-20 nM, simultaneous with blockade of bicuculline-sensitive inhibition, and depressed it at > 50 nM (IC50 = 0.09 μM). DFP, phystostigmine and pyridostigmine only depressed the MSR (IC50 = 80, 0.45 and 2 μM, respectively). Atropine and benactyzine (0.5 μM), but not p-tubocurarine (10 μM) or mecamylamine (0.5 μM), completely reversed the depression while pretreatment with low doses of atropine (10-30 nM) prevented OP-induced depression. Protective carbamylatation (> 20% inhibition) or prior inhibition of AChE by DFP failed to alter the depressant effect of sarin. Reversal of sarin-induced depression by 2-PAM, diethyldiamine and THB4 was not accompanied by regeneration of AChE. Thus, AChE inhibitors depressed the MSR by direct muscarinic receptor activation unrelated to inhibition of AChE and potentiated the MSR by blocking bicuculline-sensitive inhibition. (Supported by U.S. Army Medical Research and Development Command Contract DAMD17-86-C-6030.)
LYMOPHOCYTE DOPAMINE RECEPTORS AS MARKERS OF CNS RECEPTORS: A PILOT STUDY IN STYRNE-EXPOSED WORKERS. Teresa Cocca, Harvey Checkoway, Luigi Manzo, Stephen M. Rappaport, and Lucio G. Costa. Department of Environmental Health, SC-34, University of Washington, Seattle, WA. Institute of Pharmacology, University of Pavia Medical School, Pavia (Italy) and University of California School of Public Health, Berkeley, CA.

The biochemical and pharmacological characteristics of the dopamine D2 receptor on circulating lymphocytes were investigated in order to determine whether these could serve as surrogate markers of the same receptors present in brain (striatum). The dopamine D2 antagonist H-123 was found to bind specifically to lymphocytes isolated from rat spleen and rat and human blood. The binding was linear, saturable (Kd=4-16 nM) and was inhibited by various dopaminergic drugs (quipazine, haloperidol, spiperone, ICG values 6-60 nM). The observation that exposure of rats to styrene causes an increase in dopamine receptor binding in striatum (Agrawal et al. 1982) prompted us to investigate whether a similar alteration could be observed in lymphocytes of boat yard workers, occupationally exposed to styrene. Comparisons among 38 boat yard workers were generally consistent with Agrawal's hypothesis. Mean H-123 binding values (fmol/mg lymphocytes) were 54.3, 91.5 and 79.4, respectively in workers exposed to <2, 2-19 and 20 ppm (TWA) of styrene. These preliminary results suggest that lymphocyte D2 receptors might be useful in monitoring certain nervous system effects due to styrene exposure (Supp. in part by grants from NIEHS [P42-ES-04696 and P42-ES-04705], and Fondazione Clinica del Lavoro [Pavia]).


Lindane and type II (α-cyano containing) pyrethroids have been shown by several investigators to inhibit [35S]TBPS binding in rat membrane preparations and GABA-stimulated chloride flux into mouse brain vesicles. To determine if these insecticides also alter GABA-activated chloride currents, we tested the actions of the type II pyrethroid fenvalerate and lindane on GABA/C1 currents in rat hippocampal neurons in cultures using the whole cell patch clamp technique. To retard current rundown, internal solutions contained 10 mM EGTA and 4 mM Mg-ATP. GABA/C1 currents exhibited distinct early transient (10-20 sec) and late sustained components (>20 sec). Lindane (1-50 μM) attenuated both components when applied concurrently with GABA (15 μM); however, block of the early component was more pronounced. Lindane also delayed the onset of GABA/C1 currents when the early transient phase was completely blocked. Of note, lindane pretreatment (20-30 sec) enhanced a subsequent GABA challenge. Fenvalerate (50 μM) did not suppress but enhanced GABA/C1 currents either when applied concurrently or prior to GABA. Both insecticides produced small transient inward currents independent of GABA administration. Supported by NIH grant NS14143.
Exposure to 1,3-dinitrobenzene (1,3-DNB) induces methemoglobinemia, nausea, headache, and symptoms of nervous dysfunction. Single daily oral doses of 25 mg/kg 1,3-DNB produces ataxia in conventional male F-344 rats after 5-7 days. Plantar paralysis of the forelimbs and displacement of hindlimbs are observed. In contrast, a single oral dose of 20 mg/kg produces ataxia in germ-free male F-344 rats. Peak concentration of 1,3-DNB in the blood of germ-free are more than twice those of conventional rats after a single oral dose of 25 mg/kg; the rates of clearance are comparable. In either case, light and electron microscopy of brains from treated animals reveal focal bilaterally symmetrical, edematous lesions in the brainstem and inferior colliculi. Frequent petechial perivascular hemorrhage, usually confined to the enlarged Virchow-Robin space, occasionally infiltrates the surrounding parenchyma. The primary targets of 1,3-DNB neurotoxicity appear to be the macroglia and vasculature with secondary neuronal involvement. (Supported by NIMH grant).

The choroid plexus (CP) functions as the major producer of cerebrospinal fluid (CSF). Several neurotransmitters in CSF have been shown to be produced elevations in the second messenger cGMP in CP. Intraventricular application of these compounds causes inhibition of CSF production. This suggests a link between cGMP production and the inhibition of CSF formation.

Serotonergic application to CP tissue slices activates a 5HT1c receptor found on the epithelium, and receptor occupancy triggers a second messenger pathways: phosphoinositide (PI) turnover and cGMP production. Ethanol blocked cGMP production in a dose-dependent manner, with complete inhibition at 650uM. This concentration of ethanol does not compromise cell integrity as PI turnover is only slightly inhibited (20%). The IC50 for cGMP inhibition occurred at 54uM. Sodium nitroprusside (100uM), a stimulator of the soluble form of guanylate cyclase, elevated CP cGMP by nearly 4.4 fold basal levels, and this production was also completely inhibited by 650uM ethanol.

Ethanol can inhibit serotonin induced as well as direct activation of guanylate cyclase, and by blocking guanylate cyclase may alter the ability of various neurotransmitters to inhibit CSF production.

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The separation and identification of muscarinic receptor subtypes in calf caudate for neurotoxicity studies. L. S. Katz and J. K. Marquis. Boston University School of Medicine, Boston, MA.

Homogonates of calf caudate nuclei were found to contain at least two distinct subclasses of cholinergic, muscarinic receptors. These subtypes, labeled with 3H-1-quinoxalindinyl benzilate (QNB), can be separated by rapid filtration with the use of selective ligands. Binding assays demonstrated that these homogenates consist of a subpopulation of pirenzepine-sensitive receptors (sometimes designated M1) as well as a subpopulation of AFDX16-sensitive receptors (M2). The purpose of these initial studies was to define and quantify the ratios of receptor subtypes in order to permit neurotoxicity studies in this system. This heterogeneous receptor preparation is now being used to distinguish the effects of acetylcholinesterase inhibitors, specifically organophosphates binding to muscarinic subtypes. In an earlier study, our laboratory found that organophosphates such as paraoxon and dichlorvos significantly altered muscarinic receptor binding properties, but we were unable to identify the mechanism of this effect. It is anticipated that additional experiments in a more precisely defined receptor population such as that described here will allow further elucidation of these effects. (Supported in part by the Eppeley Foundation.)

NAD(P)H: quinone reductase (QR) detoxifies quinones via an obligatory two-electron reduction limiting the generation of active oxygen species by redox-cycling quinones. Exposure of cultured hepatic (Hepa1C1) or fibroblastic (3T3-L1) cell lines to toxic doses of menadione, produces a rapid 70% loss of QR in 2 hr with cell death in 24 hr. Nontoxic doses of menadione also produce a rapid decay of QR, however, recovery to normal values occurs in 24 hr. Paraquat, diquat, nitrofurantoin, and H2O2 produce similar results. Losses of glutathione (GSH) (50-70%) occur in 2 to 4 hr during oxidative stress. In the N18RE115 cells a loss of QR is seen with 10uM glutamate, a specific neurotoxin for this line (DeLong, et.al., Neuro. Abst. 1988). Induction of QR levels 2 to 4-fold by tert-butyldihydroquinone (3-30uM) for all cell types prevented cell death, protected against cell death by 50 to 100% over control cells. Dicumarol, a specific inhibitor of QR increases cell death. We speculate that the initial rapid loss of QR and GSH potentiates cell death by perturbation of the redox homeostasis they regulate. These in vitro data further suggest the loss of QR and GSH may be a common mechanism of cellular death with application in neuronal degenerative diseases.
The 21-aminosteroids U-74006F and U-74500A represent a novel class of lipid peroxidation inhibitors being developed for the acute treatment of CNS trauma and ischemia. The possibility that these compounds might also protect brain neurotransmitter proteins (NFP) from oxidative damage has been examined. Brain homogenates prepared in Kreb's-Ringer solution were incubated for 30 min at 37°C with the following additions: 1) none; control 2) xanthine/xanthine oxidase (x/xo; 1 mM/0.03 U/ml or 0.1 mM/0.003 U/ml) or 3) x/xo + drug (U-74006F: 100, 300, 1000μM; U-74500A: 10, 30, 100μM). Following incubations samples were analyzed by SDS polyacrylamide gel electrophoresis. NFP bands stained with Coomassie blue were identified by standards and quantified by band optical density. Incubation with the high x/xo level resulted in complete degradation of NFP bands 210K, 160K and 68K. Studies using superoxide dismutase, desferrioxamine and mannitol indicated that x/xo-induced NFP degradation was dependent upon superoxide radical and iron, but did not involve hydroxyl radical. Neither U-74006F or U-74500A prevented x/xo-induced degradation of the 210K or 160K proteins, however, both compounds caused a dose dependent preservation of the 68K protein. At the lower x/xo level, U-74006F protected both the 160K and 68K proteins, but not the 210K. U-74500A protected both the 150K and 68K proteins and in addition provided partial protection of the 210K protein. These results indicate that in addition to their previously demonstrated protective effects on lipid, the 21-aminosteroids may protect NFP from oxygen radical damage as well.

ANTIOXIDANTS FAIL TO PROTECT AGAINST MPTP-INDUCED DOPAMINE DEPLETION IN MICE. D.D. Monte, M Galbo, N Hooper, M T Smith, L DeLanney, T Irwin, J W Langston. Institute for Medical Research, San Jose CA; University of California, Berkeley, CA.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a neurotoxin which produces a parkinsonian syndrome in human and non-human primates. It also induces selective depletion of dopamine in the striatum of mice. The effectiveness of antioxidants as protective agents against MPTP-induced neurotoxicity remains controversial. Some studies have shown a protective effect in mice while others have not. The purpose of the present study was to re-assess the effects of ascorbic acid and alpha-tocopherol on MPTP-induced striatal dopamine depletion in C57Bl/6 mice. Specifically, we investigated the role of different routes of MPTP administration, age of the mice, and time of survival allowed after MPTP exposure. Data obtained in all of the experimental conditions showed that the dopamine depletions induced by MPTP were not significantly altered by treatment with either alpha-tocopherol or ascorbic acid. Furthermore, the levels of these two antioxidants in several areas of the brain (striatum, ventral mesencephalon and cerebellum) remained unchanged after exposure to MPTP. In fact, our data suggest that systemic administration of these antioxidants does not significantly increase their concentrations in the striatum. These results confirm our previous data obtained in vitro, and suggest that mechanisms other than oxygen radical production are involved in MPTP-induced toxicity.
ADUCT FORMATION BY BENZOQUINONE AND DEOXYCYTIDINE: A Computer Model and Simulation. Robert L. Guy, and R. Snyder, Joint Graduate Program in Toxicology, Rutgers University/UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ.

Deoxyctydine-3′ monophosphate (dCP) was labeled at the 5′ position using polynucleotide kinase (PNK) and gamma-32P-ATP in 50 mM glyc-NaOH, 10 mM DTT, 5 mM MgCl2 at pH 9.5. The labeled compound (dCP) was reacted with p-benzoquinone (BQ) and adduct formation followed by radiometric fractions produced by HPLC. The resulting peak was resolved and quantified by implementing the inverse diffusion method for data peak separation (Anal. Biochem.167:15(1987)) as a computer program in BASIC for the IBM PC. A kinetic model was constructed, and optimal rate constants determined by use of the Nelder-Mead modified Simplex (Computer J. 7:308(1965)).

The fundamental system of differential equations was solved by an implicit RUNGE-KUTTA algorithm. (Supported by ES02931)

PROSTAGLANDIN H SYNTHASE-CATALYZED OXIDATION OF HYDROQUINONE TO A REACTIVE DNA BINDING METABOLITE. M. J. Schlosser, R. D. Shurma, and G. F. Kalf, Department of Biochemistry and Molecular Biology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA.

Hydroquinone (HQ), a metabolite of the myelotoxic benzene (BZ), can be activated to toxic compound(s) by peroxidases. The narrow stroma which regulates hematopoiesis contains prostaglandin H synthase (PHS)-peroxidase. Since inhibitors of the cyclooxygenase component of PHS prevent in vivo BZ toxicity, we decided to study HQ metabolism by purified PHS. PHS oxidized HQ to p-benzoquinone (BQ) in an arachidonate (AA) and time-dependent manner. The presence of cysteine (CYS) yielded the mono-CYS conjugate of BQ at rates similar to BQ formation during incubations without CYS. Oxidation of [-14C]HQ in the presence of mtDNA resulted in a BQ-deoxyguanosine adduct after digestion of adducted mtDNA with nuclease. The AA-dependent (but not the H2O, driven) metabolism of HQ to BQ or its conjugates was completely prevented by 10 μM indomethacin (ID), an inhibitor of cyclooxygenase, whereas 10 μM ID had no effect on the ability of purified human myeloperoxidase to oxidize HQ to BQ. These results demonstrate that PHS oxidizes HQ to a reactive DNA binding metabolite and, together with in vivo studies utilizing PHS inhibitors to prevent BZ toxicity, suggests that PHS of narrow stroma may be involved with BZ myelotoxicity. (NIEMS grant ES03724).

ADUCT FORMATION RESULTING FROM THE REACTION OF 2′DEOXYGUANOSINE-3′- MONOPHOSPHATE AND 1,4-BENZENEDIOL. H. BAUER AND R. SNYDER Joint Graduate Program in Toxicology, Rutgers University and UMDNJ/Robert Wood Johnson Medical School, Piscataway, NJ.

We have previously reported on adduct formation between 2′deoxyguanosine-5′- monophosphate and 1,4-benzenediol (HQ) (Toxicologist 8,71(1988). Here we report our studies on the formation of adducts between 2′deoxyguanosine-3′- monophosphate (3′-dGMP) and HQ. 3′-dGMP was incubated with HQ and FeCl3 to facilitate oxidation of HQ. Products were analyzed by PEI-cellulose TLC and HPLC reversed phase chromatography. Fractions were separated and UV and fluorescence spectra were determined. To obtain the [32P] post labeled 3′dGMP-HQ adduct the reaction mixture was reacted, without further purification, with nuclease P-1 to modify the non-adducted 3′dGMP. The modified 3′dGMP was reacted with gamma-[32P]ATP and T-4 polynucleotide kinase to obtain a [32P] label at the 5′ position. TLC revealed two major spots which appear to correlate with benzene related DNA adducts formed in vivo inomers of benzene treated male Wistar rats and New Zealand rabbits. (Supported by ES02931)

ALKYLATION PREFERENCES OF S-(2-CHLOROETHYL)-GLUTATHIONE AND S-(2-CHLOROETHYL)CYSTEINE TOWARD SPECIFIC SITES WITHIN MODEL Dipeptides and NUCLEIC ACIDS. F. A. Jean and D. J. Reed. Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR.

S-(2-Chloroethyl)glutathione (CEG) and S-(2-chloroethyl)-L-cysteine (CEC) are putative metabolites of glutathione-mediated 1,2-dichloroethane (DCE) bioactivation and are direct-acting alkylating agents that covalently bind nucleic acids. We characterized the alkylation of a series of dipeptides and nucleic acids by both conjugates in terms of identifying alkylation sites and rates of reaction utilizing HPLC, NMR and MS techniques. CEG and CEC share identical alkylation preferences but varied in the extent of reaction with different nucleophilic sites. The alkylation of cys-tyr >> his-tyr > lys-tyr, gly-tyr, gly-tyr, 2′-deoxyguanosine > 2′-deoxyadenosine, 2′-deoxycytidine and thymidine. Cysteinyd chlal alkylation by CEC or CEG occurred at rates 72 and 54 times that for the N9-position of guanine and >10 and 16 times that for histidine imidazole nitrogen, respectively. CEG and CEC have demonstrated a selectivity for chlal alkylation over other amino and nucleic acid functional groups that should be of toxicological importance when assessing the in vivo role of alkylation events in DCE toxicities.
THE ROLE OF AN EPSULFONIUM-RING CYSTEINE CONJUGATE IN 1,2-DICHLOROETHANE (DCE)-INDUCED TOXICITY. K Tulp, D H Marchand, and D J Reed, Oregon State University, Corvallis, OR.

Incubation of freshly isolated hepatocytes with toxic doses of DCE produced a time and concentration dependent leakage of cellular lactate dehydrogenase and an increase in lipid peroxide formation. Cellular glutathione (GSH) was severely depleted within one hour and the remaining GSH could be accounted for by that present in mitochondria. Depletion of mitochondrial GSH preceded the loss of cell viability, but mitochondrial GSH depletion was concomitant with the loss of viability. The loss of GSH was partially due to the formation of S-(2-carboxymethyl)GSH, formed presumably from the reaction of a DCE metabolite with GSH. No increase in GSSG levels was observed intracellularly or in the medium of isolated hepatocytes treated with DCE. Protein thiois were not depleted in viable cells after exposure to DCE. It has been proposed that direct conjugation of DCE with GSH leads to the formation of a reactive episulfonium ring-GSH conjugate. Because of the reactivity of the episulfonium ring-GSH conjugate, one approach to studying its role in the bioactivation of DCE has been to examine the putative precursor of the episulfonium ring-GSH conjugate, S-(2-chloroethyl)GSH (CEG) and the more toxic cysteine conjugate, S-(2-chloroethyl)-L-cysteine (CEC). Treatment of isolated hepatocytes and kidney cells with toxic doses of CEC produced a more gradual depletion of cellular GSH than that observed with DCE. Cells treated with CEC also underwent a loss of protein thiois in viable cells in contrast to hepatocytes treated with toxic doses of DCE in which protein thiois were not depleted. Lack of evidence for the formation of cysteine conjugates of DCE and the observations presented here suggest that CEC may not be a good model for the acute toxicity of DCE in isolated cell systems, and that oxidative pathways may account for some of the metabolism and toxicity of DCE. (Supported by NICHES ES00040.)

EFFECTS OF SULFUR-CONTAINING ANALOGS OF 2,4,5,6-TETRACHLOROISOPHTHALONITRILE ON HEPATIC AND RENAL MITOCHONDRIA. H C Savides, J P Marciniszyn, J C Killeen Jr., G L Ellrich*, Riceca, Inc., Toxicology and Animal Metabolism, Paintsville, OH.* Fermentation Plant Protection Company, Mentor, OH.

Respiration by liver and kidney mitochondria was evaluated polarographically. Oxygen consumption rates were determined in the presence of ADP (State 3) and after the added ADP had been consumed (State 4). Mitochondria were incubated in the presence or absence of sulfur-containing analogs of 2,4,5,6-tetrachloroisophthalonitrile, a compound which has been shown to produce nephrotoxicity in rats following long-term dietary exposure at high dose levels. The sulfur-containing analogs, which have been detected in urine or bile, were studied in an attempt to elucidate the mechanism of site-specific toxicity. The data indicated that the 4-mono- and 4,6-di-glutathione conjugates of 2,4,5,6-tetrachloroisophthalonitrile were not directly toxic to mitochondria, but that the 4,6-dithiol analog was a potent mitochondrial toxin. Cleavage of glutathione conjugates to form thios can occur in the kidney and, thus, may explain the tissue-specific nephrotoxicity of 2,4,5,6-tetrachloroisophthalonitrile.

SPECIES DIFFERENCES IN RENAL γ-Glutamyl TRANSEPTIDASE ACTIVITY AND SUSCEPTIBILITY TO 2-BROMOHYDROQUINONE MEDIATED NEPHROTOXICITY. S S Jau, B A Hill, P Pinon and T J Monks, Div. of Pharm., The Univ. of Texas at Austin, TX and The Univ. of Texas M.D. Anderson Cancer Center, Science Park-Research Division, Smithville, TX.

The nephrotoxicity of 2-bromohydroquinone (2-BHQ) is mediated by the formation of 2-BH2(diglutathionyl-Syl)H2Q. The selective accumulation of 2-BH2(diglutathionyl)-H2Q into proximal tubular cells is mediated by γ-glutamyl transpeptidase (GGT). We now report that significant species differences exist in the susceptibility to 2-BHQ and that differences in GGT activity between species may contribute to these differences. Administration of 2-BHQ (0.8 mmol/kg i.p.) to Sprague Dawley (S.D.) rats and BALB/c mice caused elevations in blood urea nitrogen (BUN) of 101.5±12.4 and 41.3±12.2 mg% respectively. Hamsters and guinea pigs appeared relatively resistant to 2-BHQ nephrotoxicity (BUN levels of 20±10.9 and 22.9±17 mg% respectively). With the exception of the hamster (0.77 U/µg) susceptibility to 2-BHQ mediated nephrotoxicity correlated with GGT activity, which was highest in the S.D. rat (3.79 U/µg), intermediate in BALB/c mice (0.62 U/µg) and lowest in the guinea pig (0.18 U/µg). Variations also existed between species in the inhibition kinetics of GGT by AT-125 in vitro. GGT from rat was the most sensitive to inhibition by AT-125 whereas GGT from hamster was the least sensitive. The data suggest that species differences exist in the activity and regulation of GGT which may have important toxicological consequences. (Supported by USPHS awards GM 39338 and ES 04662)

EFFECT OF GLUCOCORTICOID ON MICROCYSTIN INDUCED RELEASE OF ARACHIDONIC ACID METABOLITES IN RAT HEPATOCYTES. S M Nasem and H S Hines, USAAMRID, Pathophysiology Division, Fort Detrick, Frederick, MD. Sponsor: R W Wannemacher.

We previously showed that microcystin-LR (MCT) is associated with elevated levels of eicosanoids. The study is further extended to include therapeutic effect of fluocinolone (FL) on MCT-induced release of arachidonic acid (AA) metabolites. The effect of FL on AA metabolism was determined by stimulating the 14-AA labeled hepatocytes monolayer with 1 µM of MCT after 16 hr preincubation in the presence or absence of 1 µM of FL. Treatment of cultures with MCT or FL did not alter the cell viability, and the protein content remained unaffected. Total radioactivity release into the incubation medium was not affected by FL. MCT-induced total radioactivity release increased by 240% in 2 hr incubation. Pretreatment of such cultures with FL marginally reduced the release by 21%. Whereas, AA release as determined by TLC of the medium was inhibited by 31% (p<0.025). FL pretreatment also inhibited the synthesis and release of prostacyclin (PC 6-keto F2α) by 24±2.5% (p<0.05) and thromboxane (TXB2) by 34±3% (p<0.025). Under these experimental conditions, the quantities of prostaglandin F2α and PGD2 were not significantly different when compared to control or MCT treated cultures. These results demonstrate that FL may protect against MCT toxicity.
Naphthalene (NAP), a pulmonary cytotoxicant in mice, is preferentially metabolized to 1R,2S-naphthalene oxide by murine pulmonary microsomes, but not by microsomes isolated from nontarget tissues. We have previously shown that MA are the predominant urinary metabolites derived from GSH conjugates of 1,2-naphthalene oxide. Since urinary MAs appear to be appropriate indicators of in vivo epoxide formation, we have measured MAs in mice treated IP with toxic and nontoxic doses of NAP. Excretion of MA isomers was dose dependent. The ratio of MA derived from the 1R,2S-oxide vs the 1S,2R-oxide varied from 2.6-12.5 to 1. Relatively more MA derived from 1R,2S-oxide was recovered in urine from 100 and 200 mg/kg NAP treated animals. Pretreatment of mice with 225 mg/kg NAP (24 hours before microsome preparation) resulted in a significant and selective decrease in pulmonary microsomal metabolites of NAP to the 1R,2S-oxide (as assessed by GSH conjugate formation); there was no significant effect on hepatic microsomal metabolism. In NAP pretreated animals, the total amount of MA was similar to controls, but the relative amount of 1R,2S-oxide derived MA decreased by nearly 40% in animals dosed with 19c-NAP (200 mg/kg, IP) indicating that alterations of pulmonary metabolism are reflected by changes in diastereomeric MA formation. Supported by NIEHS 04699.

Eugenol (4-allyl-2-methoxyphenol) is a principal component of clove cigarettes, which have recently been associated with acute pulmonary toxicity in humans. We have begun studying the metabolism of eugenol to reactive intermediates by both microsomal and peroxidase enzymes. We report here the oxidation of eugenol by horseradish peroxidase. Eugenol was oxidized via a one electron pathway to a phenoxy radical and a quinone methide. The eugenol phenoxyl radical was detected using fast-flow electron spin resonance. The radicals and/or quinone methide further reacted to form an insoluble polymeric material. Glutathione (GSH) or ascorbate prevented the appearance of the quinone methide and also prevented the presence of the parent compound. In the presence of GSH, a thiol radical was detected and increases in oxygen consumption and in the formation of oxidized GSH were observed. GSH also reacted directly with the quinone methide resulting in the formation of eugenol-GSH conjugates. Using 4-eugenol extensive conjugation (GSH and GSSG) was observed. Finally, the oxidation products of eugenol/peroxidase were cytotoxic to isolated rat hepatocytes.
BIOACTIVATION OF 3-METHYLINDOLE(3MI) BY ISOLATED RABBIT LUNG CELLS. W K Nichols, D N Larson, and G S Yost, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.

The pneumotoxic effects of 3MI were evaluated using alveolar macrophages (AM), type II alveolar epithelial cells (type II), and Clara cells. AM were obtained by alveolar lavage of New Zealand white rabbits. Perfused lungs were digested with protease and centrifugal elutriation was utilized to obtain enriched fractions of type II and Clara cells. A dose dependent decrease in cell viability of all three cell types was demonstrated using 3MI (0.1-1.0 mM). The order of susceptibility was AM < type II < Clara cells. Inhibition of cytchrome P-450 by the suicide substrate, aminobenzyltriazole (0.25 mM) decreased the susceptibility of all three lung cell populations to the toxic effects of 0.5 mM 3MI. Trideuterated-(methyl)-3MI incubations with Clara cells caused significantly less toxicity. This demonstrated that decreased cell viability correlated with the metabolism of 3MI via cytchrome P-450-mediated methyl oxidation. These results support the concept that bioactivation of 3MI via cytchrome P-450 is associated with its selective pneumotoxic actions on rabbit Clara cells. (Research supported by USPHS Grant HL13645. GSY is a USPHS Research Career Development Awardee, HL02119).

THE RELATIONSHIP BETWEEN OXYGEN RADICALS AND THROMBOXANE (TXB2) RELEASE IN ISOLATED LUNGS PERFUSED WITH PHORBOL MYRISTATE ACETATE (PMA) AND NEUTROPHILS (PMN). L C Dego and R A Roth. Michigan State Univ., E. Lansing, MI.

In isolated rat lungs perfused with PMA and PMN for 30 min, edema occurs which is attenuated by co-perfusion with superoxide dismutase (SOD) and catalase (CAT) or with a thromboxane synthetase inhibitor. To examine the possibility that TXB2 release was linked to O2- or H2O2 production, lungs were perfused with the presence or absence of SOD or CAT. Samples of effluent medium were analyzed for TXB2 by radioimmunoassay. Perfusion with either SOD or CAT attenuated the increase in lung weight and lavage fluid albumin caused by PMA and PMN. At 10, 20 and 30 min of perfusion, TXB2 in effluent from lungs perfused with PMA and PMN was greater than from lungs perfused only with PMA. Co-perfusion with SOD or CAT did not significantly reduce effluent TXB2 concentration until 30 minutes of perfusion. These results suggest that in lungs perfused with PMA and PMN, TXB2 release occurs independently of O2- or H2O2 at early time points, whereas it is partially dependent on the production of these species at later time points. Also, neither SOD nor CAT apparently protected these lungs from injury by inhibiting TXB2 production. (Supported by NIH grant ES04139.)

ISOLATION AND IDENTIFICATION OF THE MURINE 3-METHYLINDOLE URINARY METABOLITE 3-HYDROXY-3-METHYLMETHOXYINDOLE. G L Skiles and G S Yost, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT.

The organ- and species-selective toxicant 3-methylindole (3MI) is formed in the gastrointestinal tract as a cryptophan degradation product. Man is also exposed to 3MI as a pyrolysis product of tobacco. The identification of 3MI metabolites, in a species susceptible to pneumotoxicity, was undertaken to aid in the elucidation of the bioactivation mechanism. Mice were administered 400 mg/kg of 3MI (LD50 575 mg/kg) and the urine from 0 to 24 hours was partitioned with ethyl acetate. The major non-polar metabolite was isolated and purified by reverse- and normal-phase HPLC of the organic extract. A structural assignment of 3-hydroxy-3-methylindolone for the metabolite was achieved by high-field carbon and proton NMR, high-resolution direct-probe E1 MS, and FT IR. This unique metabolic degradation product is consistent with a pneumotoxic mechanism that requires oxidative bioactivation of 3MI to an electrophilic alkylating intermediate. Supported by USPHS Grant HL13645. GSY is a USPHS Research Career Development Awardee (HL02119).

DOxorubicin-stimulated lipid peroxidaTion and Generation of Microsomal Aldehyde Des. K B Wallace, Dept. of Pharmacology, School of Medicine, University of Minnesota, Duluth, MN.

Reactive aldehyde products of lipid peroxidation elicit several biological responses associated with doxorubicin cytostasis and may play an important role in the mechanism of toxicity of the drug. The objective of this investigation was to determine whether aldehydes are generated during the NADPH-dependent stimulation of microsomal lipid peroxidation by doxorubicin and to determine if oxygen free radicals are involved in the process. Rat liver microsomes were incubated with 100 µM doxorubicin and an NADPH-regenerating system. Lipid peroxidation was assessed from the production of thiobarbituric acid-reactive substances. Dinitrophenylhydrzone-derivatives of lipid aldehydes were extracted from the reaction mixture and quantified spectrophotometrically. Doxorubicin-stimulated microsomal lipid peroxidation and aldehyde generation were functions of time, dose, and protein concentration. Although aldehyde production by microsomes required NADPH and oxygen, superoxide dismutase, catalase, and various scavengers of hydroxyl radicals were without effect. In contrast, metal chelators and sulphydryl reagents were potent inhibitors of drug-stimulated microsomal aldehyde generation. The data suggest that biologically-reactive aldehydes, derived from drug-induced lipid peroxidation, may play an important role in mediating doxorubicin cytotoxicity. The metal requirement and lack of involvement of oxygen free radicals is consistent with the occurrence of a drug-perferryl ion coordination complex. (This work was supported in part by BRSG S07 RO8590)
MEMBRANE LIPID PEROXIDATION INDUCED BY LINOLEIC ACID HYDROPEROXIDE AND HEMATIN. E H Kim and A Sevanian, Institute for Toxicology, University of Southern California, Los Angeles, CA.

Propagation of lipid peroxidation (LP) in membranes is largely responsible for perturbation of membrane structure and function. Several methods are used to induce membrane LP, including iron-dependent propagating reactions. Initiation and propagation of LP in liposomes as model membranes were examined using organic and lipid hydroperoxides. The influence of an iron-catalyst hematin (H), and hydroperoxide concentrations was examined using polarographic and spectrophotometric methods. Addition of cumene- (CuOH) or linoleic acid hydroperoxide (LOOH) to H results in rapid oxidation of H and decomposition of peroxide as measured by oxygen consumption. This occurs more rapidly with LOOH with a closer stoichiometric relationship between LOOH decomposition and H oxidation. The redox state of H is responsible for initiation of LP when reactions proceed in the presence of soybean PC liposomes. On a molar basis, LOOH is, by many fold, more efficient in propagating LP than CuOH. This supports long held view that initiation reactions are influenced by iron catalysis (e.g. hematin) concentration whereas propagation rates are determined by hydroperoxide concentrations. Manipulation of H and LOOH concentrations provides an efficient and reproducible method for producing LP. Supported by NIH Grant ES0316.

COMPARISON OF HYDROGEN PEROXIDE AND ORGANIC HYDROPEROXIDE EFFECTS ON PROTEOLYSIS IN HUMAN RED BLOOD CELLS. R E Novak, M Runge-Moriz, and A Mortensen, Institute of Chemistry, Wayne State University, Detroit, MI and Dept. Molecular Biology, Northwestern University Medical School, Chicago, IL.

H$_2$O$_2$, cumene hydroperoxide (CHP) and tert-butyl hydroperoxide (BHP) effects on the rate of proteolysis in human RBCs and RBC ghosts have been examined with the goal of differentiating the role of organic free radicals versus reactive oxygen species in producing protein damage. Proteolysis was monitored using a fluorescence assay for tyrosine release and by HPLC amino acid analysis. CHP and BHP, at 3 mM, stimulated proteolysis by 2.5- and 2.0-fold, respectively after 2 h. In contrast, H$_2$O$_2$ addition (100 mM) to RBCs alone or in the presence of 3-aminoo-1,2,4-triazole (3AT) (50-100 mM), which inhibited catalase activity by 80-97%, failed to stimulate the rate of proteolysis. Inclusion of 3AT with CHP or BHP failed to stimulate additionally the rate of proteolysis. Antioxidant addition resulted in complete inhibition whereas inclusion of N-acetyl cysteine (30 mM) resulted in 50% inhibition of CHP- or BHP-stimulated proteolysis. These results suggest that organic free radicals generated from CHP or BHP are the proximate toxic species which damage protein and result in an elevated rate of protein degradation. Supported by grants ES 02521 (RFN) and ES 00170 (NSH).

RADICAL-INDUCED INACTIVATION OF A Na$^+$,K$^+$-ATPase AND PROTECTION BY VITAMIN E. C E THOMAS, and D J REED. Oregon State University, Corvallis, OR.

Na$^+$,K$^+$-ATPase (ATPase) is a membrane bound, protein thiol whose potential susceptibility to oxidative inactivation may play a role in oxidant-induced cell injury. Treatment of ATPase-containing liposomes with Fenton's reagent (Fe$^{2+}$/H$_2$O$_2$) led to extensive lipid peroxidation (LP), as assessed by malondialdehyde (MDA) formation, and to total loss of ATPase activity. At 150/75 uM the lipophilic antioxidant vitamin E (VE, 5 mol%) prevented MDA formation but not loss of ATPase activity. At 25/12.5 uM, VE (1.2 mol%) prevented MDA formation and loss of ATPase activity, suggesting that radicals produced from LP inactivated the enzyme. Accordingly, in the absence of liposomes there was no loss of ATPase activity with 25/12.5 uM but total inactivation with 150/75 uM. Cumene hydroperoxide, tert-butylhydroperoxide and linoleic acid hydroperoxide (all at 75 uM) also inactivated the ATPase in the presence of Fe$^{2+}$. LP initiated by 150uM Fe$^{2+}$/150uM Fe$^{3+}$, a reputed alternative initiator of LP, inactivated the enzyme and VE prevented both MDA formation and loss of ATPase activity. These data indicate that the ATPase is susceptible to radical induced inactivation and that the ability of VE to protect ATPase depends upon the nature and concentration of the initiator. (Supported by USPHS ES05422 and ES01978)

COMPARISON OF HYDROGEN PEROXIDE AND ORGANIC HYDROPEROXIDE EFFECTS ON PROTEOLYSIS IN HUMAN RED BLOOD CELLS. R E Novak, M Runge-Moriz, and A Mortensen, Institute of Chemistry, Wayne State University, Detroit, MI and Dept. Molecular Biology, Northwestern University Medical School, Chicago, IL.

H$_2$O$_2$, cumene hydroperoxide (CHP) and tert-butyl hydroperoxide (BHP) effects on the rate of proteolysis in human RBCs and RBC ghosts have been examined with the goal of differentiating the role of organic free radicals versus reactive oxygen species in producing protein damage. Proteolysis was monitored using a fluorescence assay for tyrosine release and by HPLC amino acid analysis. CHP and BHP, at 3 mM, stimulated proteolysis by 2.5- and 2.0-fold, respectively after 2 h. In contrast, H$_2$O$_2$ addition (100 mM) to RBCs alone or in the presence of 3-aminoo-1,2,4-triazole (3AT) (50-100 mM), which inhibited catalase activity by 80-97%, failed to stimulate the rate of proteolysis. Inclusion of 3AT with CHP or BHP failed to stimulate additionally the rate of proteolysis. Antioxidant addition resulted in complete inhibition whereas inclusion of N-acetyl cysteine (30 mM) resulted in 50% inhibition of CHP- or BHP-stimulated proteolysis. These results suggest that organic free radicals generated from CHP or BHP are the proximate toxic species which damage protein and result in an elevated rate of protein degradation. Supported by grants ES 02521 (RFN) and ES 00170 (NSH).

FACTORS INHIBITING LIPID PEROXIDATION IN LIVER AND MUSCLE OF RAT, MOUSE AND CHICKEN. L Kehrer and M E Murphy. Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX.

Glutathione (GSH) or sulfhydryl-dependent antioxidant factors that act to prevent lipid peroxidation have been reported in both microsomes and cytoplasm from rat liver. The cytoplasmic factor has been identified in several tissues and species, but the distribution of the microsomal factor has not been reported. Lipid peroxidation was initiated in microsomes with 10uM Fe$^{2+}$/0.4mM ADP and 0.44mM ascorbate, and assessed by measuring the formation of thiobarbituric acid reactive substances (TBARS). Chicken and mouse livers had much lower activities of the GSH-dependent membrane-associated and cytoplasmic antioxidant factors than rat liver. Neither the chicken, mouse, nor rat had significant activities of the cytoplasmic or microsomal antioxidant factors in muscle. Peroxidative damage to membranes has been hypothesized as a mechanism of tissue damage in muscular dystrophy. However, the lack of these antioxidant factors in muscle and the absence of significant differences between normal and dystrophic chicken or mouse livers in the activity of the factors associated with the microsomes or cytoplasm, indicates they are not involved in the pathogenesis of this disease. The mechanism underlying the lag times before TBARS production is not yet certain. Microsomal vitamin E levels decreased about 70% before the onset of peroxidation under all conditions and this decrease was prevented by GSH. However, the total depletion of vitamin E was never evident and it is also possible that differences in the rate and extent of reduction of iron by ascorbate and/or GSH might be involved in the mechanism of these lipid peroxidation protective factors. These results raise questions as to whether GSH-dependent lipid peroxidation inhibitory factors are physiologically important in species other than rat or in tissues other than liver. (JPK is the recipient of Research Career Development Award HL01435. This work was supported by BRSG grant RB07091.)
CHEMICALLY INDUCED HEPATIC VITAMIN E DEPLETION IN VIVO. D. L. Warren and D. B. Reed, Oregon State University, Corvallis OR.

Vitamin E is a chain-breaking antioxidant that protects tissues against damage due to the lipid peroxidation that may result from oxidative insult. Although many reports have utilized rodents receiving no dietary vitamin E as a model to investigate in vivo actions of this vitamin, no model is available to produce a rapid and reproducible depletion of liver vitamin E. We observed a 30-35% lowering of liver vitamin E content from control values (±SD: 0.561 ± 0.055 nmol E/μmol phospholipid, n = 22) after single treatments of fasted rats with either 1,2-dibromoethane (DBE; 0.10 or 0.40 mmol/kg) or methyl ethyl ketone peroxide (MEKP; 40 mg/kg). Furthermore, a similar extent of hepatic vitamin E depletion was observed from groups treated daily for 3 days with either DBE or MEKP, or for 7 days with DBE. Liver vitamin E content was not altered 4 hours after treatment with either compound. Also assayed from treated rats were the hepatic content of reduced glutathione (GSH; control values were: 23.2 ± 3.71 nmol GSH/mg protein, n = 40) oxidized glutathione and total reduced protein sulfhydryls, the serum activities of alanine and aspartate transaminases, and the plasma content of vitamin E (control values were: 8.07 ± 0.91 nmol E/ml, n = 30). Comparisons of treatment induced alterations in these parameters suggest a two-phased response pattern: primary responses, such as GSH depletion and elevations in serum enzyme activities, that show maximal alterations shortly after treatment, and secondary responses, as identified by vitamin E depletion, that may be dependent upon primary responses. Given the timing of these responses it is unlikely that the observed vitamin E depletion results from a direct reaction between DBE or MEKP and vitamin E. This model for inducing a rapid depletion in liver vitamin E may assist in various investigations concerning the physicochemical properties and toxicologic activities of vitamin E in vivo. (Supported by NIH ES-01978 and # F32 ES-05389).


TH reacts with peroxyl radicals to form stable peroxytocopherones, which may be reduced to TH by ascorbate to complete a 2-electron TH redox cycle. We synthesized 8α-(2'4'-dimethylnitrilopent-2'-yl) peroxytocopherone (1), a model peroxytocopherone, by heating TH in CH3CN with 2,2'-azo-2-is(2,4'-dimethyl-5-oxo-1-nitroanil). In acetonitrile-buffer at pH 4, 1 hydrolyzed via a tocopherone cation (T+) to 8α-hydroxytocopherone (2), which irreversibly rearranged to α-tocophereryl quinone (TQ). The rate of TQ formation first increased, then decreased. This kinetic pattern reflected the first order formation of 2 from 1 followed by the first order rearrangement of 2 to TQ. Both rates increased with decreasing pH. The rate constant for the terminal phase was equal to the first order rate constant for the rearrangement of authentic 2 to TQ. Inclusion of ascorbte with 1 or 2 resulted in TH formation, which increased with decreasing pH and with increasing ascorbate concentration. The data suggest that 1 hydrolyzed via T+ to 2, which, in rapid equilibrium with T+, then rearranged to TQ and then ascorbate reduced only T+ to TH. 1 did not hydrolyze to TQ when incorporated into phosphatidylcholine liposomes in neutral, aqueous buffer. The marked stability of 1 to hydrolysis and rearrangement at neutral pH suggests that enzymatic catalysis may be required to regenerate TH from 1 in biological membranes. (Supported by USPHS Grant CA47943).


A soybean phosphatidylcholine liposome system was used to model oxidative TH turnover in biological membranes exposed to reactive free radicals. Azobis-2,4-dimethylvaleronitrile (AMVN) was used to generate peroxyl radicals in the bilayer by thermolysis at 37°C. Apparent zero-order TH depletion in the TH concentration range 0.05-0.5 mole % reflected the slow thermolysis of AMVN and suggested that TH was depleted entirely through reaction with AMVN-derived radicals. Phospholipid conjugated diene formation was suppressed during TH depletion. A peroxytocopherone product formed was identified as 8α-(2'4'-dimethylnitrilopent-2'-yl)peroxytocopherone (1) by UV spectroscopy, mass spectrometry, and chromatography with authentic 1 produced by reaction of TH with AMVN in CH3CN. Because 1 readily hydrolyzed to α-tocophereryl quinone (TQ) in dilute acid, 1 was routinely assayed as TQ following acid treatment. TQ was not otherwise detected. During bilayer oxidation, the fraction of TH lost that was accounted for as acid-releasable TQ declined from approximately 50% to 25% between 2.5 and 5 hours. However, acid-releasable TQ remained a constant when authentic 1 was incubated with AMVN in liposomes under identical conditions. The fractional yield of 1 thus appeared to decrease as TH oxidation progressed. Although peroxytocopherones account for much of the lost TH, other products remain to be identified. (Supported in part by USPHS Grant CA47943).

STRUCTURE-ACTIVITY RELATIONSHIPS (SAR) OF N-ALKOXYACETIC ACIDS ON RAT BLOOD IN VITRO. B. I. Ghanayem, L. T. Burke, and H. B. Matthews, NIH/NIHIS, Research Triangle Park, NC.

Alkoxycetic acids are major metabolites of glycol ethers and are considered the proximate toxic species. SAR studies revealed that the reproductive and developmental toxicity of these acids is inversely proportional to the length of the n-alkyl chain. Present studies were undertaken to investigate the SAR of n-alkoxyacetic acids on rat blood in vitro and indicated that alkoxycetic acids cause concentration- and time-dependent swelling of erythrocytes followed by hemolysis. This effect was associated with a parallel decrease of ATP concentration in blood. The ranking of the activity of these acids was as follows: butoxyacetic acid (BAA) > propoxyacetic acid = pentoxycetic acid > methoxycetic acid. In other studies, heptanonic, butyroxpropiolic, and propoxypropionic acids caused no significant effect on rat erythrocytes indicating that both the presence and position of the ether linkage are critical for development of hemotoxicity by these acids. Studies of C-BAA partition between erythrocytes and plasma vs. time showed that the concentration of C-BAA in plasma remained relatively constant while that in erythrocytes increased in a manner parallel to swelling. Incubation of BAA with erythrocytes, for 30 min, followed by washing and continued incubation revealed that swelling was not reversible, however, the rate of swelling declined significantly.

The rules of molecular geometry for predicting carcinogenic activity of PAH, have been applied to 53 compounds and predicted activity is in good agreement with the results of testing. The rules were developed from a unified hypothesis that the first step in the metabolic activation of unsubstituted PAH is the biochemical introduction of an alkyl group. This reaction: 1) Takes place between certain PAH and S-adenosyl-L-methionine and is catalyzed by cytosolic methyltransferase 2) is a means of probing for nuclear reactive centers in PAH, 3) provides a biochemical link between unsubstituted preprocarcinogens and more potent alkyl substituted procarcinogens, and 4) makes it possible to include carcinogenic compounds of aromatic type ArX and ArC6H4 in a homogeneous theory of metabolic activation. Strong carcinogenic activity is predicted in PAH with a single sterically unhindered meso-anthracenic reactive center or two such isolated centers that are equivalent by symmetry. Weak to moderate activity is predicted in PAH with a sterically hindered L-region. Inactivity is predicted in PAH if all of the reactive centers of the anthracene or naphthacene nucleus are abolished by ring fusion. (Funded by UK and NCI CA45823)

MITOCHONDRIAL EFFECTS OF PENTACHLOROHUANTHRENE-GLUTATHIONE (PCBG) STUDIED WITH ISOLATED RAT RENAL EPITHELIAL CELLS (IREC). C.E. Knoblock and T.W. Jones. Dept. of Path., Univ. of MD Sch. of Med., Balto., MD.

Previous studies have suggested that inhibition of mitochondrial respiration plays a role in PCBG-induced toxicity (Jones et al.: Arch. Biochem. Biophys. 251:504, 1986). In the present study, IREC were used to further elucidate the effect of PCBG on mitochondrial function. IREC were incubated in a Krebs-Henseleit buffer with or without the addition of substrates (5 mM glucose + 4 mM lactate) and treated with 100 μM PCBG. Samples were collected for the determination of viability, KCN-inhibitable cellular respiration (QO2), and mitochondrial accumulation of [3H]-triphenylmethyl phosphonium iodide (used as an indicator of mitochondrial membrane potential (Ψ)). The presence or absence of substrates had no effect on the rate of cell killing. In both cases, cell death was preceded by a dramatic reduction of Ψ. PCBG effects on QO2, on the other hand, were substrate dependent. In the absence of substrates, QO2 was impaired prior to cell death. However, in the presence of substrates, QO2, which was higher initially, was maintained until cell death occurred. These results suggest that the earliest and perhaps most critical effect of PCBG involves damage to the mitochondrial inner membrane leading to dissipation of the mitochondrial membrane potential.

EXPRESSISS OF NADPH-CYTOCHROME P-450 REDUCTASE IN COS-1 CELLS AND ITS USE TO EVALUATE QUINONE TOXICITY. R.N. Wistrom and R.W. Estabrook. Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX. Sponsor: D.J. Clardy.

NADPH-Cytochrome P-450 reductase is stated to catalyze the one electron reduction of quinones to semiquinone free radicals. These semiquinone radicals can undergo autoxidation by a one electron process in which molecular oxygen is reduced to generate superoxide anion radicals. This redox cycling is believed responsible for the oxidative injury caused by quinones. Rat liver NADPH-cytochrome P-450 reductase has been expressed in transformed monkey kidney (COS-1) cells using a plasmid vector containing the full-length cDNA for the enzyme (the cDNA was originally obtained from C.Kasper, University of Wisconsin, and inserted into an expression vector by David Russell, Dept. of Molecular Genetics, UTSWMC). An increase of greater than tenfold in cytochrome c reduction was observed 3 to 4 days following transfection of COS-1 cells with 5 μg/ml of plasmid containing the cDNA for the reductase. By varying the plasmid concentration in the transfection procedure, intermediate levels of P-450 reductase expression were obtained. COS-1 cells (exhibiting different levels of P-450 reductase expression) when incubated with 100 μM menadione for 1 hour showed a direct relationship between the level of cytochrome c reductase activity and the observed cytotoxicity (as determined by trypan blue exclusion). This expression system should provide a very useful tool for evaluating the role of NADPH-cytochrome P-450 reductase in quinone toxicity (Supported by grant 1-959 from the Robert A. Welch Foundation).

ALLYLAMINE (AAM)-INDUCED PHENOTYPIC MODULATION OF AORTIC SMOOTH MUSCLE CELLS (SMC). L.R. Cox and K. Ramon. Philadelphia College of Pharmacy and Science, Philadelphia, PA and Texas Tech University Health Sciences Center, Lubbock, TX.

Vascular lesions in rats induced upon subchronic exposure to AAM (3-aminopropene) are characterized by SMC proliferation and fibrosis. As these alterations could result from modulation of SMC from a quiescent to a phenotypic state, studies were conducted to evaluate the phenotypic expression in primary culture of SMC isolated from AAM-treated animals. Sprague-Dawley rats (175–200 g) were gavaged daily with AAM (70 mg/kg) or tap water for 20 days. SMC obtained from AAM-treated rats exhibited a round morphology, numerous ribosomes and a well-developed rough endoplasmic reticulum network. In contrast, SMC from control animals were elongated and contained numerous myofilaments. Contractile responsiveness to norepinephrine (10 μM) was observed only in control cultures. Confluent cultures of SMC from AAM-treated rats exhibited 180% (n=5–6) and 304.3% (n=6–8) greater 3H-thymidine and 3H-proline incorporation into DNA and collagen, respectively than cultures of control cells. Exposure of cells obtained from AAM-treated rats to dexamethasone (0.1 mM) and insulin (5.0 mM) reversed the morphologic and proliferative changes induced by AAM. Collectively, these data suggest that AAM induces a shift from a quiescent to synthetic phenotype in aortic SMC.
ANTIDOTAL EFFECTS OF THE OPTICAL ISOMERS OF CYSTEINE AND N-ACETYLCYSTEINE ON ACUTE ACRYLONITRILE TOXICITY. F W Benz, D E Nerland, C Babiuk, and W M Pierce. Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY and BP America, Inc., Cleveland, OH.

The acute toxicity of acrylonitrile (AN) has been proposed to be due to the parent molecule and/or its metabolism to cyanide. Thiols, which covalently react with the AN molecule have been proposed as antidotes. The only thiols effective in vivo are cysteine and its precursors. We examined the ability of the optical isomers of cysteine and N-acetylcysteine to act as antidotes against AN toxicity. The LD50 of AN was determined in male Sprague-Dawley rats and compared to the LD50 determined after treatment with 2 mmole/kg of thiol antidote. A protective index (LD50 with antidote / LD50 without antidote), was determined for each thiol. The protective indices of L-cysteine, D-cysteine, N-acetyl-L-cysteine, N-acetyl-D-cysteine were 2.03, 1.97, 1.76, and 1.25 respectively. The time course of cyanide concentrations in the blood was also determined. All of the antidotes, except N-acetyl-D-cysteine, lowered blood cyanide levels. Conversely, both D- and L-cysteine were able to elevate blood thiosulfate concentrations five-fold. These data suggest that part of the antidotal mechanism of the cysteines is the detoxification of cyanide.

CYTOSKELETAL INJURIES INDUCED BY HALOGENATED NITROBENZENE SENSITIZERS. I N Chou, M F Leung AND K Geoghegan-Barek. Boston University School of Medicine, Boston, MA.

The sensitizing capacity of several halogenated nitrobenzenes has been tested in guinea pigs and later reproduced in humans. To understand the mechanisms of cell injury by these sensitizers, we have studied by fluorescence microscopy their effects on the cytoskeletal organization of mouse 3T3 cells. Control cells contained numerous microtubules (MT) distributed in a network fashion throughout the entire cytoplasm and extending to the cell periphery. Microfilaments (MF) were less numerous and distributed irregularly, often appearing in cable-like fashion. Exposure of 3T3 cells for 3 h to 3 µM halogenated nitrobenzenes known to induce allergic contact dermatitis in guinea pigs, resulted in a complete disassembly of MT and a marked increase in MF density and alterations in their distribution. In sharp contrast, exposing 3T3 cells for 3 h to 5 or 100 µM halogenated nitrobenzene derivatives which do not sensitize guinea pigs, had no discernible effect on the organization of either MT or MF. Thus, the sensitizing capacity of these halogenated nitrobenzenes toward guinea pigs correlates well with their ability to induce cytoskeletal perturbations. These results suggest that an in vitro assay based on cytoskeletal perturbations may be developed for screening other potential halogenated nitrobenzene sensitizers.

CYTOSKELETAL PERTURBATIONS INDUCED BY 1-CHLORO-2,4-DINITROBENZENE (CDNB): METABOLISM OF NON-PROTEIN THIOLS AND INHIBITION BY GLUTATHIONE MONOETHYL ESTER. M F Leung and I N Chou. Boston University School of Medicine, Boston, MA.

We have shown that exposure of 3T3 cells to micromolar CDNB, a substrate for glutathione-S-transferase, results in depletion of total cellular glutathione (GSH) accompanied by disassembly of microtubules (MT). Prolonged incubation resulted in cellular recovery as evidenced by increases in GSH accompanied by MT reassembly. In this study, we have performed HPLC analysis of whole cell non-protein thiols in CDNB treated cells. Exposure of 3T3 cells to 2.6 µM CDNB for 1/2 h resulted in decreases in GSH and cysteine, but an increase in cystine content. The GSH content reached the lowest level at 2 h, then began to recover at 3-4 h and exceeded the control level at 7 h. The cysteine content continued to decrease and was undetectable at 7 h. The amount of cystine was maximal at 1 h. Both cysteine and cystine contents appeared to approach the control levels at 3 h. The oxidized glutathione (GSSG) remained at very low or undetectable levels. Exposure of 3T3 cells to GSH monoethyl ester, a cellular GSH delivery system, resulted in inhibition of MT disassembly induced by CDNB. These results suggest that maintenance of an appropriate level of GSH is important in protecting the cells from cytoskeletal injuries induced by CDNB.
637 EFFECT OF MICROCYSTIN-LR ON CULTURED RAT ENDOThelial Cells. R Solow, K Merelish, G W Anderson, Jr.* and J Hewetson. Sponsor: R W Wannemacher, Jr. Pathophysiology Division and Disease Assessment Division, USAMRIID, Fort Detrick, Frederick, MD.

Primary cultures of adult rat, hepatic, sinusoidal endothelial cells were used to investigate the effect of microcystin-LR. Microcystin-LR at a concentration of (4 μM), which induces necrosis in cultured rat hepatocytes, did not produce either permeability changes or cytotoxicity in endothelial cell monolayers. Supernatants derived from cultured rat hepatocytes treated with 4 μM microcystin-LR, however, induced significant permeability changes, as indicated by the release of [14C]adenine nucleotides, and a small reduction of cell density in endothelial cell monolayers. Silamin (SM) at 0.2 mM, but not dithioerythritol (DTE) at 2.5 mM, partially protected changes produced in endothelial cells by supernatants derived from microcystin-LR-treated hepatocytes. Thus the effect of microcystin-LR on liver sinusoidal endothelial cells in vitro was an indirect one; hepatocytes treated with microcystin-LR produced either an activated metabolite(s) or factors which effected permeability changes in endothelial cells. Indirect endothelial cell injury may contribute to microcystin-LR induced liver hemorrhage observed in vivo.

639 EFFECTS OF QUINOLONE ANTIBACTERIALS UPON PROTEOGLYCAN AND PROCOLLAGEN SYNTHESSES IN RAT LIMB BUD MICROASSAY CULTURES. D E Amacher, S J Schomaker, T D Gootz, and P R McGuirk. Drug Safety and Immunology & Infectious Diseases Departments, Pfizer Central Research, Groton, CT.

A common toxicological feature of quinolones is the occurrence of articular cartilage lesions in the load-bearing joints of juvenile animals. The mechanism is unknown but could involve perturbations of either proteoglycan or procollagen syntheses, or both, in developing cartilage. We compared pipemidic and nalidixic acids (Penna, NAL), norfloxacin (NOR), and ciprofloxacin (CIP) for inhibition of either cartilage component in developing rat limb bud cultures. Micromass spot cultures, 4 x 10^6 cells each, were prepared from the limbs of 13 day CD rat embryos. The next day, cultures were exposed to test drugs (40-500 µg/ml) for 48 hours and maintained another 4 days. Representative cultures were fixed stained with either alcian blue (proteoglycan synthesis) or exposed to neutral red (cytotoxicity). In other similar cultures, drug was removed after 48 hours, medium containing 1.0 µL/ml [14C]proline added for 6 hours, medium collected, precipitated with TCA, and the collagenase-solubilized fraction assayed for proline incorporation into collagen. II. Proteoglycan synthesis was inhibited as follows: NURS (CIP) PPA NAL at concentrations producing 10% cytotoxicity. In contrast, procollagen synthesis was inhibited as follows: NAL NAL (PPA) NAL CIP at concentrations producing 15% cytotoxicity. Thus, the effects of these quinolones upon the synthesis of either cartilage component may be independent.


Adriamycin (ADM) is a potent antineoplastic agent, but its therapeutic agent is limited by cumulative cardiotoxicity. A potential intracellular target of ADM is cardiolipin (CL), an acidic phospholipid which with the drug interacts strongly. In eukaryotic cells, CL is found exclusively in the inner mitochondrial membrane with phosphatidylcholine (PC) and phosphatidylethanolamine (PE): PC/PE/CL (CA 4:4:1, mole/mole). We have recently reported Ca2+ uptake by liposomes composed of PC/PE/CL (4:4:1) formed by extrusion and containing 3 µM Arsenazo III (Aphysol, L, 1985) 33:75s. The hypothesis that ADM can modify CL-mediated Ca2+ transport was tested with the following results: (1) ADM (100 - 400 nM) altered Ca2+ uptake in a concentration-dependent fashion. (2) In the presence of 6 mM Ca2+ and below 35°C, ADM enhanced Ca2+ uptake; above 35°C, Ca2+ uptake was inhibited and Ca2+ release was induced. (3) Inhibition of Ca2+ uptake was associated with ADM-induced efflux of Arsenazo III. (4) In the presence of 2 mM Ca2+, ADM stimulated Ca2+ uptake at all temperatures. These findings are consistent with a role for altered mitochondrial Ca2+ homeostasis in ADM cardiotoxicity. [Supported by NIH (HL 32615) to EMS and the Grad Sch, Univ MD, Baltimore.]


Hydrogen peroxide is one of many toxic compounds found in cigarette smoke which may play a role in cell injury, tumor promotion and carcinogenesis of the bronchus. Because these processes may be influenced by increases in cytosolic Ca2+ ([Ca2+]i), we have studied the response of [Ca2+]i of transformed human bronchial epithelial cells (HEASEB) treated with H2O2. Pura 2-loaded BEASEB were examined by fluorescence microscopy utilizing a Tracor-Northern 8500 imaging system. Cells were grown in LHC8, a chemically defined medium; other procedures were performed in LHC8-Basal without phenol red, oystein, riboflavin and folate acid. The 340 nm and 380 nm images were collected at 0 t, and at 1, 3, 5, 10, 15, and 20 min after H2O2 was added. 0.1 mM H2O2 had little effect on [Ca2+]i; 1.0 mM H2O2 caused a 4- to 5-fold elevation followed by recovery; 10 mM caused a similar pattern. These doses of H2O2 did not cause cell blebbing or cell killing for at least 60 min. The observed increases in [Ca2+]i suggest that H2O2 may play a role in tumorigenesis. [Supported in part by #90-CP-51000.]
641 "MULTIPLE METABOLITE - MULTIPLE TARGET" HYPOTHESIS AS APPLIED TO BENZENE AND ACETAMINOPHEN TOXICITY. S. Ji, Dept. of Pharmacol. and Toxicology, Rutgers University, Piscataway, N.J.

Existing experimental data on benzene(BZ) and acetaminophen(AA) toxicity support the general concept that the toxicological consequences of these compounds are derived not from one but many reactive or stable molecular species related to them and that these toxic species interact with not one but multiple molecular targets ("toxicological receptors"). In addition, the kinetics of the interactions between toxic metabolites and their respective targets is critical in the expression of the toxic potential of these xenobiotics. There are at least six possible toxic benzene metabolites (phenol, hydroquinone, p-benzoquinone, catechol, trihydroxybenzene and mucosaldehyde), two target cell groups in bone marrow (stroma and stem cells) and two kinds of kinetics (one fast enough to effectuate toxic manifestations and the other too slow to do so), so that there are at least 6x2x2 = 24 possible mechanisms for benzene toxicity. Similarly, there are at least two toxic species for AA-acetylhydrazine (benzoquinoneimine), three target sites (hepatocellular membrane, mitochondria, Kupffer cells), and two types of kinetics (effective and ineffective), thus giving rise to 2x3x2 = 12 possible mechanisms of AA toxicity. Such multiple mechanistic possibilities for AA and BZ toxicity are not surprising in view of the complex living systems with which they interact.

642 COMPARISON OF OXIDANT-DEPENDENT REACTIONS BY PMNs FROM HUMAN, RAT AND MOUSE: IMPLICATIONS FOR THE INVOLVEMENT OF PMNs IN TOXICOLOGICAL REACTIONS. M.A. Trush, L E Twerdok and R L Esterline. Division of Toxicological Sciences, Johns Hopkins Univ. Baltimore, MD.

PMN-derived oxidants have been shown to damage macromolecules and to activate xenobiotics. In this study, we have compared the SOD-inhibitable superoxide generation and myeloperoxidase (MPO) activity of PMNs from human, rat and mouse. Additionally, we examined the following MPO-dependent reactions: luminol-amplified chemiluminescence; BP-diol-derived chemiluminescence; and covalent binding of BP-diol to DNA. With all of the monitored parameters, human PMNs exhibited the greatest activity. Significant differences in superoxide generation and MPO activity were noted between rat and mouse PMNs, although with the three MPO-dependent reactions these differences balanced resulting in equivalent activities in the two species. Addition of azide inhibited BP-diol chemiluminescence with PMNs from all three sources, whereas SOD demonstrated various effects depending on the source of PMNs. SOD (50 ug/ml) was totally inhibitory with mouse PMNs, was slightly inhibitory with human PMNs and with rat PMNs either had no effect or was stimulatory. These results suggest that humans may be at greater risk to toxicological insults mediated and/or potentiated by inflammatory cell-derived oxidants. Supported by ES 03760, CAAX and Amer. Cancer Soc. SIG-3.

643 REVERSIBLE OXIDATION OF THE THIOL OF GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE (GPD) IN HUMAN LUNG CARCINOMA CELLS (A549). A E Brodie and D J Reed. Oregon State University, Corvallis OR.

A thiol-redox change of GPD, caused by an oxidative stress to A549 cells, resulted in inactivation of enzymatic activity and inhibition of binding by iodoacetic acid (Biochem. Biophys. Res. Commun. 143:120, 1987). The A549 cells recovered quickly with no media supplements. Buthionine sulfoximine inhibition of glutathione (GSH) synthesis decreased recovery at 30 min. Reactivation of enzymatic activity was also achieved by incubation of a sonicated cell mixture with cysteine, cysteine, cysteamine, or GSH, whereas GSSG had no effect. S-hexyl-GSH and S,S'-bis(1,2-ethyl)GSH did not compete with GSH during GPD recovery. Two cellular enzyme systems have been identified which reduce disulfide bonds in proteins (Bioch. J. 213;519, 1983). These are thioltransferase, which requires GSH and glutathione reductase for activity, and thio-redoxin reductase which requires thioredoxin and NADPH. Purified GPD after inactivation recovered activity with addition of A549 cell fractions, with a dose dependence on NAPDH, glutathione reductase inhibitor, sodium arsenite, inhibited recovery of cellular GPD activity. This suggests that both GSH and NADPH-dependent reductase activity may be involved in GPD recovery in cells. (Aided by ACS grant #CH-109.)

644 THE ROLE OF OXIDATIVE STRESS IN THE ISOLATED RAT RENAL EPITHELIAL CELL TOXICITY OF MENADIONE AND ITS N-ACETYLCysteine CONJUGATE (MNAC). P C Brown and T M Jones, University of Maryland Toxicology Program and Department of Pathology, University of Maryland School of Medicine, Baltimore, MD.

Previous studies have shown that menadione and MNAC are equally toxic to isolated rat renal epithelial cells. Unlike menadione, MNAC is unable to alkylate protein thiols. However, MNAC can reduct cycle and therefore retains the potential to produce oxidative stress (Brown and Jones: Toxicologist 8:13, 1988). In the present study, the role of oxidative stress in the toxicity of menadione and MNAC was further investigated. Both menadione and MNAC cause a rapid depletion of NAPDH with a concomitant increase in NADP presumably through activity of glutathione reductase. Furthermore, the toxicity and depletion of soluble thiols induced by menadione and MNAC were potentiated by N,N-Diethyl(2-chloroethyl)-N,N-dimethylamine, an inhibitor of glutathione reductase. These results provide further evidence that the toxicity of menadione and its thioether conjugate, MNAC, involves oxidative stress. [Supported by ACS BC-570 and NM-Drf.]
Phenelzine (PZ) and hydralazine (HZ) stimulated ATP-independent proteolysis in human red blood cells (RBCs) (3% HCT) and in hemolysate. Proteolysis was measured using a fluorescence assay for tyrosine release and by HPLC amino acid analysis. PZ (1 mM) stimulated proteolysis in RBCs by 16% after 7 h, while HZ, increased the rate of proteolysis by 15% relative to controls. Similar changes were monitored in hemolysate. HPLC analysis revealed the release of 16 amino acids. Spectroscopic studies showed a concentration- and time-dependent decrease in the Soret absorbance maximum from 414 nm to 409 nm at 30 min in the presence of 1 mM PZ; HZ at 1 mM showed a lesser but significant decrease, 414 to 411 nm, in the Soret absorbance. Comparable changes in α, β bands. No significant alteration of the Soret or α, β bands occurred in controls under identical conditions. These results correlate with the reported ability of PZ and HZ to form reactive 2-phenylethyl and 3-phthalazyl free radicals, respectively upon interaction with HBO2 and suggest that these organic free radicals produce protein (hb) damage which results in an increased rate of proteolysis. Supported by NIH grants ES 00170 (MWD) and ES 02521 (RFN).

The percutaneous absorption and evaporation of [2,3-14C]ethyl acrylate (EA) was determined in vivo with sections of hairless rat skin. The skin was mounted on Franz cells with calf serum at 30°C serving as the receptor solution. The top cell contained an activated charcoal trap to adsorb volatile EA. Within 35 min after dosing 35 µl of neat EA over a 1.8 cm 2 area, 88% of the dosed radioactivity had evaporated from the skin and was trapped in the charcoal, adsorbed onto the glass top cell, or remained as vapor above the skin. Over the next 6 hr, the EA vapor in the top cell was absorbed by the skin or by the charcoal trap. At the end of the 6 hr exposure period, 88% of the exposed radioactivity was in the charcoal or adsorbed onto the top cell, 6% was in the skin and 21% was in the receptor solution. Repeating the study without a charcoal trap or top cell resulted in evaporation of 95% of the exposed EA. These results indicate that non-occluded dermal exposure to EA results in very low levels of percutaneous absorption.

As part of the Exxon product safety evaluation program, we probed the skin sensitization (S) in guinea pigs, the primary dermal irritation (PDI) and repeated (28-Day) dermal toxicity (RDT) in rabbits of a range of paraffinic lube base oils. The sample testing matrix comprised of highly refined, solvent-extracted paraffinic base stocks that varied in their molecular weight/viscosities evaluated. PDI studies yielded PII scores ranging from 0.5 - 1.8, with the lower viscosity oil producing the highest scores. The RDT consisted of various degrees of erythema and edema with the low viscosity oil producing the highest scores. All samples produced dermal responses that were below those required for warning labels in the USA, EEC and Canada. These data in conjunction with previous studies indicate that these highly-refined paraffinic lubricating oils are not skin sensitizers and are minimally irritating with a low order of dermal toxicity.
METHYL ETHYL KETONE PEROXIDES (MEKP) TOXICITY IN F344 RATS AND B6C3Fl MICE FOLLOWING 13-WEEK DERMAL APPLICATION. K M Abdo', M R Elwell1, J C Pickenham2, M R Moore3, N L Henry Net, RTP, NC. 'Emerson, RTP, NC. 'Hazelton Laboratories, Rockville, MD. Sponsor: R S Chhabra

Toxicity of MEKP is used as a curing agent for unsaturated polyester resins. Toxicity studies were conducted by applying a solution of various concentrations (0, 0.3, 1.0, 3.0, 10.0, or 30.0% MEKP in dimethylphthalate) on a shaven intrascapular area of the skin. Groups of 10 rats and mice of each sex received 0.3 ml (rats) or 0.1 ml (mice) of the solution per animal/day for up to 13 weeks. All rats and mice in the top two doses died or were sacrificed because of severe skin lesions prior to the end of the study. Body weight (P<0.05) depression occurred in rats only at the 3.0% dose level. In rats and mice the site of application was the primary target of MEKP-induced toxicity. Coagulation necrosis of the skin was present in rats and mice at the top three doses. Acneosis with hyperkeratosis occurred at the 0.3% and 1.0% dose levels. Degeneration/necrosis of the olfactory epithelium and acute inflammation of the respiratory epithelium was present in rats at the top dose. Splenic extramedullary hematopoiesis and bone marrow myeloid hyperplasia seen in dosed rats and mice were considered secondary responses to the necrosis and inflammation in the skin. Based on the severity of the skin lesions, rats appear to be more susceptible than mice to the toxic effects of MEKP.

COuMPARATIVE ACUTE EFFECTS OF 5-ACETYLSALICYLAMIDE (5-ASA) AND 5-BROMOACETYLICILVAMIDE (5-BraSA). D E Rodwell1, J C Siglin1, AND C P Chengelis2. 'Spireborn Life Sciences, Inc., Spencerville, OH and 'C. D. Searle & Co., Skokie, IL.

Two closely related synthetic chemical intermediates, 5-ASA and 5-BraSA, were evaluated and compared for acute oral toxicity in rats, photoallergic potential in guinea pigs (Modified Armstrong Method) and delayed contact sensitization in mice (Mouse Ear Swelling Test). The oral LD50 of 5-ASA was > 2.0 g/kg in males and approximately 1.7 g/kg in females. 5-BraSA was more toxic with a calculated LD50 in males and females of 2.0 and 0.8 g/kg, respectively. 5-BraSA was found to be dermally irritating during preliminary range-finding. Additional dermal and eye irritation studies with 5-ASA in rabbits proved that this chemical was non-irritating. Neither 5-ASA nor 5-BraSA produced phototoxicity or photosensitization. Non-light mediated contact sensitization was observed with 5-BraSA in the photosensitization study. Neither chemical produced contact sensitivity in the mice. Results indicate that despite the structural similarities, 5-ASA and 5-BraSA have a different spectra of toxicity. 5-BraSA is more topically irritating, acutely toxic and is a positive contact sensitizer. Neither chemical has phototoxic or photoallergic properties.


Hydrogen fluoride (HF) is capable of causing serious and progressively destructive skin burns. Since there are different therapies currently being used to manage HF dermal burns and because previously reported animal studies attempting to evaluate HF dermal burn treatments are somewhat controversial, testing has been conducted to develop a reliable animal model for efficacy assessment of various treatments for HF dermal burns. Several species and different dosing regimens (HF concentration, time of exposure, and application technique) were investigated for consistency and reproducibility of dermal responses that appeared most characteristic of HF burns caused by accidental exposure in humans. A concentration of 38% HF applied under a Hilltop Chamber® patch at exposure periods of 9, 12 and 15 minutes to the skin of anesthetized pigs produced the most suitable results. Testing is in progress to assess the therapeutic efficacy of a number of clinically applicable treatments: 10% calcium chloride, 2% calcium gluconate solution (s.c.), 2.5% calcium gluconate gel (topical), iced aq. Hyamine soak and iced aq. Zephiran soak.

ALTERED EPIDERMAL MORPHOLOGY SECONDARY TO LIDOCAINE IONTOPHORESIS: IN VITRO AND IN VIVO STUDIES. NA MONTEIRO-RIVIERE. COLLEGE OF VET. MEDICINE AND TOXICOLOGY PROGRAM, N. C. STATE UNIV., RALEIGH, N. C.

Iontophoresis is the process of delivering ionic drugs across the skin using electric current. Iontophoresis of lidocaine hydrochloride in 30 pigs in vivo and in 32 in vitro isolated perfused porcine skin flap (IPPSF) preparations produced a drug specific alteration in epidermal structure > 10 min after dosing. This alteration was characterized by the appearance of flattened dark staining nuclei in the stratum granulosum and spinosum layers, occasionally extending to the stratum basale in severe cases. The stratum cornium was normal. This morphology resembled parakeratosis but was distributed in a dyskeratotic pattern. Severity of this change, graded on a scale of 0-3 (no change to severe), was best correlated to total transcutaneous flux (mg/hr) of lidocaine measured in IPPSF studies and to flux as measured by current (mA-hrs) in vivo. In conclusion, lidocaine iontophoresis induced dose dependent epidermal changes both in vitro and in vivo, thereby eliminating an immune mediated etiology. (Supported by Becton Dickinson Research Center).
Multifocal retinal dysplasia was diagnosed clinically in twenty-four of twelve hundred (2.0% incidence) LAK:1VG(STI) Syrian Hamsters. The affected hamsters were part of twelve hundred animals to be used in carcinogenicity studies. The dysplastic retinas were detected during preliminary ophthalmoscopic examinations of hamsters as young as 6 weeks and as old as 9 months of age. Ophthalmoscopically, the dysplastic foci varied from retinal streaks, sometimes called veriform, to small, generally circular areas of cream-colored depigmentations. Blindness or other apparent visual defects were not seen, nor were the dysplasias associated with a generalized syndrome of disease. Histologic examination of the affected hamsters revealed focal dysplasias limited to the retina and were manifested as invaginations and rosette-like structures composed of elements of the photoreceptor layer, outer limiting membrane and outer nuclear layer. In addition, a procedure for maintaining prolonged mydriasis for funduscopic photography is presented.

IN VIVO SKIN DECONTAMINATION OF 42% PCBs IN RHESUS MONKEY, RC Weaster, DAW Backs, J McMeaster, and HI Malbarch, Dept. Dermatology, University of California, San Francisco, CA.

Skin decontamination involves the ability of a washing system to remove chemical from skin and the dynamic time lapse for a chemical between initial skin contact time and skin absorption (irreversible removal). [14C]-42% PCBs in trichlorobenzene or mineral oil (vehicles in transformers) was applied to rhesus monkey skin for a 15 minute interval. Dosing was 2 ul vegetable/cm² with 4ug/cm² PCBs. Each group was four monkeys. Skin was washed five successive times with soap and water (20% v/v Ivory liquid) or the solvents ethanol, trichlorobenzene, and mineral oil. At a 0 h wash, removal of PCBs applied in trichlorobenzene vehicle were 102+9% (mineral oil), 102+8% (trichlorobenzene), 93+7% (soap and water) and 63+3% (ethanol). Removal of PCBs applied in mineral oil were 90+10% (mineral oil) and 71+18% (soap and water) at 0 h. As skin contact time increased to 10 min., 1, 3, 6 h, the ability to decontaminate skin decreased. At 24 h post skin application, only 25-45% of PCBs could be removed from skin regardless of decontamination solvent. This model provides a practical method of developing guidelines for worker and consumer decontamination.

IN VIVO NUCLEAR MAGNETIC RESONANCE (NMR) AS A TOOL TO STUDY DERMAL ABSORPTION, C D Carrington, C T Burt, and M B Abu-Donia, Duke Univ. Med. Cntr., Durham, NC and NIEHS, RTP, NC.

The rate of disappearance of hexafluorobenzene (HFB) from a dermal administration site was monitored using 31F-NMR. Doses of 15 to 50 mg HFB were applied to a 2 cm² area on the abdomen of 400-600 gm Sprague-Dawley rats. The dosing site was sealed by glass and cyanoacrylate glue. When HFB was applied undiluted, the signal was found to decrease with approximate zero order kinetics to less than 10% of its initial level in 1-2 hours. The remainder was absorbed much more slowly. The frequent appearance of a second minor peak was probably a consequence of a chemical shift resulting from penetration of the chemical into the skin. The second peak was more persistent than the original peak. When HFB was dissolved in petroleum jelly (PJ), the rate of absorption was greatly decreased. A dose of 50 mg HFB in 50% (w/w) PJ was absorbed at a rate of about 5 mg/hr. A dose of 50 mg HFB in 25% PJ was absorbed at a rate of 2-3 mg/hr. Extraction of the tissues surrounding the dosing site followed by analysis using in vitro NMR indicated that HFB diffused within the skin from the application site and accumulated in the fat associated with the dermis. These results indicate that NMR is a promising non-invasive tool for monitoring dermal absorption in vivo without the use of radiolabeled compounds. Supported in part by NIOSH Grant No. GHO 9823.
Age dependence in percutaneous absorption (PA) of 14-C-folpet was assessed in 33 and 52 day old Fischer 344 rats. Folpet was applied (0.29 μmol/cm²) in acetone to the previously clipped back skin and radioactivity in treated skin, tissues, urine and feces was determined at 6, 24, 48, 72 and 120 hours. Dose-absorption was determined at 72 hrs using three dosages (0.1, 0.54, 2.7 μmol/cm²). In-vitro PA was measured in the same aged rats by static and flow-through methods. PA at 120 hrs averaged 9.5% in young and 7.8% in adults. Most of the absorbed folpet derived radioactivity, about 91%, appears in urine by 120 hrs. Although the body burden is 41% of the dose at 120 hrs, it is increasing which suggests that the body has not reached steady state. Kidney and liver each contain 10% of the body burden. The static and flow-through in-vitro systems gave slightly lower PA than was observed in-vivo; 3.63, 3.26, 1.35% in in-vivo, static, and flow, respectively. A physiological compartmental model was optimized to the distribution data. Membrane resistance or limited organ uptake from blood is suggested. PA was independent of age, but dependent on dosage. Age dependent retention and excretion were noted. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

The influence of sodium hydroxide on skin barrier function was investigated in 10 female volunteers. Only 5 minutes of topical application of a 0.2n NaOH solution using a 3.5x2.0x1.3 cm plastic block to achieve uniform distribution resulted in reversible destruction of skin barrier function for 10 minutes as evaluated by transepidermal water loss (TEWL). Five minutes after the end of application TEWL was 23.2±2.7 g/sem/h (mean ± S.E.) as compared to 4.0±0.3 before treatment (p<0.0001). Only 10 minutes later baseline values were reached (6.1±1.7).

There was a high correlation between this short term NaOH test and the irritant response to 24 hours application of 1.0% sodium lauryl sulfate (SLS) patches (r=0.743; p<0.01). Use of this model may help to investigate skin barrier function as well as testing protective devices and barrier creams. Its major advantages over currently used models are the very short test period to achieve stratum corneum barrier disruption, its quick reversibility and the lack of pain or irritation to the volunteer subject.

The dermal absorption of 1,2,3-trichlorobenzene (TCB) was investigated in the rat by measurements of areas under the blood concentration-time curve (AUC), and of the cumulative excretion. AUC data indicated that the dermal absorption rate of TCB at a dose of 4.0 mg/kg b.w. ranged from 1-3% of those observed for the intravenous route. When the absorption was assessed by the cumulative dose which was excreted following administration of the test material, the dermal rate was approximately 7-8% of the amount that was produced by an intramuscular dose. The effect of vehicle and use of occlusive pad were also investigated. Use of occlusive pad and acetone as a vehicle increased the rate of dermal absorption. Data of the present study indicated that both the AUC method and measurements of excreted dose proved to be reliable methods to assess dermal absorption of TCB.


The HRSF model, recently developed at the University of Utah, allowed the study of initial absorption (0-8 h) of three C-14 labeled compounds through human skin by appearance of labeled penetrant in the femoral vein draining the flap. This model was compared to the SDR model, where initial absorption through abdominal rat skin was monitored by appearance of labeled penetrant in the ipsilateral femoral vein. Systemic blood levels were determined by sampling from the contralateral femoral vein in both models. Percutaneous absorption was calculated by dividing the percent of the applied radioactive dose excreted in the urine over 48 hours by the fraction excreted after subcutaneous injection. For benzoic acid, percutaneous absorption was similar in the two models (40%) and blood levels of radioactivity followed a similar pattern. Percutaneous absorption of N,N-diethyl-N-ethylurea was 2 fold higher and triethanolamine 6 fold higher in the SDR vs the HRSF. Percutaneous absorption results in the HRSF model were in good agreement with previously reported human data and demonstrated the importance of human skin in the model. For certain compounds (e.g., benzoic acid), the simpler SDR model may provide a useful approximation of the HRSF model.
Chemical contamination of the skin is a complex problem of occupational and environmental relevance. An important question is: Can skin permeability of a given compound be predicted from simple experiments? Absorption literature identifies two key observations: (a) the SC, the skin's outermost layer, is the major barrier to chemical transport, and (b) qualitative correlations exist between penetrant permeability and oil/water partition coefficients (PC). To obtain more quantitative predictions of permeation, we evaluated SC/water and SC/isopropylmyristate (IPM): A model lipophilic vehicle) PC for substituted phenols of diverse physicochemical properties (p-acetanido [AC], p-cyano [CY], p-iodo [I] and p-pentoxyl [PO]). The results, independent of SC preparation technique, after 24 h equilibration were:

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<th>AC</th>
<th>CY</th>
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<tr>
<td>SC/water</td>
<td>4.6±0.7</td>
<td>8.5±2.6</td>
<td>5.4±4.4</td>
<td>6.7±14.0</td>
</tr>
<tr>
<td>SC/IPM</td>
<td>32.4±8.4</td>
<td>6.2±0.9</td>
<td>2.2±1.2</td>
<td>1.5±0.2</td>
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The data demonstrate that reproducible partitioning can be obtained using the biological tissue of greatest relevance, and that the pattern of behavior observed for the 2 different vehicles studied is compatible with physicochemical expectations. Further, permeation work in man suggests that the PC measured may be useful predictors of in vitro and in vivo skin transport and valuable assets, therefore, in the evaluation of risk.

DERMAL ABSORPTION OF DRINKING WATER CONTAMINANTS.
R R Vanderlance and E V Chianan, US EPA, Office of Drinking Water, Washington, DC.

Under the Safe Drinking Water Act, the US EPA Office of Drinking Water (OW) evaluates both the toxicity and exposure data in setting maximum contaminant level goals (MCLGs) and Health Advisories (HAs). OW is currently re-evaluating its methodology for setting MCLGs/HAs. Normally, when chemical specificity data show >20% of the exposure to a contaminant is assumed to come from air and food; the remaining 20% from ingesting 2 liters of drinking water per day. Concern has been expressed that other exposures to drinking water contaminants, e.g., dermal exposure while bathing, should also be considered. A simple dermal absorption model was developed based on a contaminant's dermal permeation coefficient (PC). In addition, values for Fc were compared to the contaminant's octanol/water partition coefficient (Kow). No precise correlation was found, but Kow could be used to establish an upper limit for the expected Fc. The model predicts that dermal absorption accounts for at most, 25% of the exposure expected from ingestion of 2 liters of water each day. Lower estimates of dermal absorption are predicted for contaminants with a value of Kow < 500. Instances in which the model may underestimate dermal exposure to drinking water contaminants are also explored.

COMPARISONS OF K⁺ LEAKAGE AND INHIBITION OF PROTEIN SYNTHESIS AS INDICES OF ADVERSE CELLULAR RESPONSES TO CHEMICAL TOXINS. J.M. Frazier and T.N. Vozar. The Johns Hopkins University, Baltimore, MD.

Inhibition of cellular protein synthesis (PS) and K⁺ leakage (KL) have been used as experimental endpoints to indicate adverse cellular responses to chemical toxins. A modified protocol from that previously reported for toxicity evaluations in isolated rat hepatocytes [The Toxicologist 5:212 (1983)] includes the following modifications: a rapid membrane filtration technique to measure 4-h isoleucine incorporation, an assay for cellular K⁺ by atomic absorption spectrophotometry and normalization of data by DNA content. Two positive controls are included in all toxicity studies - cycloheximide for protein synthesis and sodium azide for cytotoxicity. The method was used to evaluate the toxicity of cadmium, copper and zinc. Based on three indices, viability (trypan blue exclusion), PS inhibition and KL, the three metals exhibited the same order of toxicity: Cd more toxic than Cu more toxic than Zn. In all cases, KL was the most sensitive endpoint. However, different patterns of response were observed for each metal. Thus, the toxicity assay protocol proposed can be used to evaluate adverse responses in isolated rat hepatocytes. Furthermore, these data indicate that the mechanism of toxic action of the three metals is different.
T-2 MYCOTOXIN EFFECTS ON SWELLING AND RESPIRATION OF ISOLATED RAT LIVER MITOCHONDRIA. G A Miura and J C Pace. USAMRIID, Pathophysiology Division, Fort Detrick, Frederick, MD. Sponsor: H W Wannemacher.

T-2 toxin (48,15-diacetoxy-8a-(3-methylbutyryloxy)-3a-hydroxy-12,13-epoxytrichothec-9-ene) (0.25 to 2 mM) promoted in vitro swelling of mitochondria isolated from Fischer 344 rat liver. Swelling was assayed spectrophotometrically at 520 nm. Metabolites of T-2, HT-2, triol, and tetraol (1 mM) also produced swollen mitochondria. Swelling was reversed with 5 mM ATP and was inhibited by dinitrophenol or potassium cyanide. When electron transport was assayed in vitro with succinate, 2 mM T-2 (3 min incubation at 28°C) reduced the ADP/O ratio and respiration control index by 13% and 70%, respectively. In contrast to mitochondria isolated in a mannitol medium (Toxicon 21:675, 1983), mitochondria isolated in a sucrose medium, which permitted the organelle to swell when assayed, displayed a loss of coupling, which was typical of the hydrophobic state. Electron micrographs of cultured rat hepatocytes exposed to T-2 showed mitochondria with translucent foils (Toxicon 24:413, 1986), which indicate the hydration of mitochondrial matrices. Thus, in conformity with the commonly described mechanism as a protein synthesis inhibitor, T-2 may also disrupt cellular processes through an impaired oxidative phosphorylation attributed to altered mitochondria morphology and/or blocked electron transport chain.

PREVIOUSLY a concentration- and oxygen-dependent chronic injury of CCL, rat liver slices was demonstrated. The effect of biotransformation inducers and inhibitors was examined in this model. Precipitate liver slices were incubated in a roller culture system in Waymouth's media (+gentamycin) at 37°C for 9 hr. The slices were exposed by vaporizing CCl4, producing a toxic concentration of 0.6 mM in the media. Intracellular K+ levels, isocitrate dehydrogenase (ICDH), and the alanine aminotransferase (ALT) were used as indices of cytotoxicity. Liver slices from rats pretreated with 50 mg/kg isonicotinic acid demonstrated a 450% of K+ and 423% in ICDH relative to controls following a 6 hr exposure to CCL. By 9 hr ICDH was 421% and intracellular K+ was 465%. Intracellular K+ in slices from rats treated with 80 mg/kg phenobarbital was 417% at 6 hr and ICDH was 437%. By 9 hr intracellular K+ was 475% and the loss of ICDH was 39%. While ICDH levels decreased, the levels of ALT were unchanged indicating a centrilobular lesion. Slices from allylisopropylacetonate rats were refractory to CCL toxicity indicating that the toxicity was not a direct "solvent effect." The manipulation of CCL-induced injury in the liver slices by altering biotransformation will allow a mechanistic investigation of the toxicity in the target cells. (GM 38290)


Oxometidine (OXMET) is cytotoxic to isolated rat hepatocytes. The mechanism of OXMET-induced hepatocyte injury may be related to sustained inhibition of mitochondrial oxidative phosphorylation leading to a reduction in cellular ATP content and cell death. This investigation was undertaken to determine the site of inhibition of rat liver mitochondrial (RLM) electron transport. OXMET did not significantly inhibit succinate supported oxygen consumption in isolated RLM at concentrations up to 500 μM. With beta-hydroxybutyrate or isocitrate supported respiration, OXMET significantly inhibited oxygen consumption at 0 μM and 25 μM, respectively. In RLM electron transport particles (ETP), OXMET inhibited NADH-oxidase and NADH-CoQ reductase activity with an IC50 of 3.5 and 2.5 μM, respectively. OXMET up to 200 μM did not significantly affect NADH-FeCN reductase. SK&F 9205S, a thiourea analog of OXMET, approximately 2 fold more toxic to hepatocytes, produced similar inhibition of NADH-oxidase and NADH-CoQ reductase with an IC50 of 800 μM and 600 μM, respectively. SK&F 9205S did not significantly inhibit NADH-FeCN reductase activity at 3.0 mM. These data suggest that OXMET and SK&F 9205S inhibit RLM electron transport in a manner functionally similar to rotenone and pyridinidin.

ERYTHROMYCIN ESTOLATE (EE) STIMULATES THE RELEASE OF SUPEROXIDE (O2-) FROM ACTIVATED RAT NEUTROPHILS (PMNs) IN VITRO. J A Hewett and R A Roth, Mich. State Univ., E. Lansing, MI.

The objective of the present study was to test the hypothesis that the hepatotoxicant, EE, stimulates the extracellular release of O2- from glycogen-elicted, rat peritoneal PMNs in vitro. EE caused a concentration-dependent increase in O2- release from PMNs at concentrations greater than 30 μM. 100 μM EE induced the release of 9.6±2.2 nmoles of O2- from 2x106 PMNs when the cells were incubated at 37°C for 10 min. Preincubation of 2x106 PMNs for 5 min at 24°C with 2 ng/ml PMA, a concentration which induced only marginal O2- release by itself, greatly enhanced the stimulatory effect of EE. Significant enhancement of O2- release was exhibited by PMA-pre-treated PMNs following incubation with 3.15 μM EE, and 41±2.0 nmoles of O2- were released in 10 min from PMA-pre-treated PMNs following incubation at 37°C with 100 μM EE. EE is the lauryl sulfate salt of the propionyl ester of erythromycin base. Erythromycin base did not stimulate O2- release from PMNs. Incubation of 2x106 PMNs with equimolar amounts of sodium lauryl sulfate (SDS) did not stimulate O2- release from the cells by itself. However, PMNs preincubated with 2 ng/ml PMA and exposed to SDS released similar quantities of superoxide as equimolar concentrations of EE. These results suggest the possibility that stimulation of O2- release from PMNs by EE may contribute to its hepatotoxic effects in vivo. (Supported by USPHS grant ES04139.)
ADMINISTRATION OF POLYETHYLENE GLYCOL (PEG) COUPLED-CATALASE (CAT) AND SUPEROXIDE DISMUTASE (SOD) TO RATS DOES NOT ALTER α-NAPHTHYLISOThIOCYANATE (ANIT)-INDUCED HEPATOTOXICITY. L. J. Dahm and R. A. Roth. Michigan State Univ., E. Lansing, MI.

When administered to rats, ANIT causes cholestatic liver injury characterized by an infiltration of neutrophils (PMNs) into perportal regions of the liver. We have observed recently that ANIT stimulates PMNs to release superoxide anion (O2-) in vitro, raising the possibility that PMN-derived, reactive oxygen species might be involved in ANIT-induced hepatotoxicity. To address this question, agents which metabolize O2- and its dismutation product, hydrogen peroxide, were employed. Rats were treated intravenously with either a PEG-CAT/PEG-SOD combination or PEG-bovine serum albumin (BSA) control and either ANIT 150 mg/kg, p.o., or corn oil vehicle. Activities of PEG-CAT in rat serum 12 and 24 h after ANIT treatment were 106.5 and 142.6 IU/ml, respectively, and PEG-SOD activities in serum at 12 and 24 h were 76.4 and 121.5 IU/ml, respectively. Liver injury was assessed 24 h after ANIT treatment. Reduced bile flow and serum elevations in γ-glutamyl transferase activity, aspartate aminotransferase activity, total bilirubin, and total bile acids occurred in rats treated with PEG-BSA and ANIT. Administration of PEG-CAT/PEG-SOD to ANIT-treated rats did not alter any index of liver injury, suggesting that extracellular release of reactive oxygen species from PMNs is not involved in ANIT-induced hepatotoxicity. (Supported by USPHS grant ES04139.)

THE ROLE OF α,β- UNSATURATED AMINO ACIDS IN THE TOXICITY OF MICROCYSTIN-LR AND NONODULARIN; TWO HEPATOTOXINS FROM CYANOBACTERIA. A. M. Dahlem, V. R. Beasley, K. I. Hanada, M. Matsuzawa, C. A. Harvis, K. L. Rinkehart, and W. W. Cervenka. 1Colleges of Veterinary Medicine and 2Chemical Sciences, Univ. of Illinois, Urbana, IL; 3Faculty of Pharmacy, Meijo University, Nagoya, Japan; 4Dept. of Biological Sciences, Wright State University, Dayton, OH.

Some species of cyanobacteria (blue-green algae) sporadically produce monomeric peptide hepatotoxins which can adversely affect the health of animals ingesting algal cells or toxin-containing water. A uniform characteristic feature of these peptide toxins is the presence of α,β-unsaturated amino acids. Unsaturated amino acids of this type have been found in other peptides, usually of microbial origin, and are closely linked to their associated bioactivities. Reaction of two different cyanobacterial peptides with sodium borohydride resulted in saturation and yielded dihydroproducts. The dihydro-products were compared with the parent toxins to assess the role of the α,β-unsaturated moieties in the hepatotoxicity and overall lethality of the peptide toxins. Saturation of the dihydro-products caused a 2 to 4 fold reduction in lethality as compared with parent toxins in mice, but did not alter the specific hepatotoxic lesions characterized of this group of toxins. These studies demonstrated that while the α,β-unsaturated amino acids play a role in the observed toxicities of these peptides, they are not essential for bioactivity.

DOSE AND TIME DEPENDENT EFFECTS OF THE HEPATOTOXIN MICROCYSTIN-LR IN MICE. S. J. Hermanovsky, R. S. Markin, and S. J. Stobes. University of Nebraska Medical Center, Omaha, NE.

Microcystin-LR, one of several cyclic heptapeptides produced by the blue-green algae Microcystis aeruginosa, produces rapid death in laboratory animals following microgram doses. However, no detailed dose and time dependent studies have been performed. Thus, female Swiss Webster mice were treated with 0, 12.5, 25, 50, or 100 μg microcystin-LR/kg. Liver, heart, lung, and kidney weights, liver histology, serum lactate dehydrogenase (LDH), serum aspartate amino transferase (AST) and serum alanine amino transferase (ALT) as well as hepatic hemoglobin content were determined with time after treatment. No lethality or changes were observed in any of the measured parameters at doses of 50 μg/kg or less. However, 100% lethality occurred in less than 2 hours in mice treated with 100 μg/kg. In these animals, significant increases in liver weight and hemoglobin content were present within 45 min post-treatment. Liver histology showed loss of hepatic architecture and necrosis 30 min after treatment. All serum enzymes increased 5 to 10 fold by 60 min post-treatment. The LD50 for microcystin-LR was 71 μg/kg. Microcystin-LR exhibits a sharp dose-response curve with no observable changes with doses approximately 20 μg/kg below the LD50.


Pretreatment of rats with the insecticide lindane has been shown to increase the level of aminoparatation recovered from the G.I. tract one hour after peroral administration of parathion. To determine whether lindane induces GI enzyme activity, weanling male and female rats received daily p.o. injections of 20 mg/kg lindane, in peanut oil, for 2 or 5 weeks while controls received the vehicle. After four weeks, half the remaining animals were transferred to isolation cubicles and received antibiotics in addition to their regular treatment. At autopsy, small intestine, large intestine and cecum were aseptically excised, homogenized under a stream of CO2, and incubated with a DMSO mixture of p,p'-DDT, 3,4-dichlorodibenzo-p-dioxane, p-nitrophenol-β-D-glucuronide, and methyl orange. After two weeks, lindane induced significantly higher β-glucuronidase activity in the small intestine and significantly higher dechlorinase activity in the cecum. After 5 weeks of treatment lindane significantly increased nitroreductase and azoreductase activities in the small intestine. Enzyme activity in the small intestine was primarily of tissue origin whereas that of the cecum was primarily bacterial. Abstract does not reflect EPA policy.
Bilirubin (BIL) is the end product of heme metabolism, and if not eliminated can produce CNS toxicity. Elimination of BIL from the body requires glucuronidation, forming either a mono- (BMG) or diglucuronide (BDG) prior to its excretion into bile. The purpose of this investigation was to determine the relative importance of the co-substrate, UDP-glucuronic acid (UDP-GA), and the enzyme, UDP-glucuronosyltransferase (UDP-GT), in the conjugation of BIL to BMG and BDG. Chemicals used to pretreat rats were selected based on their ability to alter hepatic UDP-GA levels and UDP-GT activity. Administration of 3-methylcholanthrene (3-MC), phenobarbital (PB), and trans-stilbene oxide (TSO) resulted in an elevation of hepatic UDP-GA concentration, whereas cadmium (Cd) administration resulted in a decrease in UDP-GA levels. An increase or decrease in UDP-GA concentration of 50% did not alter either BDG or BMG excretion in bile. UDP-GT activity was induced by treatment with pregnenolone-16α-carbonitrile (PCN), clofibrate (CLO), or isoflavone (IF). A 2-fold induction of UDP-GT activity by PCN and CLO resulted in a 2-fold increase in BDG/BMG ratio in bile. These results suggest that the formation of bilirubin-conjugates is independent of UDP-GA levels within 50 to 150% of control values, and that UDP-GT activity regulates the relative concentrations of BDG and BMG excreted into bile. (Supported by USPHS Grants ES-03192 and ES-07079).

THE PERSISTENT EFFECT OF 1-CYANO-2-HYDROXY-3-BUTENE (CHB) ON PANCREATIC GLUTATHIONE (GSH) LEVELS. M Wallig and E H Jeffery. University of Illinois, Urbana, IL.

CHB, a component of cruciferous vegetables, is pancreatotoxic and significantly elevates hepatic and pancreatic GSH. We have determined the temporal relationship between toxicity and GSH elevation. Male rats (6/group) were treated once with CHB, 200 mg/kg po, and histological evaluation and GSH determination carried out 1, 2, 4, 6, 12, 24, 48, 72, and 96 h after dosing. Pancreatic GSH was depressed at 2 and 4 h, then rose to a maximum at 12 h (5.4-fold), and remained elevated through 96 h (2.75-fold). In liver, GSH was first depleted approximately 50%, then elevated 1.5-1.8-fold at 48 and 72 h, returning to normal by 96 h. In pancreas, diffuse loss of zymogen and vacuolation in acinar cells was seen by 6 h, and apoptosis of acinar cells was widespread by 12 h. Signs of recovery were present by 24 h, with 15% of rats at 72 h and 50% of rats at 96 h exhibiting normal pancreas. No lesions were observed in the liver at any time at this dose. While this pattern of depletion of pancreatic GSH followed by an elevation is similar to the consumption and "rebound" of hepatic GSH observed after exposure to many hepatotoxins, the persistent elevation of pancreatic GSH after recovery of lesions suggests an additional mechanism at play. Disclosure of this mechanism may lead to development of a method to raise GSH levels clinically.

MORPHINE-INDUCED DEPRESSION OF HEPATIC GLUTATHIONE: RELATIONSHIP TO HYPOXIA, HYPOTHERMIA, TOLERANCE, AND GLUTATHIONE STATUS IN OTHER TISSUES. N P Skoulis, R C James, R D Harbison and S M Roberts. University of Arkansas for Medical Sciences, Little Rock, AR and Center for Environmental Toxicology, University of Florida, Gainesville, FL.

Previous studies in our laboratory have indicated that morphine is capable of depressing hepatic glutathione (GSH) levels through an effect that is initiated in the central nervous system. In the present study, the hepatic GSH depression resulting from the systemic (i.p.) administration of morphine (100 mg/kg) was completely blocked by the intracerebroventricular (i.c.v.) administration of the opioid receptor antagonist naloxone (250 μg), indicating that all of the effect of morphine on GSH at this dose originates within the CNS. Consistent with many other central opioid effects, tolerance developed to the depression of hepatic GSH after repeated doses of morphine. The depression of hepatic GSH was found to not be secondary to morphine-induced hypoxia or hypothermia, nor could it be attributed to hepatic intracellular oxidation of GSH. When morphine (100 μg) was administered by the i.c.v. route, GSH concentrations in liver and plasma were significantly altered while heart and kidney were resistant. Variable responses to i.c.v. morphine were obtained in spleen, stomach, and lung. The results of this study suggest the potential for enhanced susceptibility to some hepatotoxic compounds as a consequence of a central pharmacologic action of morphine.

DIMINISHED GLUTATHIONE IN MICE SUBJECTED TO COLD-RESTRAINT STRESS. H F Simmons, R C James, R D Harbison, and S M Roberts. University of Arkansas for Medical Sciences, Little Rock, AR and Center for Environmental Toxicology, University of Florida, Gainesville, FL.

Male mice of the ICR, NIH, ND/4 and B6C3F1 strains each had significantly diminished hepatic glutathione (GSH) concentrations following 2-3 hours of cold-restraint. The average decrease in hepatic GSH varied with the strain, ranging from approximately 15% (ICR) to 40% (ND/4). This depression of hepatic GSH was not related to hypothermia, as cold-restraint produced no change in body temperature in the most responsive strain (ND/4). Further experiments with the ND/4 mouse indicated that the extent of hepatic GSH depression was a function of the duration of cold-restraint the mouse endured. Catecholamines have been previously shown to depress hepatic GSH, and total serum catecholamines in ND/4 mice (and mice of other strains) were elevated by cold-restraint. However, the temporal relationship between changes in catecholamines and hepatic GSH did not support a role for catecholamines as mediators of the cold-restraint induced decrease in hepatic GSH levels. Cold-restraint was also found to diminish GSH levels in plasma and kidney, but not in spleen, stomach, heart, or lung. These studies suggest stress may compromise glutathione-mediated detoxification reactions of the liver, plasma and kidneys.
GLUTATHIONE DEPLETION AND POTENTIATION OF CARBON TETRACHLORIDE HEPATOTOXICITY BY SYMPATHOMIMETIC AMINES. J F Seng, R C James, R D Harbison, and S M Roberts. University of Arkansas for Medical Sciences, Little Rock, AR and Center for Environmental Toxicology, University of Florida, Gainesville, FL.

Epinephrine has been previously reported to diminish hepatic glutathione (GSH) concentrations. In this study we sought to determine whether pharmacologically-related sympathomimetic drugs also decreased GSH levels in the liver. Amphetamine (AMP; 1-20 mg/kg), phenylpropanolamine (PPA; 10-200 mg/kg), pseudoephedrine (PSE; 10-200 mg/kg), and phenylephrine (PHE; 10-200 mg/kg) each caused a dose-dependent loss of hepatic GSH in ICR mice comparable to that produced by epinephrine (0.1-1.0 mg/kg). The maximum decrease in GSH was similar for all drugs tested, and was approximately 30-40% of total hepatic GSH. Because epinephrine has been reported to potentiate the hepatotoxicity of carbon tetrachloride (CCL4), PPA and PHE were tested for their effects on CCL4-induced hepatic injury. PPA (200 mg/kg) co-treatment caused a significant enhancement of hepatic injury from CCL4, as measured by serum ALT activities, while PHE was without effect on CCL4 hepatotoxicity. These results indicate CCL4 hepatic injury may be potentiated by some, but not all, sympathomimetic drugs, and that this potentiation appears to occur by a mechanism independent of GSH depletion.

FAILURE OF PROSTAGLANDIN E1 (PGE1) AND 16, 16-DIMETHYL PROSTAGLANDIN E1 (dMPEG1) TO PROTECT AGAINST CCL4-INDUCED LIVER DAMAGE IN THE RAT. M J Derelanko. Allied-Signal Inc. Dept. of Toxicology, Morristown, NJ.

The prostaglandin analog dMPEG1 has been reported to protect the liver of rats against cell necrosis induced by CCL4. The studies reported herein were performed to determine if PGE1 shares hepatoprotection with its analog. 4F-344 rats were administered PGE1 s.c. in saline at a gastric cytoprotective dose (0.5 mg/kg) various times ranging from 30 min. prior to 24 hours following an s.c. injection of CCL4 (10 ml/kg). The rats were sacrificed 48 hrs. later. Liver damage was evaluated by gross appearance, elevation of SGPT levels and microscopic examination. PGE1 failed to prevent or reduce the increased SGPT and necrosis found in rats only receiving CCL4. A number of the PGE1-treated rats showed significantly higher SGPT levels than the untreated CCL4 rats. Subsequent studies utilizing dMPEG1 at a gastric cytoprotective dose (5 µg/kg) gave similar results.

EFFECTS OF A HIGH CARBOHYDRATE DIET ON THE HEPATOTOXICITY OF ACETAMINOPHEN, BROMOBENZENE AND CARBON TETRACHLORIDE IN MALE AND FEMALE GSH MICE. C S Boyer and D R Peterson. Molecular and Environmental Toxicology Program and School of Pharmacy, University of Colorado Health Sciences Center, Denver, CO.

We have identified a high carbohydrate (CHO) diet which increases hepatic pyridine nucleotides by approximately 30% but does not increase reduced glutathione. The purpose of this study was to evaluate the effect of this diet on the toxicity of three well-documented hepatotoxins. Male and female C57/2IBg mice were fed the liquid diet for six days. On day five of the pretreatment, a single dose of carbon tetrachloride (CCL4) (500 ul/kg), bromobenzene (BB) (600 ul/kg) or acetaminophen (AA) (400 mg/kg) was administered ip. Blood samples for the determination of serum ALT levels were taken 24 hours after dosing. Control animals were allowed to consume normal lab chow (Wayne Sterilizable Lab Blox). Pretreatment of both male and female mice with the high CHO diet had no effect on the hepatotoxicity of CCL4. The ALT activity following BB was decreased 80% in males and 65% in females maintained on this diet. The high CHO diet also decreased post- AA ALT activity 50% and 75% in males and females respectively. This data points to the possibility of the importance of reduced pyridine nucleotides in the maintenance of cellular defense and homeostasis following a toxic insult.

454-INDUCED INHIBITION OF HEPATIC MICROSOMAL AND MITOCHONDRIAL CALCIUM SEQUESTRATION IN RATS PRETREATED WITH CHLORODECONE. P R S Kodavanti, and N M Mehdalkar. Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS.

Earlier reports from our laboratory indicated increased hepatocellular Ca2+ levels in rats receiving CCL4 after pretreatment with chlorodecane (CD). In the present studies, we tested the ability of mitochondria and microsomes in sequestering the Ca2+ 45Ca-uptake by mitochondria and microsomes was measured using four Ca2+ concentrations (0.01 to 20 µM) in Ca-EGTA buffered medium at different timepoints after CCL4 administration (100 µl/kg). Male SD rats were maintained for 15 days either on normal (N) or on 10 ppm CD diet prior to CCL4 injection. Cd pretreatment alone significantly inhibited the 45Ca-uptake by mitochondria and microsomes when incubated at 10 µM and higher, but not at lower concentrations of Ca2+. CCL4 administration to both normal and Cd pretreated rats resulted in significant inhibition of microsomal and mitochondrial 45Ca-uptake as early as 1 h at all concentrations of free Ca2+. While the extent of inhibition was greater and irreversible after Cd+CCL4 treatment, it was reversible after NaCl treatment. Irreversible perturbation of Ca2+ homeostasis in CD-potentiated CCL4 toxicity might be a critical event in the progressive and irreversible liver injury. (Supported by APOSE-88-0009).
The pathogenic mechanism underlying anesthetic-induced hepatitis may include a role for the immune system. In addition, individuals sensitized to one halogenated anesthetic may produce antigenically similar moieties upon exposure to other chemically similar anesthetic. Guinea pigs were exposed to halothane, enfurane, or isoflurane (3 or 1 exposures at 1%) in a 40% O₂ atmosphere. Guinea pigs were also given halothane IP (1 exposure) or enfurane/isoflurane (3 exposures). Antibody to halothane metabolite antigenic moieties were determined by a hemagglutination immunoperoxidase assay using anti-trifluoroacetyl (TFA) antibody. Following inhalation or IP exposure to halothane, TFA antibodies were localized in the centrilobular area. No TFA antibodies were detected in animals following enfurane or isoflurane exposures. One isoflurane–exposed guinea pig contained circulating antibodies reactive with TFA–albumin detected by indirect ELISA. Although isoflurane and enfurane can generate antigens that cross-react with anti-TFA antibodies, only in halothane–exposed animals could these adducts be demonstrated. A possible cross sensitization between isoflurane and halothane as suggested by anti-TFA antibodies in one guinea pig exposed to isoflurane requires further exploration. (NIH GM 34788)

Differential potency of the enantiomers of 2,2',3,4,4',6-hexachlorobiphenyl as inducers of cytochrome P-450 in chick embryo hepatocytes. L E Rodman, A Mannschrack, M Püttermann, L W Robertson, A T Swim and J I Shedlofsky. Graduate Center for Toxicology, Univ. of Kentucky, Lexington, KY and Univ. of Regensburg, Regensburg, FRG.

To assess the effects of the enantiomers of the polychlorinated biphenyl, 2,2',3,4,4',6-hexachlorobiphenyl (HCB) on induction of hepatic cytochrome P-450, cultured chick embryo hepatocytes were treated with (-)-, (+)- or racemic (rac)-HCB (0.034 - 10 μM) for 18 hours. Cytochrome P-450 concentrations, benzphetamine N-demethylase (BPD), and ethoxyresorufin O-deethylase (EROD) activities were assayed. HCB elevated cytochrome P-450 and BPD activity in the order (+)-HCB > (rac)-HCB > (-)-HCB. Although HCB is a phenobarbital-type inducer of cytochrome P-450 in the rat, HCB also increased EROD activity in chick embryo hepatocytes, in the same rank order (+)-HCB > (rac)-HCB > (-)-HCB. A cell culture system was selected to minimize pharmacokinetic influences on the induction of cytochrome P-450. Nevertheless potent differences were seen. These data indicate 1) a basic difference between the rat and this avian system in the mode of induction of cytochrome P-450 by HCB and 2) chirality in the recognition events associated with the induction of cytochrome P-450 in chick embryo liver. (Supported by Veterans Admin. Funds.)

Mechanistic studies of the synergistic hepatotoxicity produced by carbon tetrachloride and chloroform in male F-344 rats. D R Steup, M Tokars and L G Sipes. Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ.

Possible mechanisms by which chloroform (CHCl₃) potentiates the hepatotoxicity of carbon tetrachloride (CCl₄) were studied using fasted male F-344 rats. Compounds were administered IP in 10% Emulphor and hepatic injury was evaluated by means of plasma alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) activities and histologic examination. Influence of the total quantity of xenobiotic given was evaluated by comparing rats dosed with CCl₄ (0.52 mmol/kg) + CHCl₃ (0.49 mmol/kg) to rats injected with an equimolar dose (1.01 mmol/kg) of each chemical alone. The combination was significantly more toxic (8-15 fold) in each case. In another study, deuterated chloroform (CDCl₃) (0.49 mmol/kg, ip) potentiated the toxicity of CCl₄ to the same extent as an equal dose of CHCl₃. Since CDCl₃ is metabolized at roughly half the rate for CHCl₃, this suggests that CHCl₃ metabolism is not a critical factor in the genesis of synergistic toxicity. Studies now in progress will extend these results to a wider range of compounds and to other routes of administration. (EPA CR812557: Abstract does not necessarily reflect EPA policy).

The isomers of dichlorobenzene produce different hepatotoxic effects. J W Allits, E Berman, D E House, B L Robinson and J E Simmons. Health Effects Research Laboratory, USA Environmental Protection Agency, Research Triangle Park, NC.

The hepatotoxic potency of the three isomers of dichlorobenzene (DCB) differed in male F344 rats 24 hrs after gavage with a single dosage up to 2.2 ml/kg. For each isomer, 24 different doses were given, one animal per dose. The ortho isomer proved the most hepatotoxic causing centrilobular necrosis and increased serum ALT and AST at 0.14 ml/kg. The meta isomer of DCB was less toxic, with necrosis and increased ALT and AST beginning at 0.35 ml/kg. For p-DCB treated rats these endpoints were unaffected, but vacuolar degeneration occurred at 0.375 ml/kg. Further, the cytochrome P-450 system responded differently to each isomer. For o-DCB, P-450 levels decreased steadily above 0.04 ml/kg. For m-DCB, P-450 increased 20% at 0.08 ml/kg, but at 0.3 ml/kg began decreasing. For p-DCB, P-450 increased 30% at 0.3 ml/kg and higher doses. The doses where P-450 increased correlated with the onset of vacuolar degeneration; the doses where P-450 decreased correlated with the initial changes in necrosis, ALT and AST. Based on the varying response of cytochrome P-450 and the different thresholds for the other endpoints, we conclude that each isomer has a unique mechanism for toxicity in the liver. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)
ULTRASTRUCTURAL CHANGES ASSOCIATED WITH 1-NITRONAPHTHALENE INDUCED HEPATOTOXICITY IN THE RAT
namazo, MI, & IRDC, Mattawan, MI.

1-Nitronaphthalene (1-NN), a component of diesel exhaust particulate, has been shown to cause
liver and lung toxicity in rats. Recent evidence suggests that injury is due to metabolites formed
through cytochrome P-450 mixed-function oxidase activity. Cellular damage in the lung (Clara
cell necrosis) and liver may be due to lipid peroxidation, possibly mediated by the formation of
reactive oxygen species or N-hydroxy radicals. This study focuses on ultrastructural changes in
hepatocytes over a series of time intervals follow-
ing a single 1-NN injection (100 mg/kg ip)
with & without phenobarbital (PB) pretreatment
in male Sprague-Dawley rats. Preliminary ev-
dence shows that PB pretreatment enhances hepa-
totoxicity with the centrilobular region more
severely affected. Within 8 to 12 hours, cells
show several changes, including cellular swell-
ing, mitochondrial distension, vacuolization,
and notable clumping of nuclear material. After
24 hours, nuclear disruption is more pronounced,
and widespread phagocytosis of mitochondria is
evident. Nuclei are severely pyknotic by 48 hours,
and mitochondria are largely replaced by a
post-phagocytic state containing large hya-
line inclusions. These findings support the
notion that reactive intermediates formed at
the site of injury are responsible for the hepato-
toxicity.

IDENTIFICATION OF d-LIMONENE METABOLITES THAT
BIND TO α2u-GLOBULIN (α2u). LD Lehman-
McKeeman, PA Rodriguez, R Takigiku, D Caudill,
and ML Fey. Miami Valley Laboratories, Procter &
Gamble Co, Cincinnati, OH.
d-Limonene or its metabolites bind in a rever-
sible manner to α2u and produce a male rat-spe-
cific nephrotoxicity. In this study, the metab-
oxides responsible for this binding were identi-
cified by gas chromatography with simultaneous
radioactivity and mass spectrometric detection.
α2u was isolated from male rat kidneys 24 hr
after oral [4C]d-limonene treatment (3 mmol/kg;
2.5 mCi/kg) by gel filtration or reverse-phase
HPLC and extracted with methylene chloride at pH
7.4 or pH 2. Following extraction at pH 7.4, 3
peaks of d-limonene-derived radioactivity were
detected and identified as cis d-limonene-1,2-
oxide (1,2-oxide; 82%), d-limonene (13%) and
d-limonene-1,2-diol (1,2-diol; 5%). With acid
extraction, 1,2-oxide was hydrolyzed to the diol
and only d-limonene and 1,2-diol were detected.
1,2-Oxide was detected in female rat kidney cy-
tosol, but at levels 50-times less than that
associated with α2u in male rat kidney. Thus,
the major d-limonene metabolite that binds to
α2u is 1,2-oxide, which because of its suscepti-
bility to acid hydrolysis, can be isolated only
with neutral extraction. Previous studies have
indicated reversible binding of d-limonene
metabolites to α2u, suggesting that the inter-
action between α2u and 1,2-oxide does not
directly involve the epoxide moiety.

CHRONIC OCCUPATIONAL TOXICITY OF JP8. C L
Alden, T K Newell, and D L Mattie. Toxic
Hazards Division, Wright Patterson AFB, OH.

A kerosene-type jet fuel, JP8, consists of a
complex mixture of aliphatic and aromatic
hydrocarbons. Because of the utility of JP8
studies have been conducted to identify the
potential long-term consequence of occupa-
tional exposure. Fischer 344 rats and C57BL/6
mice of both sexes were exposed to JP8 by
aerosol at 0, 500, and 1000 mg/ml for 90 days
then followed until approximately 24 months of
age. The male rat kidney developed a revers-
ible ultrastructural increase in size and pro-
portionality for crystalline change of phago-
lysosomes and proteinic reabsorption droplets
in proximal convoluted tubular epithelium. A
specific triad of persistent light microscopic
renal lesions occurred but functional change
was limited to a reversible decrease in urine
concentration. The response is comparable to
the chronic effect of lifetime exposure of the
male rat to unleaded gasoline, d-limonene, and
paradichlorobenzene, except for the absence of
tubular tumorigenesis. Thus, the active
toxicologic response presumably must occur over
a greater proportion of the male rat's life
span for the tumor component of this male rat
hydrocarbon nephropathy syndrome. Since the
pathologic response to JP8 involved only one
tissue in one sex of mice, and since the male
rat response may be linked to an inherent
renal protein peculiarity, the predictiveness
for humans must be questioned.

Renal Toxicity of Carbon Disulfide (CS2) in Three Strains of Mice. R J Rubin and R B Kroll, Johns Hopkins University, Baltimore, MD.

We have previously shown that CS2 is renotoxic in rats. It is our hypothesis that this effect is due to the metabolic activation of CS2 in the kidney. Different strains of mice have been shown to have varying toxic effects to chloroform associated with varying capacities to form a reactive metabolite. Thus, we chose to study CS2 toxicity in three strains of mice with widely different renal sensitivities to chloroform. DBA/2J (D), C57BL/6J (C), and ICR (I) mice were administered CS2 (30-1250 mg/kg, ip) and effects on BUN and on 14C-PAH accumulation by renal cortical slices were assessed. At 4 hours post CS2, all three strains had comparable increases in BUN; however, by 24 hours markedly greater increases were seen in the D and C mice. With regard to the effect of in vivo CS2 on PAH uptake, the relative sensitivities at 4 hours post-treatment (PT) were: D (47% inhibition) > C (26%) > I (no effect). At 24 hours PT, the effects were D (42%) = C (48%) > I (no effect). These data indicate that D and C mice are sensitive to CS2-induced renal toxicity, while ICR mice are relatively resistant. Subsequent experiments will be designed to evaluate the relative abilities of these strains to metabolically activate CS2. Supported by NIEHS ES09297.
Effects of dichlorobenzenes (DCB), liver and kidney toxic agents, on freshly isolated liver and kidney cells were examined. The cells were prepared from male rats by collagenase perfusion in vitro. Isomers of DCB (o-, m-, p-) did not have any effects at 0.5 mM on the glutathione (GSH) contents and the viability of hepatocytes but decreased them at 1 mM. The potencies were: o-DCB = 1 mg/mL > p-DCB. On the other hand, those of hepatocytes obtained from phenobarbital pretreated rats were decreased by DCB at 0.5 mM. The potencies were: o-DCB < m-DCB = p-DCB. Effects of DCB were suppressed by methylpyrone (0.5 mM). Addition of GSH (1 mM), methionine (1 mM), and albumin (1%) to the cell suspensions did not protect the hepatocytes from the toxicities of DCB (1 mM). Analysis of GSH analogues by HPLC indicated the increase of suspected GSH conjugates of o-DCB and m-DCB outside of the cells. The amounts of oxidized GSH were not different from control experiments. In the case of renal cells, decreases in GSH contents and viabilities by DCB (1 mM) appeared without phenobarbital pretreatment. The potencies were: o-DCB = m-DCB = p-DCB. It appears that metabolic activation by liver is not necessarily required for the renal toxicity of DCB.

The onset of cephaporadine nephrotoxicity was compared between normoglycemic and diabetic male Fischer 344 rats. Rats (200-250 g) were injected with 35 mg/kg STZ (ip) to induce a diabetic state. The animals were divided into the following groups (N=4-8): normoglycemic vehicle (NV), normoglycemic-cesphoradine (NC), diabetic-vehicle (DV), and diabetic-cesphoradine (DC). Normoglycemic and 14 day diabetic rats were injected with 1500 mg/kg cephoralidine or vehicle (ip). Urine volume was increased in the NC group 24 hr after cephoralidine administration (p<0.05). Kidney wt. was increased (p<0.05) 30 in the NC group within 24 hr. Renal cortical slice uptake of p-aminohippurate (PAH) and tetraethylammonium bromide (TEA) were diminished in 3-5 days post cephoralidine treatment in the NC group (p<0.05). PAH and TEA uptake were not altered in the DC rats. Cephoralidine also did not increase kidney wt. in the DC group. These results indicate diabetes protects against the nephrotoxicity of cephoralidine. Additionally, the protection mediated by diabetes cannot be attributed to an alteration in the onset of cephoralidine toxicity. (Supported in part by NIH RR05870).

Glutathione (GSH) depletion and lipid peroxidation are key events in cephoralidine (CPH) nephrotoxicity. These studies were designed to test the hypothesis that CPH inhibits activity of GSSG Rx, a key enzyme of the GSH redox cycle, and that inhibition of GSSG Rx contributes to CPH-induced GSH depletion and toxicity. Renal cortical slices from naive male Fischer-344 rats were incubated at 37°C in bicarbonate buffer containing 0.5 mM CPH for 30-120 min. Toxicity was evaluated as LDH leakage and lipid peroxidation estimated as malondialdehyde (MDA) production. CPH inhibited activity of GSSG Rx in a time and concentration related manner, and preceded CPH-induced MDA production and LDH leakage. Deferoxamine and promethazine did not block CPH inhibition of GSSG Rx or CPH-induced GSH depletion. In contrast, treatment of renal cortical slices with GSH or dithiothreitol (DTT) blocked CPH inhibition of GSSG Rx and GSH depletion. These data suggest that inhibition of GSSG Rx activity by CPH may contribute to the progression of CPH-induced GSH depletion, lipid peroxidation and toxicity. Furthermore, protection against CPH inhibition of GSSG Rx by DTT and GSH suggests that this inhibition may be related to oxidation of critical sulhydryl groups of this enzyme.

Cephaporadine nephrotoxicity: in vivo alterations of the drug metabolizing enzyme activities and of the transport systems of the rat renal brush border membrane.
ROLE OF INTRACELLULAR REACTIVE OXYGEN SPECIES AND CALCIUM IN GENTAMICIN CYTOTOXICITY USING DICHLOROFLUOROSCEIN AND FURA-2 IN PRIMARY RENAL CORTICAL EPITHELIAL CULTURES. J Swann and D Acosta. University of Texas, Austin, TX.

We examined two aspects of gentamicin cytotoxicity reported in the literature: production of intracellular reactive oxygen species and increases in intracellular free calcium. Rat renal cortical epithelial cells were grown in primary culture on coverslips and changes in fluorescence of two intracellular probes were monitored photometrically. Generation of reactive oxygen species was monitored using the sensitive intracellular fluorescent probe, 2',7'-dichlorofluoroscein (DCF). Gentamicin (2-4 mM, 6-24 hours) did not cause increased fluorescence of intracellularly trapped DCF, while tert-butylhydroperoxide (500 μM, 1 hour) caused a 774% increase and cephaloridine (1 mM, 1 hour) caused a 56% increase in DCF fluorescence within the cells. It has been reported that gentamicin causes a rapid increase in cytosolic calcium, and that this was central to the mechanism of toxicity. However, perfusion of 4 mM gentamicin for one hour, in calcium-free or calcium-containing medium (1.25 mM Ca²⁺), failed to cause any increase in cytosolic calcium, as determined by measuring fura-2 fluorescence. In conclusion, within the specified treatment periods, gentamicin does not cause the generation of reactive oxygen species, or increased cytosolic calcium levels. This suggests that reactive oxygen species do not play a role in gentamicin-induced cellular injury, and that any increases in intracellular calcium must occur later in the time-course of this injury.


Initial preclinical toxicology studies with Fostriecin, an inhibitor of Topoisomerase II, indicated that the kidney was a target organ. The objective of this study was to investigate renal clinical biochemical, in vitro functional, and histopathological changes following acute IV administration of 0, 10, and 20 mg/kg of the drug. Daily measurements of serum BUN, creatinine, and 24 hr urinary excretion of glucose, Na⁺ and K⁺ were made for one week. In a second experiment, renal sections were sequentially taken for histopathology and renal cortical slices prepared for measuring organic ion transport. At 20 mg/kg, increased BUN (384%), creatinine (58%) and 24 hr glucose excretion (338%) peaked 24 hrs postdose and returned to control values by 168 hrs. Twenty-four hr excretion of Na⁺, K⁺, and urine osmolality decreased 24 to 72 hrs postdose (50 to 80%) and approached control values by 168 hrs. Renal lesions consisted of vacuolization and necrosis of proximal and distal tubular epithelium at the corticomedullary junction extending into the medulla, were most severe from 24 to 48 hrs, and were absent by Day 28. There was no decrease in organic ion transport by slices obtained from the outer cortex. Except for decreased 24 hr Na⁺ excretion, alterations were consistent with reversible acute renal failure due to localized effects on tubular epithelium.

PROTECTIVE EFFECT OF PYRIDOXL-5'-PHOSPHATE AGAINST GENTAMICIN-INDUCED NEPHROTOXICITY IN RAT. S Kacew, Department of Pharmacology, Univ. of Ottawa, Ottawa, Ontario, Canada.

Gentamicin (GEN) is widely used antibiotic is known to produce nephrotoxicity as evidenced by an increased urinary excretion of β-galactosidase (GAL), β-glucuronidase (GLU) and β-N-acetylglucosaminidase (NAG). The observed enzymeuria is accompanied by renal phospholipidosis and a decrease in Na⁺-K⁺ ATPase and alkaline phosphatase. Since pyridoxal-5'-phosphate (PLP) was previously found to provide protection against GEN-induced nephrotoxicity, neuromuscular paralysis and death, the objective of this study was to determine whether the effects of GEN on rat kidney metabolism and function could also be prevented by PLP. GEN (60mg/kg/day) administered (sv) daily for 14 days to male Sprague-Dawley rats increased the urinary excretion of GAL, GLU and NAG; elevated renal phospholipid levels; and decreased PLP content. Simultaneous GEN and PLP (250mg/kg/day) administration (ip) daily for 14 days prevented the GEN-induced metabolic and functional alterations in kidney. Data show that depletion of renal PLP may play a role in the development of aminoglycoside-induced nephrotoxicity. (Supported by the Medical Research Council of Canada).

EFFECT OF POLYASPARTIC ACID PRETREATMENT ON VANCOMYCIN-INDUCED NEPHROTOXICITY. M A Smith, D L Kaplan and R Hildebrandt. The University of New Mexico College of Pharmacy, Albuquerque, NM. Sponsor: G B Corcoran.

The glycopeptide antibiotic vancomycin (VAN), produces a dose-related increase in total kidney weight and blood urea nitrogen concentrations (BUN). VAN-induced myeloid body formation resembles that produced by the aminoglycosides. The polyaminoacid, polyaspartate (PAA), has been used to prevent aminoglycoside-induced renal damage in the rat. PAA appears able to prevent aminoglycoside-induced renal damage without altering the renal concentrations of the aminoglycoside. The purpose of this study was to determine the effects of PAA on VAN-induced nephrotoxicity. VAN was administered i.p. to female Sprague-Dawley rats (750 umole/kg). PAA was administered s.c. one hour prior to VAN administration. Nephrotoxicity was assessed 48 hrs later by BUN determinations and kidney weight. Renal cortical VAN concentration was determined by HPLC. Treatment with VAN alone resulted in a 10-fold increase in BUN concentrations and a 2-fold increase in total kidney weight. Pretreatment with PAA resulted in no significant change from control in BUN concentrations or kidney weight. Renal cortical VAN concentrations decreased by 60% following pre-treatment with PAA. PAA-induced protection of VAN-induced nephrotoxicity may be related to decreased cortical concentration of VAN.
CISPLATIN (CP) TOXICITY STUDIED WITH PRIMARY CULTURES OF RAT PROXIMAL TUBULAR CELLS (PTC). J. Y. Lee, R P. Trump, J. W. Jones. Department of Pathology, University of Maryland School of Medicine, and Maryland Institute for Emergency Medical Service Systems, Baltimore, MD

Studies were undertaken to establish an in vitro model of CP nephrotoxicity using primary cultures of rat PTC. CP (50-400 μM) resulted in dose-dependent toxicity over 24 h as determined by the neutral red assay. Morphological alterations consisting of nuclear segmentation, polyribosomal dispersion, and formation of aggregates of smooth and rough ER, observed as early as 2 h post-CP, are similar to those described following CP administration to rats (Jones et al.; Lab Invest 52:236, 1985). In addition, the role of non-protein thiol in CP toxicity was investigated by pretreating PTC with either 0.2 mM buthionine sulfoximine (BSO) for 6 h (thiols = 4% of control) or 1 mM 2-oxothiazolidine-4-carboxylic acid (OTC) for 9 h (thiols = 12% of control). BSO pretreatment resulted in potentiation of CP toxicity, while OTC pretreatment provided significant protection. These results suggest that primary cultures of rat PTC are useful for studies of CP toxicity and that renal cells non-protein thiol (i.e., glutathione) play an important role in defense against CP toxicity. [Supported by NIH GM-19440 and ACS BC-5701]


Whole body hyperthermia (WBH) has been shown to increase the nephrotoxicity of cisplatin (CDDP) chemotherapy. To study the effects of WBH on CDDP disposition, 18 beagle dogs received 20, 50, or 80 mg/m2 of CDDP by constant IV infusion for 60 min. under normothermic or hyperthermic conditions. Blood was collected during infusion and whole blood, plasma, and ultrafiltered plasma (Centricon-10 and -30 filters) collected. At the end of infusion, dogs were sacrificed and all major tissues were sampled. CDDP was determined by atomic absorption. CDDP in blood of WBH dogs were lower than those of normothermic dogs. The area under the concentration-time curves (AUC) of CDDP in C-10 and C-30 plasma were similar across treatments. The correlations between dose and AUC were highly significant in both normothermic and WBH dogs (R^2=0.93 and 0.96; p<0.0005, resp.) indicating linear pharmacokinetics at both temperatures. Tissue levels, corrected for AUC, inhibited tissue specific concentrations with most sites from WBH dogs being greater (esp. GI organs). These data indicate that WBH significantly changes CDDP disposition, a factor which should be accounted for in designing CDDP dosage regimens in WBH. (Supported by PHS grant CA42745)

EFFECTS OF COPPER OR BISMUTH PRELOADING ON DDTCE RESCUE ON CISPLATIN NERPHOTOXICITY AND KIDNEY METAL CONCENTRATIONS. R.S DeNardo and JF Riviere. Toxicology Program, North Carolina State Univ., Raleigh, NC.

Metallothionein inducing metals and metal chelators have been proposed as protective agents to lessen the nephrotoxicity of the chemotherapeutic agent, cis-diamminedichloroplatinum (CDDP). Bismuth has been previously shown to protect against CDDP toxicity in mice. Kidney metal concentrations and serum creatinine and urea nitrogen concentrations(SUN) were measured in F344 female rats 4 days after an intraperitoneal (ip) dose of CDDP alone or with pre-administration of bismuth, copper, or cadmium, or post-administration of diethylthiocarbamate (DDTCE). Either 20 μmol bismuth, 40 μmol bismuth, 10 μmol copper, or 5.5 μmol cadmium was administered subcutaneously, daily for three days prior to a 5 mg/kg CDDP treatment. Bismuth was also administered prior to a 10 mg/kg CDDP dose. DDTCE was given at 750 mg/kg ip 1 hour after a 5 or 10 mg/kg CDDP dose. Kidney platinum concentrations and weight loss were not lowered by any of the pretreatments, although weight loss was lower in the 10 mg/kg CDDP group with DDTCE. DDTCE rescue in the 5 mg/kg CDDP group lowered kidney platinum concentrations from 13.2 mg to 6.2 mg/kg wet tissue. Increased CDDP decreased the kidney copper concentrations and the kidney bismuth concentration. Copper pretreatment had no effect on kidney copper levels at day 4, but creatinine concentrations were not significantly increased by the 5 mg/kg CDDP treatment at 4 days post-dosing, but were by the 10 mg/kg dose. Bismuth did not protect against this nephrotoxicity, however, the DDTCE rescue resulted in a significantly lower SUN. These results do not support the use of bismuth or copper as an effective protective agent to lessen the nephrotoxic effects of CDDP. (Supported by PHS Grant CA42745)


Quinapril is an angiotensin converting enzyme (ACE) inhibitor being developed as an antihypertensive drug. Other ACE inhibitors produce hypertrophy of the juxtaglomerular apparatus (JGA) and may produce renal tubular lesions in rats. To assess the effect of quinapril on renal function and structure, a 4 week time course study was conducted in male Wistar rats with daily oral doses of 0, 10, 100, and 400 mg/kg. Glomerular filtration rate (creatinine clearance) and fractional electrolyte excretion values were derived from urinalysis and blood biochemistry data obtained at Days 1, 7, 14, and 28. Renal sections were collected on days 2 and 4 weeks for histopathologic evaluation and cortical slices were obtained to assess organic ion transport in vitro. Expected pharmacologic effects of an ACE inhibitor were seen at all doses and included decreased serum aldosterone (65 or 25% of control at 10 or 400 mg/kg, respectively, on Day 28), increased plasma renin activity (2x control at Day 28), and JGA hypertrophy/hyperplasia. However, there were no functional alterations or renal tubular lesions suggestive of renal toxicity seen with daily administration of quinapril for 4 weeks at doses up to 400 mg/kg (400X the efficacious dose in the spontaneously hypertensive rat).

Hepatotoxic doses of APAP also induce nephrotoxicity in mice and rats. In the Fischer rat, APAP deacetylates nephrotoxicity. This study was undertaken to determine if deacetylation similarly mediates nephrotoxicity in the mouse. Fasted (18hr) 3 month old male CD-1 mice were given APAP 600mg / kg or vehicle, po. and killed 12 hr later for assessment of hepato- and nephrotoxicity. Mean plasma sorbitol dehydrogenase and BUN levels were 10.898 U/ml and 46 mg/dl, respectively, after APAP and 33 U/ml and 34 mg /dl in controls. There was marked hepatic centrilobular and renal cortical necrosis in treated mice. Treatment with bis [nitro phenyl] phosphate [BNPP] inhibited kidney homogenate deacetylas [alpha naphthyl acetate hydrolysis] activity by 1.3 hr after 100 mg/kg, ip. Similar pretreatment of mice with BNPP, 1 hr before APAP, did not alter APAP’s hepato- or nephrotoxicity. These results indicate that deacetylation of APAP to PAP may not be required for nephrotoxicity in the CD-1 mouse. (Supported by NIH GMJ460, ES0713 and the Center for Biochemical Toxicology)

HEPATIC AND RENAL DRUG METABOLIZING ENZYMES (DME) FOLLOWING CHRONIC DIETARY RESTRICTION IN RATS. E Graichen, K Tyrrell, R Goldstein, and T Leonard. Smith Kline & French Labs., Swedeland, PA.

Dietary restriction increases life-span and decreases age-related disease in rodents. The effects of reduced food or protein consumption on hepatic and renal DME were studied in male Fischer-344 (F) and Sprague-Dawley (SD) rats. From 2.5 to 1.68 months of age, rats were allowed Purina 5002 rat chow (20% protein) (HP), or an isocaloric reduced protein (12%) chow (LP), both ad libitum, or Purina 5002 chow for only 6.5 lighted hours daily (RES). At 6 and 12 months of age, hepatic cytochrome P450 content was similar in all diet groups in both strains, but began declining by 18 months. At 24 months, hepatic P450 content was 4.6 (HP), 5.4 (LP), and 7.1 (RES) nmol/g liver in F rats, and 5.2 (HP), 4.6 (LP), and 6.6 (RES) in SD rats, compared to 7.8 and 9.8 nmol/g liver in 2.5 month old F and SD rats, respectively. Renal P450 content was more stable in aging animals, and at 24 months was 0.89 (HP), 0.91 (LP), and 0.99 (RES) nmol/g kidney in F rats, and 1.02 (HP), 0.98 (LP), and 1.05 (RES) in SD rats, compared to 0.93 and 1.05 nmol/g kidney in 2.5 month old F and SD rats, respectively. Ethoxyresorufin metabolism reflected these trends. No age or diet-related changes in hepatic or renal glutathione content, hepatic glutathione-S-transferase, or hepatic microsomal epoxide hydrolase were observed. Renal epoxide hydrolase was slightly reduced in 24 month animals. These data demonstrate that hepatic P450-dependent metabolism is decreased in aging F and SD rats, while renal metabolism is less affected. Dietary restriction, however, has minimal effects on hepatic or renal DME activities.

SALICYLATE-INDUCED NEPHROTOXICITY IN MIDDLE-AGED RATS. PA Glascott, RS Goldstein2 and JJ Kocis1 1Dept. of Pharmacology, Thomas Jefferson Univ., Philadelphia, PA. 2Dept. of Investigative Toxicology, SmithKline & French Laboratories, King of Prussia, PA.

Previous studies suggest an age-dependent increase in acute salicylate-induced nephrotoxicity in middle-aged (12 mo old) rats compared to young adult (3 mo old) rats. These studies were designed to better characterize this age-dependent nephrotoxicity of salicylate. Sexually mature male 3 mo old and retired breeder (9-12 mo old) Sprague-Dawley rats were administered 0, 125, 250 or 500mg/kg sodium salicylate ip and urine was collected for 6hr post treatment. Salicylate appeared to increase total urinary protein, glucose and gamma glutamyl transferase excretion to a greater extent in middle-aged rats than in young adult rats. However, salicylate did not affect accumulation of organic cation or glomerulonephrosis by cortical slices in either age group. These data suggest that salicylate has greater effects on the tubule brush border functions in older rats than in young adult rats.

Age-related nephropathy in rats often makes it difficult to distinguish age-related from drug-related effects in chronic toxicity studies. These studies were designed to evaluate the effects of dietary restriction on lifespan and on the progression of age-related nephropathy. Male SD and F344 rats (2.5 months) were offered: (1) 20% protein, ad libitum (HP), (2) 12% protein, ad libitum (LP) or (3) 20% protein, offered for only 6.5 hrs/day (RES). Food intake, body weight and renal function and morphology were evaluated every 3-6 months through 30 months of age. RES rats consumed = 25-30% less food and weighed = 25-30% less than HP or LP rats. Lifespan of RES rats was greater than that of HP or LP rats; in SD, RES rats, 50% survival was observed at 2.5 yrs of age versus 2 yrs in HP or LP rats. Age-related proteinuria was most pronounced in the HP group and was significantly ameliorated in LP and RES rats. Similarly, the severity of age-related nephropathy was greatest in HP and markedly less in LP and RES rats. These studies demonstrate that dietary restriction improves survival and ameliorates age-related nephropathy in both SD and F344 rats.


A swine model of blood exchange was used to evaluate toxicity of a human hemoglobin (Hgb) cross-linked with bis (3,5-dibromosalicyl) fumarate. Splenectomized animals were exchange perfused with a 14 g/ml solution. Prior to euthanasia, animals were clinically monitored for 7.5 h to 15 days. Extravasated Hgb was first seen in tissues at 7.5 h and persisted for 4 days. Microscopic evaluation of liver and kidney revealed hepatocellular and renal epithelial cell damage by 7.5 h resolving by 15 days. Hepatic centrilobular necrosis was followed by renal nephrosis. Fe3+ staining occurred only at 24 h in liver vascular endothelium and some hepatocytes. Kidneys showed Fe3+ stain in tubular epithelium first at 4 days, with heavy focal localization at 7 days and reduced localization at 15 days. Ultrastructurally, liver endothelium, hepatocytes and renal proximal tubule cells were swollen and contained vacuoles with dense material. Hepatocytes also had swollen endoplasmic reticulum and fibrin-like material. We speculate that the mechanism of damage may depend on formation of Fe3+ mediated free radicals.


Bulk isolation of rabbit PCT and PST was done to investigate mechanisms underlying selective PT injury. Separate dissection, digestion, and Percoll centrifugation of outer cortex and outer medullary stripe provide enriched populations (>95%) of PCT (38 mg; 74% yield) and PST (13 mg; 26% yield) respectively. Both PT segments were enriched in the PT marker leucine aminopeptidase and de-enriched in the distal marker hexokinase (HEX). HEX and lactate dehydrogenase (LDH) activities were 30 and 37% higher in PST vs PCT. Suspensions of PST and PCT were incubated in DMEM/F12 at 37°C in air/CO2 for 1 h. Suspensions were then exposed to N2/CO2 for 40 min and allowed 1 h of recovery. Control PCT had basal O2Q of 38 nmol/min/mg, ATP of 11 nmol and LDH release was <10%. During hypoxia, PCT lost 91% of its ATP and 80% of its LDH. Following recovery, ATP and O2Q were 27 and 32% of control with no change in LDH. In contrast, PST lost less ATP (73%) and LDH (40%) during hypoxia, and recovered better with ATP and O2Q values reaching 55 and 66% of controls. Protective mechanisms in PST were then examined by resuspending PT in buffer with 5 mM glucose as the only carbon source or glucose free buffer for 30 min. In glucose buffer, PCT O2Q and ATP levels were 39 and 55% of control with 21% LDH release. In contrast, PST O2Q and ATP in glucose buffer were 79 and 73% of control with only 8% LDH release. In glucose free buffer PST and PCT responded as PCT did above. These results suggest that PCT with their increased HEX and LDH activities have a greater glycolytic capacity than PCT and thus endure longer periods of hypoxia.

RENAL EFFECTS OF U-69,951 F (LOBENZARIT): IN VIVO AND IN VITRO CHANGES IN CYSTOMOLGUS MONKEY. M O Manis, G A Elliott, K L Feenstra, D A VanderMeer, J K Schlicklin, G L Elfring, and R J Weaver. The Upjohn Co., Kalamazoo, MI.

Lobenzarit is under development as an anti-arthritic agent. Renal hypertrophy and tubular dilatation have been observed in rats. These effects were investigated further in Macaca fascicularis. Monkeys were dosed orally for 30 days with 0, 5, 15, 50, 100, or 150 mg/kg bid. Animals at the highest dose died early in the study; only clinical chemistry results and histology (Histo) were evaluated. For all others uptake of p-aminohippurate (PAH) and tetraethylammonium (TEA) into renal cortical slices was evaluated at necropsy and compared to clinical and Histo data. Slice/medium ratios of PAH and TEA were increased at all doses in both sexes, with a general dose response observed in males but not females. Glucosoneogenesis was increased at all doses in females and at two doses in males. Tubular dilatation was not detected at 10 mg/kg/day but was observed at all higher doses, with 100% being affected at 200 mg/kg/day. Dilatation, casts, and sloughed cells were observed in the animals that died. Water consumption was significantly increased in males at 100 mg/kg/day. BUN and creatinine were elevated at 300 mg/kg/day. Urinary NAG, creatinine, and NAG/creatinine ratios appeared to be unaffected. Thus, changes were observed in functional parameters measured in vitro. These were not directly reflected in the Histo or clinical chemical findings.
709 SUBCHRONIC ORAL AND DERMAL STUDIES OF 2-HYDROXY-4-METHOXYBENZOPHENONE (MOB) IN F344 RATS AND B6C3F1 MICE. MEP Goed, LE Sendelbach, HJ Escher, A Braun and J French. 'NIOSH/NTP, Research Triangle Park, NC and EG&G Mason Research Institute, Worcester, MA.

MOB is used in many products as a sunblock agent. Male and female F344 rats and B6C3F1 mice (10/sex/dose level) were given MOB in the diet for 13 weeks (50,000, 25,000, 12,500, 6250, and 0 ppm) or treated by skin application (200, 100, 50, 25, 12.5, and 0 mg/kg). Significant changes were observed only in dosed rats in feed studies. Gross lesions were urine staining, green urine, enlarged and irregular kidneys, and bladder concretions. Microscopically, 50,000 ppm males and females and 25,000 ppm males had renal tubule dilatation, interstitial nephritis, and papillary necrosis. Lesions were less prominent at lower doses; 12,500 ppm males had focal nephritis. Increased white cell numbers were seen on urinalysis in treated rats. Serum urea nitrogen and creatinine levels were normal. Glomeruli were morphologically normal in rats with renal lesions. Similar lesions are observed with non-steroidal antiinflammatory agents.


Strychnine and its less toxic dimethoxy analog, brucine, are both CNS toxins. Lack of pertinent toxicological information on these two compounds prompted U.S. EPA to conduct subchronic toxicity studies as a part of the RCRA Land Disposal Program. SD rats (20/sex/dose) were gavaged daily with 0, 50, 100, or 150 mg/kg/day brucine and 0, 0.25, 1.0 or 2.5 mg/kg/day strychnine in denitribized water for 30 to 90 days. Ten rats/sex/group were sacrificed after 30 days dosing for interim evaluations; the remaining were dosed for 13 weeks. Dose-related mortality caused by pulmonary congestion and respiratory paralysis was observed in all strychnine exposed rats; other toxicological parameters were unaffected. Dose-related changes, such as, mortality, liver hypertrophy and degeneration were observed in rats exposed to the two higher doses of brucine. At 50 mg/kg/day a trend in body weight reduction; salivation and liver hypertrophy were observed; thus, this dose was the lowest-observed-adverse-effect level for liver effects. Application of an uncertainty factor of 10,000 (10S x 10A x 10H x 10L) yields a reference dose of 5E-3 mg/kg/day for brucine. Mortality precluded derivation of a reference dose for strychnine.


CI-936 (N-(2,2-diphenylethyl)adenosine) has been identified as a potential antipsychotic agent acting via adenosine receptors. CI-936 has been tested for potential toxicity in mice, rats, Beagle dogs, and monkeys. Following single oral doses, median lethal dose values were approximately 10 fold greater in rats than in mice. Clinical signs including depression, prostration and necrosis of the tail were seen in both species. CI-936 was well tolerated up to 40 mg/kg in rats, 12.5 mg/kg in dogs, and 12.5 mg/kg in monkeys for 2 weeks. Depression, necrosis of the tail and death occurred in rats given 120 and 160 mg/kg. Pathologic changes consisted of pancreatitis, gastric ulceration, lymphocyte depletion, and pulmonary congestion at 80 mg/kg or greater. In dogs, emesis was noted at 12.5 mg/kg and greater. Significant pathologic changes included coronary arteritis and lymphocyte depletion at 25 and 50 mg/kg; pancreatic acinar necrosis at 50 mg/kg and renal tubular degeneration at 12.5 mg/kg and greater. Emesis and depression were noted at 25 and 50 mg/kg in monkeys with death at 50 mg/kg. Renal tubular changes were noted at 25 and 50 mg/kg. These studies demonstrated that CI-936 at high doses produced a wide range of adverse effects in different laboratory animal species.


Nerve conduction velocity (NCV) provides a readily accessible means of assessing neurophysiological function in peripheral nerves and has been used by several researchers to investigate the neurotoxic effects of lead in occupationally exposed subjects. Approximately 30 lead-NCV studies have been reported in the published literature, but they vary in methodology, the nerves examined, and their treatment of other variables. This paper critically reviews these studies, noting their strengths and weaknesses and the discrepancies and consistencies in their findings. Although results from different studies are mixed in some respects, lead-exposed workers generally show decreases in nerve conduction velocity, with the most consistent decrement evident in median motor nerve. Statistical meta-analytic methods are used to estimate the size of these effects and to evaluate the blood lead-NCV relationship. The meta-analytic concept of effect size offers an important aid in the synthesis of this literature. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)
Aluminum has been placed on the list of contaminants for possible regulation in drinking water by the U.S. Environmental Protection Agency. Aluminum, a ubiquitous substance, is commonly used in drinking water treatment as a coagulant. Human health risks from exposure to aluminum in drinking water are unclear. Factors that may influence aluminum toxicity include its bioavailability from various media, the chemical species of aluminum and the source of exposure. While aluminum is toxic to persons with impaired renal function, its role in neurological disorders such as Alzheimer's Disease, Parkinson's disease, or Amyotrophic Lateral Sclerosis remains controversial. Recent data from animal and human studies may provide insight into the potential toxicity of aluminum from a drinking water source.

Nickel (Ni) occurs throughout the environment and in many biological systems. Toxic effects including pneumonitis, asthma, central nervous system disorders, and immunological disorders have been reported for humans following exposure to Ni. In certain mining and industrial processes, inhalation is a primary route of exposure. Analyses of systemic toxicity data from human exposure and animal studies were used to derive a quantitative risk assessment for inhalation exposure to Ni. Animals exposed to various Ni compounds or Ni dust exhibited histological alterations of the respiratory tract epithelium, suppressed immune response, and increased mortality following inhalation exposure to <1.0 mg Ni/m^3. Toxic effects varied among species studied. Long-term exposure of humans to 440 µg Ni/m^3 resulted in increased frequency of eye and airway irritations, headaches, and fatigue. Following analysis of the data base, a quantitative risk assessment for inhalation toxicity of Ni was derived.

RISK ASSESSMENT FOR THE RECREATIONAL USE OF AN ARSENIC CONTAMINATED LAKE IN NEW JERSEY. L. Jowa and R. Hazen, New Jersey Department of Environmental Protection (NJDEP), Trenton, NJ.

Arsenic, a known human carcinogen, was detected in significant quantities in the sediments and water of Union Lake, Cumberland County, New Jersey. Exposure of arsenic-containing sediments during the lowering of the water level raised a concern over the potential human toxicity from the continued use of the lake for recreational purposes. A risk assessment was performed by NJDEP which estimated acute exposure to a child for one day, and to an adult for an entire swimming season, using inhalation, dermal, inhalation, and ingestion routes. The NJDEP concluded that a significant health risk was posed to both the adult and child, and suspended recreational use of the lake during the wetdown period. An additional risk assessment was performed to examine the recreational uses of the lake upon its return to normal levels, to take into account more recent sampling data and a new arsenic carcinogenic potency factor developed by the U.S. EPA. Both risk assessments on Union Lake will be presented and compared.

USE OF RISK ASSESSMENT IN THE STATISTICAL DESIGN OF A CARCINOGENICITY BIOASSAY OF ACRYLAMIDE. V. Franko*, I. H. Dulk†, and M. A. Friedman†*Environ Corp., Washington, DC, †American Cyanamid Co., Wayne, NJ.

A carcinogenicity study of acrylamide monomer (Johnson, et al., Toxic Appl. Pharmacol. 1986 82:154-168) was confounded by high tumor incidences in control animals and unusual dose responses for scrotal mesotheliomas and mammary tumors. A new bioassay was designed to 1. Better define the dose response characteristics of tumors seen in male and female rats, 2. Clarify biological significance of CNS tumors and their background variability and 3. Establish if the true NOEL for scrotal mesotheliomas is 0.1 mg/kg/day. Statistical procedures and computer simulations using the Weibull and Multistage models were used to design the new study and evaluate the effect of this new study on risk assessment. Doses were 0, 0.1, 0.5, and 2 mg/kg/day in males and required groups of 284, 284, 182 and 75 animals, respectively. Doses in females were 0, 1, and 3 mg/kg/day and required groups of 100 animals each. Control animals were allocated to two equally sized groups in each study. Results of the new study will be compared to the predicted results.
CARCINOGENIC RISK ASSESSMENTS OF DDT AND ITS CONGENERS. C L Liao, J P Christopher and F Cavender. California Department of Health Services, Toxic Substances Control Division, Sacramento, CA, and Dynacor Corporation, Rockville, MD.

The carcinogenicity of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (DDE), 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDT), 1,1-dichloro-2,2-bis-(p-chlorophenyl) ethene (DDE) was assessed to provide health-based criteria to risk managers at hazardous waste sites. Human epidemiological studies were not adequate for carcinogenicity assessment. However, several animal bioassays have produced evidence of carcinogenicity of DDT, DDE, and DDE via the oral route. There were 10, 3, and 4 positive data sets for DDT, DDE, and DDE, respectively. Following the method of Allen et al. (1987), 95% lower confidence bounds on dose at a risk of 10⁻⁶ were estimated for each positive site using GLOBAL82. From these, the median lower bound was selected for each species. A "bias-correcting conversion factor" of 1.4 (Allen et al., 1987) was applied to extrapolate to humans on a body weight basis. The 95% lower bound on the dose associated with an incremental lifetime risk of 10⁻⁶ for human cancer induced by DDT, DDE, and DDE were estimated to be 1 x 10⁻⁵, 2 x 10⁻⁵, and 2 x 10⁻⁵ mg/kg/day, respectively.


Pentachlorophenol (PCP) has previously been regulated as a noncarcinogen. A recent NTP bioassay has shown that PCP is carcinogenic in B6C3F1 mice. The carcinogenicity of PCP was assessed to provide health-based standards to risk managers at hazardous waste sites in Calif. Using GLOBAL86 and the method of Allen et al. (1987), the 95% lower confidence limit on the dose associated with an incremental lifetime risk of 10⁻⁶ for human cancer induced by PCP was estimated to be 8.9 X 10⁻⁵ mg/kg/day, based on a response of combined hepatocellular adenomas and carcinomas in male mice. California DHS has analyzed the PCP test articles used in the NTP bioassay for 2,3,7,8-chlorinated dioxins and furans to determine the contribution of low levels of these contaminants to the observed tumorigenic response. Comparisons of the tumorigenic activity of the PCP test articles with the tumor sites and tumor rates observed in a NTP bioassay of hexachlorodibenz-p-dioxin in B6C3F1 mice have suggested that all of the observed tumorigenic response may not be attributable to PCP alone.

PHENOL TOXICITY: ISSUES IN RISK ASSESSMENT. R A Hoad. California Dept. of Health Services, Toxic Substances Control Division, Sacramento, CA.

Phenol is rated as a non-carcinogen based on a negative but somewhat equivocal NCI whole-life study in rats and mice. In setting levels for phenol regulation, we wish to examine some basic issues: a) Repeated dermal application of phenol to mice causes skin necrosis followed by both benign and malignant tumors; does this high local-dose treatment by definition exceed the maximum tolerated dose? b) Benzene's carcinogenic effect appears to be mediated through phenol, so is it logical to call benzene a carcinogen but not phenol? c) Does the direct toxicity of phenol simply limit the dose, so tumor incidence is too low to observe in a standard study? d) Is it acceptable to calculate phenol toxicity for drinking water based on gavage studies as the EPA does, when phenol is far less toxic when administered in drinking water? e) Should phenol in potential drinking water be regulated on the basis of toxicity of the more hazardous chlorophenols formed by water chlorination? f) Can it be justifiable to regulate phenol to exposure levels below the amounts synthesized in the body daily? g) Should phenol be regulated not on toxicity, but on the basis of objectionable taste or odor in water? Consideration of these issues leads us to a proposed phenol exposure limit of 10 mg/day, several times the EPA standard.

EVALUATION OF PARATHION AS A POTENTIAL TOXIC AIR CONTAMINANT. D Oudiz, K Klein, K Pfeifer, L Baker, California Department of Food and Agriculture and California Air Resources Board, Sacramento, CA.

The California Department of Food and Agriculture has been mandated to assess pesticides for their potential to be community toxic air contaminants. Parathion was chosen as the first pesticide to be evaluated under this program. In order to assess ambient exposure to parathion, monitoring was conducted at times and sites in California which were believed to have the highest probability of parathion exposures. Ambient levels of parathion were monitored for in the San Joaquin Valley during the winter and in the Imperial Valley during the late summer. The overall mean was 0.170 ug/cu meter, with peak value of 1.423 ug/cu meter, in the San Joaquin Valley and a mean of 0.028 ug/cu meter, with a peak of 0.150 ug/cu meter in the Imperial Valley. Additionally, literature values of immediate offsite data collected during an application were included. These values ranged as high as 34 ug/cu meter during the first 15 minutes of application and 3.09 ug/cu meter during the next two hours. Cholinesterase inhibition was the limiting adverse effect in acute, subchronic, and chronic health effects studies. The NOEIs identified in these studies which were used for the margin of safety calculations were 1.21 mg/cu meter (rat), 0.01 mg/cu meter (dog) and 0.05 mg/kg/d (human), and 0.01 mg/kg/d (dog), respectively. The margins of safety for both adults and children ranged from 12 to 2200.
WATER QUALITY CRITERIA FOR COLORED SMOKES.
K A Davidson, P S Hovatter, and R H Ross.
Health and Safety Research Division,
Oak Ridge National Laboratory,* Oak Ridge, TN.
Sponsor: P Y Lu.

Colored smokes, which include red, violet, green, and yellow, are used by the military for communication and signaling. Some of the dyes that have been used in these smokes are Disperse Red 9 (red and violet), 1,4-diamino-2,3-dihydroanthraquinone (violet), Solvent Yellow 33 (yellow and green), and Solvent Green 3 (green). Two of these dyes, Solvent Yellow 33 and Solvent Green 3, are also approved for use in externally applied drugs and cosmetics and are referred to as D&G Yellow No. 11 and D&G Green No. 6, respectively. A review of mammalian toxicity data is presented in order to assess the quality and quantity of the database for purposes of establishing water quality criteria for the protection of human health. Using USEPA guidelines to calculate a human health criterion, priority is given to human and/or animal carcinogenicity data, which are used to estimate a criterion based on cancer risks of $10^{-7}$, $10^{-6}$, and $10^{-5}$. For noncarcinogens, the criterion, which is based on an acceptable daily intake, is derived from chronic toxicity data.


ASSESSING THE INHALATION CONTRIBUTION TO TOTAL EXPOSURE FOR ORGANIC CHEMICALS IN TAP WATER.

This project addresses the relative importance of household exposure to VOCs transferred from tap water to indoor air, and the implications for risk assessment and the regulatory process. A three-compartment model simulating the 24 hr concentration history of VOCs in the shower, bathroom, and remaining household volumes as a result of tap water use was first developed. Next, a preliminary data base for household parameters was used to derive a range of concentrations and human exposures, with a ratio of inhalation uptake to ingestion uptake calculated in the range of 1 to 6 for VOCs. Experiments were performed to validate this model in an actual dwelling to measure VOCs released from a shower and toilet. VOCs used span a range of diffusion coefficients and Henry's Law constants. The shower and toilet were plumbed both to receive an influent stream carrying VOCs at a constant concentration and so that effluent water could be collected for analysis. Loss of VOCs was calculated from the difference in influent and effluent water. (Funded by the California Department of Health Services, Toxic Substances Control Division.)

APPLIED ACTION LEVELS IN AIR AND WATER FOR TRI-CHLOROBUTYLBENZENE (TCE). J P Christopher.
California Department of Health Services, Toxic Substances Control Division, Sacramento, CA.

Applied Action Levels (AALs) are health-based allowable exposure criteria for use by risk managers at hazardous waste sites. Epidemiological studies are not adequate to estimate the carcinogenic potency of TCE, so chronic bioassays in mice are used: NCI, 1976; NTP, 1983; Fukuda et al., 1983. Metabolized doses of TCE are estimated using kinetic data (Rieben and O'Flaherty, 1985; Frout et al., 1985). Using GLOBAL82 and the method of Allen et al. (1987) with "no averaging" and median 95% lower confidence bound on dose at a reference risk of $10^{-6}$ is estimated to be $6 \times 10^{-4}$ mg/kg/day (Fukuda et al., 1983, female CD-1 mice, lung tumors). Applying a "bias-correcting conversion factor" of 1.4 for extrapolation to humans on a body weight basis (Allen et al., 1987) yields $8 \times 10^{-4}$ mg/kg/day as the 95th lower bound on dose of TCE needed for an incremental lifetime cancer risk in humans of $10^{-6}$. Radon data are used as surrogates for TCE to estimate the exposure occurring via volatilization into indoor atmospheres during domestic uses of water (Andelman, 1983; Frichard and Gesell, 1981; Hess et al., 1983). Total oral and inhalation exposure corresponds to 9 L/day of ingested water for a 70 kg human. AAL_{air} is calculated to be 7 µg/L. AAL_{air} is calculated to be 7 µg/m³ for a 70 kg human breathing 20 m³/day.

APPLIED ACTION LEVELS IN AIR AND WATER FOR CHLOROFORM (CHCl₃). R A Becker and J P Christopher. California Department of Health Services, Toxic Substances Control Division, Sacramento, CA.

Applied Action Levels (AALs) are health-based allowable exposure criteria for use by risk managers at hazardous waste sites. Epidemiological studies are not adequate to estimate the carcinogenic potency of CHCl₃, so chronic bioassays in mice and rats are used: NCI, 1976; Roe et al., 1979; Jorgensen et al., 1979. Although large doses of CHCl₃ were used in some bioassays, no corrections are made for saturation kinetics due to the poor quality of available pharmacokinetic data. Using GLOBAL82 and the "no averaging" method of Allen et al. (1987), 95% lower confidence bounds on dose at reference risks of $10^{-6}$ are estimated for each data set and median lower bounds are determined for each species: mice - $1.3 \times 10^{-4}$ mg/kg/day (Roe et al., 1979, male TCI, kidney neoplasms); rats - $2.6 \times 10^{-4}$ mg/kg/day (NCI, 1976, male Osborne-Mendel, kidney neoplasms). Selecting mice as the median species, a "bias-correcting conversion factor" of 1.4 (Allen et al., 1989) was applied to extrapolate to humans on a body weight basis. The median lower bound on a dose of CHCl₃ associated with a human incremental cancer risk of $10^{-6}$ is $2 \times 10^{-4}$ mg/kg/day. Use of simple exposure scenarios (70 kg human, 2 L/day, 20 m³/day), yields AAL_{air} of 0.6 µg/L and AAL_{air} of 0.6 µg/m³.
Dose rates received by human populations exposed by inhalation to fugitive dusts up to 100 micrometers aerodynamic diameter were computed. The equations used were based on relationships previously validated in adults. The first step was to compute the inspirability (fraction of the aerosol that enters the body) for particles including those often considered too large to be respirable. Next, deposition equations were scaled for age and used to calculate doses to the nasal, tracheobronchial, and alveolar regions. The calculations generally predict greater inhaled doses from fugitive dusts for younger people than for adults. Actual aerosol deposition data in human torso mannequins and in hollow airway models were used to verify the computational model and scaling approach. In these studies monodisperse aerosols were used and variables included particle size, body and airway dimensions and inspired flow rate.

Beryllium is a well recognized human pulmonary toxicant with approximately 900 cases of disease recorded in the beryllium case registry. The respiratory effects may occur as either a nonspecific acute disease characterized by a chemical pneumonitis which may lead to edema and even death or as a more specific chronic disease resulting in dyspnea, cough, and weight loss. Recent investigations have suggested that the current OSHA standard of 2 μg/m³ is not sufficiently protective. Animal studies have shown pulmonary effects from exposures of 35 μg/m³. These data were used as the basis for a quantitative risk assessment of atmospheric beryllium.

INHALED CIGARETTE SMOKE INDUCES DNA ADDUCTS IN RAT LUNGS. J.A. Boyce, B. T. Chen, R. G. Cuddihy, W. C. Griffith, and J. J. Mauerly. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

The purpose of these experiments was to investigate the molecular dosimetry of inhaled cigarette smoke in rat lungs. Male and female Fischer 344 rats were exposed to diluted mainstream smoke from 1R3 research cigarettes smoked in 1/min, 2-sec. 35 ml puffs. Rats were exposed to smoke by nose-only intermittent (NOI), nose-only continuous (NOC), and whole-body continuous (WBC) exposure modes. Additional rats were sham-exposed nose-only (NOS) and whole-body (WBS) to air. All groups received 1200 mg particulate/h/m6 daily 5 days/wk for 4 wk (preceded by one-half concentration for 1 wk). NOI received 10, 10-min exposures at 720 mg/m3 during 6 h/day. NOC and WBC received 200 mg/m3 for 6 h/day. Lung DNA adducts were quantitated by the 32P-postlabeling method. There was a significant increase in levels of adducts in male and female rats exposed to smoke compared to sham-exposed rats. There was no significant effect of exposure mode or sex on DNA adducts. Adduct levels for both sexes combined in NOI, NOC, WBC, NOS, and WBS were 50 ± 4, 52 ± 6, 52 ± 10, and 2 ± 4 adducts per 108 bases, respectively (± SE). These results indicate that inhaled cigarette smoke induces lung DNA adducts which may play a role in cigarette smoke-induced lung carcinogenesis. (Supported by U.S. DOE/HER Conract DE-AC04-76EV01013.)


Cells of the vasculature in normal animals exhibit a remarkably low turnover rate. Proliferative changes in small vessels were assessed by autoradiography and morphometry of lung tissue from mice exposed to chrysotile asbestos for 5 hours. Asbestos inhalation induced 3H-thymidine incorporation by endothelial and smooth muscle cells of small, 30 to 80 um diameter vessels, near the first alveolar duct bifurcations where the most concentrated asbestos deposition and alveolar macrophage accumulation occurs. 3H-thymidine incorporation by cells in both arterioles and venules was markedly increased 19 to 72 hours after asbestos exposure. As many as 20% of vessels in asbestos-exposed mice have labeled cells 24, 31, and 48 hours postexposure. No labeled endothelial or smooth muscle cells have been observed in sham-exposed controls. The time response coincides with that of labeling in bronchiolar-alveolar epithelial and interstitial cells demonstrated by autoradiography and morphometry in previously reported companion studies. We speculate that the proliferative response may be due to the release of mitogenic factors by macrophages attracted to the inhaled asbestos.


Pulmonary platelet sequestration in MCTP-induced pulmonary hypertension was examined using 111In-labeled platelets. Blood platelet number in rats was reduced to 10-25% of the normal level by anti-rat platelet serum (PAS) on days 6-8 after a single administration of 3.5 mg/kg (iv) MCTP. Lung injury, assessed from elevated lung weight and from lavage fluid total protein, an albumin concentrations and lactate dehydrogenase activity, was evident at day 8. Additionally, right ventricular hypertrophy and elevated pulmonary arterial pressure were present. Treatment with PAS did not affect the lung injury but resulted in an attenuation of the pulmonary hypertensive response. Pulmonary platelet sequestration was also reduced by PAS treatment, yet the sequestration in the lungs of MCTP-treated rats was significantly higher than that in the lungs of controls. MCTP-treated rats receiving control serum tended to sequester more 111In-labeled platelets than respective controls, but this was not statistically significant. In a second study, treatment of rats with PAS did not result in an attenuation of the MCTP-induced pulmonary hypertensive response when examined 18 days after MCTP administration. In fact, lung injury was more extensive at this time than at 14 days. These results support the hypothesis that platelets are involved in the mediation of MCTP-induced pulmonary hypertension. (Supported by NRSA HL07701 and NEHS ES02581.)

EFFECT OF CICLOFENAC ON BLEOMYCIN-INDUCED FIBROSIS IN HAMSTERS. D. Chandler, S. Burton D'Souza, P. C. McGaw, Portland, OR, and Univ. of Alberta. Sponsor: S. H. Griffin.

The mechanism of bleomycin (bleo)-induced lung fibrosis is unknown, but prostaglandins (PGs) are thought to modulate the fibrotic process. Therefore, we studied the effect of the non-steroidal anti-inflammatory drug diclofenac (DIC) on lung collagen (COL), DNA, and lipid peroxidation (LP) during bleo-induced fibrosis. Animals received either 1.1 U/ml of saline (saline) intratracheally. Hamsters studied included: 1) a saline control (C) group (gpr) given 0.1 ml saline IM BID (SS); 2) a gpr saline given 100 mcg DIC in 0.1 ml saline IM BID (DS); 3) a bleo gpr given 0.1 ml saline IM BID (SB); 4) a bleo given 100 mcg DIC in 0.1 ml saline IM BID (DB). Animals were sacrificed at 4,7,14, and 21 days. Bleo significantly (sig) increased (INC) lung COL at 7, 14 and 21 days in the SB gpr but at no time point in the DB gpr. SB COL was sig INC above DB COL at 14 and 21 days. LP was sig INC at all times in the SB gpr but only at 4, 7, and 21 days in the DB gpr. SB LP was sig INC above DB gpr at 14 days. Lung DNA content was INC in the SB gpr at 4, 14, and 21 days but only elevated at 7 and 14 days in the DB gpr. SB gpr DNA was sig INC above DB gpr DNA at 4 and 21 days. These data indicate that inhibition of PG synthesis by DIC modulates the development of bleo-induced fibrosis. (Supported by AIA and VA Med Res.)
THIOURASES APPEAR TO PROTECT AGAINST PARAQUAT TOXICITY BY REACTING WITH SULFUR OxIDe AND NOT HYDROXYL RADICALS. NJ Keener, R Bagnell, NM Alexander, University of California San Diego

Thiourea and Dimethylthiourea prevented paraquat toxicity in cell cultures, whereas ten other hydroxyl radical scavengers failed to do so. The protective effect was not due to inhibition of paraquat uptake. Thiourea and Dimethylthiourea competitively reacted with superoxide radical in three different superoxide assays, whereas other hydroxyl scavengers did not. Enzymatic or chemically produced superoxide converted Thiourea to a sulfhydryl compound that reacted with Eilman's reagent. However, oxidation of Thiourea or Dimethylthiourea with hydrogen peroxide failed to convert them to a similar product. Potassium superoxide also converted Thiourea to this sulfhydryl compound. These studies suggest that Thiourea and Dimethylthiourea protect against paraquat cytotoxicity by scavenging superoxide rather than hydroxyl radicals. While Thiourea and Dimethylthiourea have long been considered to be primarily hydroxyl radical scavengers, our studies demonstrate that they also react with hydrogen peroxide and superoxide radicals.

RESPIRATORY TRACT TISSUE CYTOCHROME P-450 ISOZYME-DEPENDENT MONOXYGENASE ACTIVITIES. S A Lacy, D A Thomas, J B Mangum, AND J I Everett. CIT, RTP, NC Sponsor: J A Poppe.

The cytochrome P-450 isozyme complement of respiratory tract tissues may affect site-specific metabolic activation and detoxication of xenobiotics. Alkoxy-O-dealkylase (AOD) activities (pmol/min/mg) using either octoxynol-9 or pentoxyresorufin (PROD) were measured to characterize monooxygenase systems in [1] rat nasal olfactory (off), nasal respiratory (resp), and pulmonary (pulm) microsomal fractions; and [2] sonicates of freshly-isolated rat lung cells enriched for pulmonary endothelial cells, alveolar type II cells or Clara cells. Constitutive off-P-450 content (0.32 pmol/mg) was >4-fold higher in pulm and resp microsomes. Relative AOD activities (PROD/EROD) for pulm, resp and off were 18.1, 21.6 and 1.0, respectively. The highest PROD and EROD specific activities were in pulm (19.9) and off (39.9), respectively. In rats pretreated with β-naphthoflavone (β-NF), pulm, resp, and off PROD were only slightly elevated (<20%), while EROD were increased 9.1-fold, 7.8-fold and 2.1-fold, and relative AOD activities shifted to 1.8, 2.7, and 0.7, respectively. P-450 content (pmol/10⁶ cells) was greater in Clara cells (3.8) than in type II cell (2.6) and endothelial cell (2.0) fractions. Pretreatment with β-NF, which induced pulm P-450 >40%, did not affect spectrally-detectable lung cell P-450. Relative AOD activities (per 10⁶ cells) in Clara cell, type II cell, and endothelial cell fractions were 94.3, 36.9, and 32.6, respectively, while β-NF pretreatment shifted relative AOD activities to 0.5, 0.4, and 1.1, respectively. These data demonstrate tissue- and cell-specific differences in respiratory tract P-450-dependent enzyme activities. β-NF pretreatment modulated individual respiratory tract tissue monooxygenases to comparable PROD/EROD AOD activities.

DEPENDENCE OF CULTURED CELL CYTOXICITY ON THE PHYSICOCHEMICAL PROPERTIES OF BERYLLIUM COMPOUNDS. G L Finch, A L Brooks, M D Roover, W F Lowther and R G Cuddihy. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM.

Beryllium is used in the nuclear, electronics, and aerospace industries. Following deposition in the respiratory tract, beryllium causes both acute and chronic toxic responses. To compare the toxicities of several beryllium compounds, cultured rat alveolar macrophages (FAM), Chinese hamster ovary cells (CHO), and rat lung epithelial cells (LEC) were exposed to beryllium for 20 hours. The beryllium compounds used were Be₂SO₄, BeO prepared at either 500 or 1000°C, or two different sizes of Be metal particles. Specific surface areas of the particles were also measured. Endpoints examined included viability and phagocytic ability of FAM and colony forming ability of CHO and LEC. LEC cultures were more sensitive to beryllium-induced cell killing than CHO or FAM cultures. The order of cytotoxicity of the compounds was Be₂SO₄ > 500°C BeO > 1000°C BeO > Be metal (small size) > Be metal (large size). However, when cytotoxic effects were expressed on the basis of surface rather than mass, the relative differences in toxicity between compounds was decreased. The order of toxicity was Be metal (small) > Be metal (large) > 500°C BeO > 1000°C BeO. These data indicate that for short-term cell toxicity assays, the factors that control beryllium toxicity are related to solubility. (Research supported by the U.S. DOE/OHIER under Contract DE-AC04-76DP001013.)

REPEATED OROPHARYNGEAL NEBULIZATION OF DRUG TO RATS AND DOGS. C F Sabalitis, J K Coombs, and B K J Leong. The Upjohn Co., Kalamazoo, MI.

Piripost Potassium solution, an experimental anti-asthmatic drug, was nebulized oropharyngeally into the lower respiratory tract of anesthetized rats and dogs. The dosages were 0, 2.5, 5 and 10 mg/kg/day for 14 consecutive days. This technique permitted the delivery of a dose equivalent to several hours of an inhalation dose in 5 to 10 minutes. Furthermore, the requirement for a large supply of the drug, as would be required for regular nose-only or chamber inhalation exposures, is eliminated. Depending on the dosage, the rats developed pharmacotoxic signs ranging from lacrimation, nasal discharge, urine stained abdomen, slight ataxia, sneezing, respiratory rales to dyspnea immediately following dosing. Subsequently, the rats receiving >25 mg/kg/day had a significantly reduced body weight gain. For the dogs the immediate responses were occasional coughing, respiratory rales and salivation. Subsequently, an increase in neutrophil count was slight for the male and significant for the female dogs, and a slight weight loss was observed for both sexes receiving >25 mg/kg/day. The histopathologic findings were hypertrophy and proliferation of the alveolar lining cells of the lungs of both species receiving >5 mg/kg/day. The no-adverse-effect-dose of the drug was 2.5 mg/kg/day for both species.

Nicotine content in tobacco smoke is hypothesized to affect elastase-induced emphysema. Animals treated with porcine pancreatic elastase (PPE) were exposed to cigarette smoke with differences in nicotine delivery. Seventy female Long-Evans rats were divided into seven groups: 1) control; 2) low nicotine smoke (KY 2R1, 2.2 mg nicotine); 4) PPE only; 5) PPE + sham-smoke; 6) PPE + 2AI smoke; and 7) PPE + 2R1 smoke. Animals were exposed to 10 puffs (35 ml) daily, 7 days/week for 14 weeks. Smoke exposures began three days after instillation of PPE (400 IU/kg). Post-exposure, pulmonary function tests were performed under anesthesia and lung tissues harvested for quantitative morphometry. Smoke exposure alone had no effects. PPE increased lung volumes, compliance, diminished gas exchange capacity, and increased mean alveolar diameter. Exposure to 2R1 but not 2AI smoke significantly augmented the functional and structural changes produced by PPE. These results suggest that nicotine delivery of cigarette smoke may be an important determinant of its ability to augment emphysematous changes. (Supported by Grant No. 124-05-7K200-4A002 from University of Kentucky Tobacco and Health Research Institute).

DEPLETION OF RAT AND GUINEA PIG TISSUE GLUTATHIONE BY CIGARETTE SMOKE AND DIETHYL MALEATE. M H Billimoria and D J Echtpichon. Pathology Institute and Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, CN.

Glutathione (GSH) levels in tissues of rats and guinea pigs have been measured after exposure to cigarette smoke using the B.-A.T.-Mason inhalation system. Exposure had to be increased to 240 puffs before significant reduction in GSH in the lung, liver and kidney were recorded. GSH levels in these tissues returned to normal within 3 to 6 hours. Smoke exposure at 480 puffs did not result in a further decrease in GSH in rat tissues. Exposure of rats and guinea pigs to sidestream smoke also showed significant reductions in GSH levels in tissues. Most of the GSH-lowering activity was present in the particulate matter, the gas phase showing little, if any, effect on GSH. Smoke exposure on DEM-treated, GSH-depleted rodents did not show an additive effect. These species appear to be well protected against the oxidant damage by tobacco smoke, possibly due to rapid resynthesis of pulmonary GSH and the availability of vitamins and other antioxidants in the animal diet.


Chronic nicotine body burden in rats is hypothesized to predispose the lung to elastolytic insult. An osmotic pump was implanted peritoneally to deliver nicotine (40 mg/kg) for 14 days before an endotracheal instillation of porcine pancreatic elastase (PPE) 400 IU/kg. Fifty-six young adult, female Long-Evans rats were divided into four groups: 1) control; 2) nicotine infusion then endotracheal saline; 3) saline infusion then endotracheal PPE; and 4) nicotine infusion then endotracheal PPE. One week after the instillations, function tests were performed under anesthesia and lung tissues harvested for quantitative morphometry. The Group 2 treatment did not produce significant changes in either function or structure. The Group 3 treatment significantly increased lung volumes, lung compliance as well as diminishing gas exchange capacity. Additionally, a significant increase in mean alveolar diameter was noted. The Group 4 treatment significantly augmented the functional and structural changes produced by PPE. These results suggest that nicotine may be an important determinant in predisposing the lung to elastolytic insult. (Supported by Grant No. 124-05-7K200-4A002 from the University of Kentucky Tobacco and Health Research Institute).


Epidemiological studies suggest an influence of cigarette smoking on the reproductive health of women. To determine if cigarette smoke exposure alters the estrual cycle, female C57Bl/6 mice were exposed twice daily for 60 consecutive weeks to mainstream (MS) and sidestream (SS) smoke from University of Kentucky reference cigarettes in a nose-only exposure system. The vaginal smears were prepared from 12 animals of each group for 12 consecutive days. The animals averaged blood carboxyhemoglobin values of 19.1% and 36.4%, for MS and SS groups, respectively. The smoke particulate intake dose for the MS and SS groups averaged 16.5±2 and 6.5±1 mg/kg body wt/exposure, respectively. These values indicated effective inhalation of smoke by the animals. An evaluation of the vaginal cytology showed that the average estrous cycle length and the relative frequency of different estrous stages in the MS and SS-exposed mice were significantly altered in comparison to room and sham-treated controls. These observations suggest an adverse effect of both types of smoke on estrual cyclicity (KTR8 5-4031).
EFFECTS OF ADRENERGIC ANTAGONISTS ON THE PULMONARY TOXICITY OF COCAINE. D J Murphy, D FrancoCaracco, D A Culp and W D Matthews, Dept. of Investigative Toxicology, Smith Kline & French Lab, King of Prussia, PA.

Cocaine-induced changes in pulmonary function were measured in anesthetized, spontaneously breathing Sprague-Dawley rats using a whole-body plethysmograph and an esophageal manometer. Carotid arterial pressure was simultaneously monitored. A dose of 10 mg/kg of cocaine HCL (i.v.) resulted in death from respiratory failure one to two minutes after dosing. Within two minutes after administering a sublethal dose of cocaine (5 mg/kg), total ventilation (minute volume) was transiently reduced by 45%. No changes in pulmonary resistance or compliance occurred. Pretreatment with the α-antagonist phentolamine (10 mg/kg) prevented the cocaine-induced respiratory depression, while the β-antagonist propranolol (1 mg/kg) potentiated it. At a dose of 0.3 mg/kg, labelatalol, a compound possessing both α- and β-antagonist activity (1:7), potentiated the respiratory depression. At higher doses (10 mg/kg), this effect was no longer evident. Thus, compounds possessing α-antagonist activities could be of therapeutic value in the treatment of cocaine-induced respiratory depression, whereas compounds with β-antagonist properties would be contraindicated.

IN VIVO TOXIC EFFECTS OF AMIODARONE AND DESETHYLMIODARONE IN RATS: ALTERATION OF AMIODARONE UPTAKE BY LUNG. U M Joshi, and H M Mehendale, Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS.

Amiodarone (A) pneumotoxicity in patients receiving this drug is well recognized. We investigated the in vivo effects of A and its major metabolite desethyamiodarone (DEA) in rats and also studied the effects on pulmonary uptake of A. Fisher 344 male rats were given A or DEA (100 mg/kg/day, p.o.) for 2, 7, or 21 days. Food consumption and body weight gain were drastically reduced by both A and DEA. The control rats therefore, were pair-fed. A and DEA increased lung/body weight ratio and number of alveolar macrophages. There was no difference between A and DEA treatments in the above parameters. Mortality was high (100%) in DEA rats by 21 days, while 20-30% of animals died during this period and live rats recovered subsequently despite continuous treatment. 14C-A uptake was increased in A and DEA treated perfused rat lungs, macrophages and surfactant material. Again, there was no significant difference in pulmonary 14C-A uptake between A and DEA-treated rats. These results therefore suggest that DEA is more toxic to rats than A, although the ability to sequester A and other parameters revealed no significant differences between A and DEA (Supported by HL-20622).

DISTRIBUTION OF AMIODARONE IN RAT LUNG. M J Reasor, C L Ogle and P R Miles, West Virginia Univ. Health Sci. Ctr. and ALOSH/NIOSH Morgantown, WV.

Treatment of humans with the antiarrhythmic drug, amiodarone (AD), can cause pulmonary toxicity. To further characterize the response of the lung to AD, the distribution of AD and its principal metabolite, desethylAD, was determined in the lungs of male F-344 rats. AD was given at 150 mg/kg, p.o., for 2 days and 1, 3, and 9 wk. Lungs were lavaged and drug levels were measured in the following compartments: lavaged lung, alveolar macrophages (AMs) and acellular lavage fluid. Proportionally higher accumulation of AD and desethylAD occurred in lavaged lung compared to the AM compartment at all times. The relative amounts present in the AM compartment were highest at 1 wk and decreased thereafter. The AD and metabolite profiles were similar in the 3 compartments. There was no preferential sequestration of one species in any compartment. At 9 wk the ratio of desethylAD to AD was 1.22 in lavaged lung and 1.18 in AM. Less than 1% of either drug was present in the acellular lavage fluid, and all of that was associated with sedimentable lipid. AD and desethylAD were present in type II cells at 2 days (14 and 12 ug/10^7 cells, respectively) and did not change significantly with longer treatment. In contrast, levels of both drugs increased in AMs with increasing treatment time. (Supported by the American Heart Association)

VO_2 MAX DECREASES INVERSELY WITH BLOOD METHEMOGLOBIN CONCENTRATION FOLLOWING THE INHALATION OF NITRIC OXIDE. D M Stefert, C Dingle, P Lehner, Los Alamos National Laboratory, Los Alamos, NM 87545; Walter Reed Army Institute of Research, Washington, D.C.

Nitric oxide (NO) can be inhaled at high mass concentrations in a variety of occupational settings where thermal combustion processes occur. Inhalation of this agent results in the formation of methemoglobin (MetHb), which decreases the oxygen carrying capacity of the blood. In this study, we examined the relationship of endurance, as indexed by VO_2MAX and MetHb concentration [MetHb] following inhalation exposure to NO. Behaviorally and physically conditioned Fischer-344 rats were exposed to 1000 ppm NO for 23 min and exercised on a treadmill contained in a metabolic chamber at 2, 60, 120, and 240 min thereafter. [MetHb] at these post-exposure times were -65, 45, 25, and 10%, respectively. At a [MetHb] of -65%, VO_2MAX was only -55 ml O_2/kg/min, but VO_2MAX increased to within control limits (-95 ml O_2/kg/min) as [MetHb] approached zero %. Overall, VO_2MAX closely scaled inversely with [MetHb], R=0.99, R<0.001. This study demonstrates that: 1) brief exposure to a relatively high concentration of NO can result in marked compromises in endurance, but 2) recovery to normal endurance capacity predictably occurs after MetHb is eliminated.
KINETICS OF METHEMOGLOBIN FORMATION AND 
ELIMINATION AS A FUNCTION OF INHALED NITRIC 
OXIDE CONCENTRATION AND MINUTE VENTILATION. 
G Rippee, T Mundie, D M Stavert, B E 
Lehnert. Walter Reed Army Institute of 
Research, Washington, D.C. 20307; Los Alamos 
National Laboratory, Los Alamos, NM 

We: 1) characterized the kinetics of formation of 
MetHb during the inhalation of NO, and the 
elimination kinetics of MetHb after exposure, and 
2) investigated the kinetics of MetHb formation 
and elimination relative to minute 
ventilation (VE). Fischer-344 rats were exposed 
to 1000 ppm NO for 23 min or 200 ppm NO for 120 
min and blood was sampled for MetHb concen-
trations (MetHb) during and after the exposures. 
The (MetHb) during the 1000 ppm biphase 
reached 55%; reductions in (MetHb) after 
exposure declined monophaseically with a t 1/2 of 
-100 min. The (MetHb) following the 200 ppm 
exposures was -28%, and its accumulation 
was also biphasic. Decreases in (MetHb) after these 
exposures also had a t 1/2 of -100 min. Rats 
were exposed to 5% CO, during and after: 15 min 
exposures to 500 ppm NO. CO increased VE 
1.6-fold; (MetHb) was also increased 1.6-fold 
over the resting VE condition. Increasing VE 
did not affect the removal rate of MetHb. Our 
results indicate: 1) the rate of MetHb formation is 
dependent on inhaled NO concentration, 2) the 
elimination rate of MetHb is independent of 
initial MetHb, 3) MetHb formation during NO 
exposure is directly proportional to VE, and 4) 
MetHb removal is independent of VE.

MECHANISTIC STUDIES OF REDUCTION OF CYANIDE 
(CN) TOXICITY BY ALPHAL-1-KETOCYANURIC ACID (OKG) 
S J Moore and A S Kyne, Dept. of Pharmacol. 
and Toxicol., Univ. of Miss. Med. Ctr., Jackson, MS. 

OKG is an effective antagonist of cyanide-
duced toxicities. Based upon in vitro studies, 
we have hypothesized that these effects are 
due to the ability of OKG to bind the CN 
nucleophile to produce a cyanohydrin, thusly, 
diminishing cyanide distribution to the vital 
tissues. This results in a reduction in the 
CN-induced histotoxic hypoxia. Male ICR mice 
(24-28 g) were injected i.p. with OKG or with 
an equal volume of isotonic saline. Ten 
minutes later the mice were challenged with a 
dose (i.p.) of potassium cyanide (5 mg/kg) 
with a specific activity of 0.2 mCi/kg (CN) 
KRN. The mice were decapitated at specific 
intervals after CN challenge and tissues were 
collected. CN plasma concentrations were signi-
ificantly (p<0.05) greater in the OKG treated 
animals than in the control animals. CN 
concentrations in the brainstem and heart tissue 
of the OKG pretreated mice were significantly 
reduced (p<0.05). Metabolic acidosis, associ-
ated with CN induced histotoxic hypoxia was sig-
ificantly (p<0.05) reduced by OKG pretreat-
ment. These results indicate that the ability of 
OKG to antagonize the toxic effects of 
cyanide is at least partially due to its ability 
to bind and retain CN in the vascular 
system. (USAMRDC contract DAMD 17-85-C-5286).

VENTILATORY CHANGES UPON THE INHALATION OF HIGH 
CONCENTRATIONS OF NITROGEN DIOXIDE. T. Mundie, 
G Rippee, D.M. Stavert, B.E. Lehnert. 
Walter Reed Army Institute of Research, 
Washington, D.C. 20307; Los Alamos National 
Laboratory, Los Alamos, NM 

Information as to how ventilatory patterns are 
altered by exposure to nitrogen dioxide (NO), 
at high concentrations is limited. Accordingly, we 
examined the ventilatory responses to high NO, 
during and after exposure. Fischer-344 rats in 
partial body flow plethysmographs were exposed 
to 100-500 ppm NO, for 1 to 2 min. Ventilatory 
parameters measured included minute ventilation 
(VE), tidal volume (V), breathing frequency 
(f), and inspiratory and expiratory times (T,
T). Only the outcomes from the 500 ppm NO 
exposures will be summarized here. f and V,
rapidly increased and decreased, respectively, 
during the exposures with VE being reduced 
overall. T, and T, also decreased concurrently. 
After the 1 min exposures, VE was slightly 
reduced from pre-exposure values for at least 15 
min; this reduction was due to persistent 
decreases in V,. After the 2 min exposures, VE 
was further reduced to 2/3 pre-exposure values 
and this reduction, which was due to decreases 
in V, persisted for 30 min. The results of this 
study demonstrate that: 1) the inhalation 
of high concentrations of NO, markedly disturb 
normal ventilatory patterns so that a greater 
fraction of VE is delivered to the conducting 
airways, and 2) some ventilatory changes 
after very brief exposures to high NO, can persist 
for a relatively prolonged period of time.

ALTERATION OF RESPIRATORY CYCLE TIMING IN MICE 
EXPOSED TO TRIMELLITIC ANHYDRIDE (TMA) AEROSOLS. 
M Schaper, M Brost, M Stock and Y Alarie. 
University of Pittsburgh, Pittsburgh, PA.

Groups of 4 non-cannulated mice were exposed 
to TMA aerosols for 30 minutes. No sensory irrita-
tion was induced in animals exposed to TMA aero-
sols. However, these animals exhibited a bre-
thathing pattern characterized by a pause (i.e., 
napelc period) between breaths. Such pauses were 
not observed during the respiratory cycle of 
control mice. This effect was seen within several 
minutes of exposure to TMA aerosols and persis-
ted throughout the exposure and often for hours 
post-exposure. The length of such pauses (TP) 
increased as TMA exposure concentration was 
raised. In contrast, the time of inspiration (TI) 
and expiration (TE) decreased as TMA exposure 
concentration was raised. These modifications 
in respiratory cycle timing resulted in changes 
in respiratory frequency (F). Increases in F 
occurred when brief pauses were seen and decrea-
ses in F occurred when longer pauses were seen. 
Groups of tracheally-cannulated mice were also 
exposed to TMA aerosols. Similar results were 
obtained in cannulated vs. non-cannulated ani-
mals. These changes in respiratory cycle timing 
were probably due to a local effect of TMA aer-
sols on vagal nerve endings in the lower respira-
tory tract. Supported by NIEHS grant ROI- 
ES02747.
749 BRONCHOCONSTRICTION IN GUINEA PIGS INDUCED BY REPEATED EXPOSURE TO TRIMELLITIC ANHYDRIDE (TMA) AEROSOLS. M Brost, M Stock, M Schaper, and Y Alarie. University of Pittsburgh, Pittsburgh, PA.

Guinea pigs were exposed to aerosols of TMA on days 1, 5, 19 and every 2 weeks thereafter or using similar protocols. On days 1 and 5, sensory irritation was present within a few seconds of exposure to TMA and was followed by coughing in all animals. These faded within a few minutes as the exposure continued for 30 minutes. On day 19 and upon further challenges at 2 week intervals, the same reactions were also seen. However, by 10 minutes of exposure, bronchoconstriction began to develop and intensified to the point of collapse (in some animals) and often persisted for hours post-exposure. In other groups of guinea pigs similarly sensitized and challenged, the intense bronchoconstriction seen on the challenge day diminished with daily challenges to the point where no reaction occurred by the 5th consecutive challenge day. We also monitored sensitized animals for the possibility of a second reaction between 6 and 12 hours post-exposure and at 24 hours post-exposure. There was no evidence of a reaction at this time. These results are the first demonstration of an immediate immunologically-mediated asthmatic reaction; induced by a small molecular weight organic chemical to both sensitize and challenge guinea pigs. Supported by NIH grant ROI-ES02747.

750 CYCLOPENTADIENYL MANGANESE TRICARBONYL (CMT)-INDUCED ELEVATION IN PULMONARY NONPROTEIN SULPHHYDRAZ (NPSH) LEVELS. R J Clay and J B Morcia, Toxicology Program, University of Connecticut, Storrs, CT.

Organomanganese compounds are used as fuel additives and are of toxicologic interest because they are selectively pneumotoxic. Pilot studies revealed dramatic CMT-induced elevations in pulmonary NPSH levels. The dose-dependence and temporal relationships of this effect were assessed in the Sprague-Dawley rat after s.c. injection of propylene glycol vehicle or CMT. Pulmonary NPSH levels were increased in a dose-dependent manner 24 hr after administration of 0.1-1.0 mg Mn/kg as CMT; maximal NPSH levels were 200-250% of control. NPSH levels were not depleted below control levels at any time. CMT was pneumotoxic, pulmonary lavage albumin was elevated 6 hr after dosing. Overt cytotoxicity (enzyme leakage) was not evident until 48 hr. Similarly, overt alveolar inflammation, as measured by lavage DNA content did not occur until 48 hr. The elevation in NPSH levels may reflect a primary response to CMT (or its metabolites) or may represent a secondary response associated with early changes in the inflammatory process. (Supported in part by NIH grant ES07163.)

751 BRONCHOCONSTRICTION IN GUINEA PIGS REPEATEDLY EXPOSED TO OVALBUMIN (OA) AEROSOLS AND ITS PREVENTION USING VERAPAMIL. K Dietzler, R D Thompson, M Schaper and Y Alarie. University of Pittsburgh, Pittsburgh, PA.

A group of 4 guinea pigs was exposed to OA aerosol for 10 minutes per day for 5 consecutive days and challenged with the same aerosol for 20 minutes every 2 weeks for 1 year. During the first 5 days of OA exposure, no changes in respiration occurred. On day 19 and after, animals bronchoconstricted after about 10 minutes of OA exposure. Reactions were often severe by the end of the challenge. The number of guinea pigs that reacted to OA increased with repeated challenges, with 2/4 reacting on initial challenges and 3/4 or 4/4 reacting after numerous challenges. Also, the intensity and duration of reaction increased in these animals. On several occasions, animals were followed for 12-24 hours post-exposure to determine if there was a second reaction; although none occurred. Finally, after months of repeated OA challenges, animals were pretreated with verapamil (aerosol or i.p. injection) and immediately challenged with OA. Little reaction was seen. However, a large reaction occurred 2 weeks later when no verapamil was administered. This data indicates the importance of conducting challenges beyond the conventional 14-21 day period after sensitization and demonstrates an approach to evaluate anti-asthmatic drugs. Supported by NIH grant ROI-ES02747.

752 REDUCTION OF EXERCISE PERFORMANCE IN GUINEA PIGS BY HISTAMINE AEROSOLS. M Iwasaki and Y Alarie. University of Pittsburgh, Pittsburgh, PA.

An ergometer was used within a whole body plethysmograph. Respiratory frequency (f), pressure change in the ergometer (ΔP) which is proportional to tidal volume (VT), and oxygen uptake (VO2) were continuously measured prior to and during exercise. The level of exercise was maintained at 26 mJ/min of running speed which resulted in a 3 times increase in f, little change in ΔP and 2.5 times increase in VO2 above resting conditions. Five guinea pigs were trained to run for a period of 40 min with clean air flowing through the ergometer. These animals were then exposed to aerosol of histamine, while running, at concentrations of 0.32 to 1.0 mg/m3 (size: 85% of particles<0.5 um MMD). From these experiments the exposure concentration of histamine which reduced the running time by 50% of a planned 30 min exercise was found to be 0.6 mg/m3 and a no-effect level was extrapolated to be about 0.35 mg/m3. In sedentary conditions the concentration which induced obvious bronchoconstriction in the same animals was 0.72 mg/m3 while it was found to be 0.52 mg/m3 under exercise. Thus we conclude that the impairment in running performance was due to this effect of histamine. The system used can begin to help define the extent of performance decrement associated with airways constriction. Supported under NIH grant ROI-ES02747.
The levels of ascorbic acid (AAH), uric acid (UA), glutathione (GSH), and alpha-tocopherol (AT) in nasal lavage (NL) and bronchoalveolar lavage (BAL) samples from humans were compared with those of rats and guinea pigs. Major differences were as follows. NL: humans had higher (>160x) UA/protein ratios than rats or guinea pigs, but only about 0.15 as much AAH. BAL cell-free fraction: humans had the highest AAH/protein values (measured from 2.5 to 4x those of the other species) and the lowest AAH levels (0.1 as much as rats). Humans had much higher AT/lipid phosphorous (LP) ratios in this fraction. BAL cells: humans had the lowest levels of all 4 antioxidants - AAH/protein was 0.66 to 0.2 that of rats and guinea pigs; GSH/protein was 0.1 to 0.2 as high, and AT/LP was 0.2 that in the other species. UA/protein in humans was 0.1 as high as in the other species and was only about 0.003 that found in human nasal lavage and BAL cell-free fraction. These data indicate generally lower levels of antioxidants in human NL and BAL as compared to rats and guinea pigs. These differences suggest that the human lung and nasal tissues may be expected to be more sensitive to the action of some inhaled oxidants. (This abstract does not necessarily reflect EPA policy.)
The long-term pulmonary effects induced by a single, sublethal dose of OCS-IMP, an impurity present in organophosphate insecticides, was examined in female C57BL/Ka mice. Animals received either vehicle or 30 mg OCS-IMP/kg body weight i.p. and were studied at the following time periods: 10 d, 30 d, 90 d, 6 mos and 1 yr. No animal died spontaneously. Significant increases in wet and dry lung weights occurred at 10-90 d in OCS-IMP treated mice. No difference was observed in percent lung water content. The predominant morphologic change was hypertrrophy and hyperplasia of type II alveolar epithelial cells with changes in the size and number of osmiophilic lamellar bodies. Interstitial morphologic changes were characterized by increased numbers of fibroblasts, increased amounts of collagen fibrils, and basement membrane alterations. These changes were accompanied by a significant increase in pulmonary hydroxyproline content at 10-30 d. The results of this study indicate that a single, sublethal dose of OCS-IMP induces intermediate-term structural and biochemical changes in the mouse lung, but that the murine lung is able to adequately repair.

The distribution of radiolabeled 3,4,3′,4′-tetrachlorobiphenyl (TCB) and induced effects on serum and lung retinoid content, and lung morphology was studied by LSC, HPLC, light microscopic autoradiography and transmission electron microscopy. Adult female C57BL/6J rats received either vehicle, 15 mg TCB or 200 mg (1.85 μCi) H-TCB/kg body weight i.p. and were killed at 1, 3, 7, and 14 days after treatment. TCB treatment resulted in a significant decrease in serum retinol without an associated reduction in lung retinol or retinyl palmitate content. There was a continuous increase in lung radioactivity over time. The majority of H-TCB in the lung was metabolized at all times. There was a selective distribution of radiolabel to the Clara cell, and to a lesser extent, the type II alveolar epithelial cell. The most pronounced morphologic alterations occurred in type II alveolar epithelial cells, and included changes in the number and size of osmiophilic lamellar bodies. These results indicate that the lung is a target organ of TCB intoxication and that there is a selective accumulation of TCB metabolites in the lung.
761 IN VITRO INHIBITION OF HEPATIC GLUTATHIONE S-TRANS-FERASES (GST) BY THE WATER-EXCRETED, Haloaceto-nitriles. J. C. Ich, S. T. Soliman, and A. E. Ahmed, Department of Pathology, University of Texas Medical Branch, Galveston, TX.

The objective of this study is to investigate in vitro the biochemical mechanisms of inhibitory effect of haloacetonitrile (HAN) on hepatic GST activity using GFP as substrate. Increasing concentrations of acetonitrile, monochloroacetonitrile (MCN), dichloroacetonitrile (DCN), trichloroacetonitrile (TCN), dibromoacetonitrile (DBN), and monochloroacetonitrile (MCN) were potent inhibitors in 50 % values 2.486, 0.343, 0.832 and 4.44 nM, respectively. At corresponding concentrations of their IC50 values for MCN, DCN, and DBN produced a decrease in Ks and Vmax, the enzyme activity (20-40% of the control) toward GST. TCN increased Ks and Vmax for GSH to 522% and 120% of the control, respectively. At concentrations equal to 0.5, 1, or 2 x IC50, MCN, DCN, DBN, and TCN tended to decrease Vmax and to increase Ks of GST toward GSH. The inhibitory activities of these four compounds are of a mixed type. The inhibitory effect of HAN is reversible. Our results indicate the inhibition of GST activity by the drinking water disinfectant, HAN. This effect may lead to decreased detoxification of other potentially reactive chemicals, thus increased incidence of cancer and injury. (Supported by NIH grant No. ES 01871).

763 RESPONSE OF HAMSTER LIVER MIXED FUNCTION OXIDASE (MFO) TO GLUTARAZ (OTP). M. A. Merrick, J. K. Sellick, M. Heidrick, D. Cook and R. G. Scholl, SC Johnson & Son, Inc., Racine, WI. Calcium, HCL, Cincinnati, OH; NIH ES 01871; FTP, NC, UCNEV Medical Ctr, Omaha, NE and ND Dakota State Univ, Fargo, ND.

OTP (5-(2-pyrazinyl)-4-methyl-1, 2-dithiole-3-thione) has been reported to ameliorate acute hepatotoxicity resulting from acetaminophen, allyl alcohol, aflatoxin or carbon tetrachloride. Metabolic elimination, via MFO, is central to each agent except allyl alcohol. These studies addressed the influence of OTP (2.0 mmole/kg, po) on in vitro MFO when given 48 hr prior to isolation of liver microsomes. SDS-PAGE suggested enhanced, as well as, additional bands in migration ranges (MW: 40-56k) of MFO-associated proteins for OTP-treated groups. However, spectrophotometrically measurable cytochrome P450 contents were similar. Of MFO determined (hydroxylation and N- and O-dealkylations), only aminoptyrine N-demethylase (143%) was different than control. Total benzo[a]pyrene (BaP) metabolism was unchanged; however, metabolite patterns were shifted by OTP. Production of BaP-7, 8-diol decreased, while BaP-6, 7-diol increased. Neither BaP, 10-diol, nor BaP-quinone appearances were altered. These data indicate hamster liver MFO undergoes modest but subtle changes in response to OTP. (Supported by a Sandusky Institute Fellowship (MID) and a Burroughs-Wellcome Toxicology Award (RCS)).


Chlorodecone (CD) amplifies the hepatoxic and lethal effects of CCl4. Since CCl4 is toxic by virtue of its bioactivation by the hepatomicrosomal cytochrome P-450 (P-450) system, which is in turn destroyed, it was of interest to determine if specific forms of P-450 were destroyed in this interaction. Hepatic microsomes from variously treated male rats were used. The treatments were CD alone (10 ppm, diet, 15 days), CCl4 alone, CD + CCl4 and with or without CoCl2 treatment on days 13 and 14 of the protocol & control receiving normal diet and corn oil. Solubilized microsomes were resolved into five peaks by HPLC. P-450 and cytochrome c reductase of each peak were determined. In CD pretreatment there was slight increase in the peak heights whereas peak heights in CCl4 treatment were similar to control. CoCl2 caused nonuniform decreases in the magnitude of all peaks, decrease of two peaks being maximal. However, in CD + CCl4 treatment absence of two peaks and decrease in the other three peaks were noticed. Microsomal proteins separated by SDS-PAGE also showed decreased staining intensity of hemoprotein bands in CD + CCl4 interaction. CoCl2 caused a nonuniform decrease of P-450. CD + CCl4 selectively decreased P-450 subpopulation. (EPA and Medical Research Command, Wright Patterson AFB, CR 814053; abstract does not reflect EPA policy)

764 EFFECT OF 2-THIOTRIAZONE (TTZ) AND DIETHYL-MALEATE (DEM)-TTZ COMBINATION ON HEPATIC MIXED FUNCTION MONOXYGENASE SYSTEMS OF RATS. M. M. Tate, W. Flory, Dept. Phys., Ph. and Toxicol. School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA.

2-Thiottiazione is a thiourea derivative that was found to be acutely toxic in adult male rats. Previous studies have shown that there are marked differences in susceptibility in female and immature rats to TTZ. Sprague Dawley rats (adult and immature) were dosed ip with a toxic dose of TTZ (10 mg/kg) or dosed with DEM (0.1 ml/kg) 30 minutes prior to dosing with TTZ (10 mg/kg). The hepatic mixed function monoxygenase systems of control and treated animals were characterized by the following: Cytochrome P-450 content, cytochrome b5 content, NADH and NADPH cytochrome-C-reductase activities, GSH activity, UDPG activity, and four cytochrome P-450 specific activities (EROD, ECOQ, APNO and AE). There were significant increases in cytochrome P-450 and NADPH cytochrome-C-reductase activity in TTZ dosed female rats. All other activities in TTZ treated animals were decreased or were unaffected. All enzyme activities in rats dosed with DEM-TTZ combination were significantly decreased except for a slight increase in GSH activities in adult and immature male rats. Results from this study show that TTZ and DEM-TTZ combination causes a decrease in biotransformation.

We have reported that deacetylated SPL (an antimineralocorticoid) selectively inactivates rat hepatic cytochrome P-450 [dexamethasone(DEX)-inducible isozymes] in a suicidal process. Hemolysis is lost stoichiometrically and it appears that ~30% of this hemolysis is found covalently bound to microsomal proteins. SPL metabolism in vitro was examined to elucidate the mechanism of such inactivation. Two major polar metabolites (separated by HPLC with UV detection) have been identified from aerobic incubations of SPL and NADPH with microsomes prepared from livers of DEX-pretreated rats or human adrenal glands. Metabolite formation was found to be not only O2, NADPH, and SPL concentration dependent, but also attenuated by the P-450 inhibitors N-oxycyamidine and CO. GSH was found to inhibit both SPL-mediated P-450 inactivation and metabolite formation. FAB/MS and Fab/MS/MS analyses of these metabolites (purified by HPLC) have shown intense ions at m/z 405(M-H) and m/z 421(M-H) which correspond to the sulfonic acid/sulfonic acid derivatives of SPL, respectively. The authenticity of the sulfonic acid was confirmed by MS comparison with the chemically synthesized compound. The detection of these metabolites indicate the oxidation of the sulphydryl moiety of deacetylated SPL by P-450. These findings implicate the formation of highly reactive thyl radicals/sulfonic acid derivatives of SPL by rat hepatic and human adrenal P-450s, which may be responsible for the unusual and selective mode of SPL-mediated inactivation of these particular isozymes. Supported by NIH grant DK26508 & SOT Hazleton graduate student award.

SELECTIVE INHIBITION OF CYTOCHROME P-450 ISOZYMES BY THE HERBICIDE SYNERGIST TRIDIPHANE. P E Levey, D E Moreland, W P Novitzky, and E Hodgson. Toxicology Program, North Carolina State University, Raleigh, NC.

Tridiphane is an experimental herbicide known to synergize atrazine toxicity in plants by inhibiting glutathione conjugation of atrazine (a detoxication reaction). It is also important, however, to look at the interactions of this compound with enzymes of mammalian species because of potential exposure of this compound in agriculture. In vitro examination of hepatic microsomal monooxygenase activities in untreated, phenobarbital (PB) treated, and 3-methylcholanthrene (MC) treated mice showed moderate inhibition of monooxygenase activities in untreated microsomes, significant inhibition in PB-induced microsomes, and very little inhibition in MC-induced microsomes. PB microsomes readily gave a type I binding spectrum with a Ks of 1.4 uM; no spectrum, however, was obtained with MC microsomes. Use of purified isozymes and reconstituted monooxygenase activity confirmed the selectivity of tridiphane inhibition with P-450 PB.

ENZYME INDUCTION DEFECT TO PHENOBARBITAL IN THE fa/fa ZUCKER RAT: RELATION BETWEEN CYTOCHROME P-450 AND UDPGT. I Chaudhary, L Robertson, and A Blausin. Graduate Center for Toxicology and College of Pharmacy, University of Kentucky, Lexington, KY.

The hepatic cytochrome P-450 in the fa/fa (obese) Zucker rat is not inducible by phenobarbital (PB). The purpose of this study was to determine if PB-inducible isozymes of cyt P-450 and UDP-glucuronosyl transferase (GT) are under similar regulatory control. PB was administered orally to lean (LZ) and obese (OZ) rats. The microsomes from livers of 6 control and induced LZ and OZ rats were prepared and analyzed for total cyt P-450 and GT activity (nmol/mg protein) using 3H-morphine as substrate. PB-treatment significantly increased total cyt P-450 in LZ induced vs control (1.08 ± 0.02 vs 0.44 ± 0.06) but no change was observed in OZ induced vs control (0.42 ± 0.09 vs 0.33 ± 0.07). Morphine-GT activity was induced in LZ rats vs control (31.61 ± 3.9 vs 13.72 ± 1.0) but no induction effect was observed in obese induced vs control (15.91 ± 4.7 vs 12 ± 2.7). Results from this study indicates that obese rats are not inducible by PB and suggest that cyt P-450 and morphine-GT may be similarly regulated in this model.
THE ALCOHOL-INDUCIBLE FORM OF CYTOCHROME P-450 IS A HIGH AFFINITY PYRIDINE N-OXIDASE. S G Kim and B P Howes. Institute of Chemical Toxicology, Wayne State University, Detroit, MI.

Pyridine (PY), prototypic of several volatile nitrogenous bases, induces cytochromes P-450 in both rats and rabbits. Recent experiments provide evidence for the role of P-450LM9 in PY N-oxide production. PY-induced rabbit hepatic microsomes exhibited a single Km value of 81 μM with an 8-fold increase in V_max relative to controls. In contrast, uninduced and isosafrole (ISF)-induced microsomes showed biphasic kinetics. Uninduced microsomes gave Km values of 85 and 973 μM and a V_max of 0.95 nmol PY N-oxide/min/mg protein, whereas ISF-induced microsomes yielded Km values of 230 and 1700 μM, with a V_max of 0.67 nmol PY N-oxide/min/mg protein. Phenobarbital induction resulted in a single high Km (949 μM) enzyme; V_max was increased by 3.5-fold over control. When rates were normalized for P-430 content, PY- and PB-induced microsomes gave a 4- and 1.4-fold increase in PY N-oxide production. p-Nitrophenol competitively inhibited (K_i = 13 μM) the production of PY N-oxide in PY-induced microsomes. PB-induced P-450LM9 was purified to homogeneity and, in the reconstituted system, was 6 to 8-fold more active than P-450LM2 and 15-fold more active than P-450LM4 in producing PY N-oxide. The results suggest that the alcohol-inducible form of P-450 is a high affinity catalyst of PY N-oxide production. Supported by NIH ES03656.

IMPRINTING OF HEPATIC P450 IN ADULT MALE RATS FOLLOWING NEONATAL EXPOSURE TO 2,4,5,3',4',5'-HEXACHLOROBIPHENYL (HCB) VIA MATERNAL MILK. B J Pavia, P E Thomas, M J Vodonaik and S A Wightgon. Medical College of WI, Milwaukee WI and Rutgers, Piscataway, NJ.

To determine whether neonatal exposure to HCB results in the imprinting of the adult hepatic P450s, pregnant rats were injected ip on d14 gestation (G) with corn oil (CO) or 100 mg HCB/kg, a PCB congener which causes a mixed-type hepatic P450 induction. Offspring were sacrificed on d19G, d4 (d1 of birth), d15 and d25. Hepatic microsomes were assayed for P450 content and activities associated with P450c (e.g. ethoxyresorufin-O-deethylation, EROD) and P450b+e (benzphetamine-N-demethylation, BeND). No differences were seen on d19G between offspring of CO and treated (T) dams. Significant increases were seen in the T group in EROD and BeND activities and P450 content on d4 and d15 (EROD > 200-fold; BeND > 6-fold; P450 > 2.5-fold). Immunoblot and testosterone metabolite profiles also indicated that P450 p, b+e and c+e were induced at d4 and d15, but not at d19G. These data indicate the transfer of HCB from the mother to the offspring was via milk and not via the placenta. EROD activity and P450 content were not different between the adult male CO and T groups while BeND and testosterone 16β-hydroxylase activities were significantly higher in the T group than the CO group (1.36- and 3.6-fold, respectively). Immunoguaniation of the levels of P450 b+e, c+e and p demonstrated that only the levels of P450b+e were elevated in the T adult group (3-fold). The data indicate that despite using a mixed-type inducer, HCB only imprints P450b+e in the adult following neonatal exposure via mother's milk.

CYTOCHROME P450 AND P448 DEPENDENT METABOLISM IN RAT, MOUSE, MONKEY, AND HUMAN TESTIS. K W DiBiasio, C D Brown, D W Wilson, C G Plopper, M G Miller, and L R Shull. Dept's of Environmental Toxicology and Veterinary Pathology, University of California, Davis, CA.

Since testicular venoceptive metabolism is poorly understood, cytochrome (c) P450 and P448 dependent metabolism was studied in human, monkey, rat, and mouse testicular microsomes. 0-Dealkylation of pentoxyresorufin (PBR) (cP450 dependent) and ethoxyresorufin (ETR) (cP448 dependent) were measured fluorometrically. Rat ETR activity was 10-300 fold greater than mouse. However, PBR activity was not detected in either rat or mouse. Similarly, neither PBR nor ETR activity was detected in human or monkey (minimum detection levels = 1.25 and 0.080 pmole/min/mg, respectively). Administration of inducing agents phenobarbital (PB) and B-naphthoflavone (BNF) to rats and mice markedly increased liver PBR and ETR activities. In the testis, BNF induced mouse ETR activity (2X), but not rat. PBR activity in rat and mouse testis was still below the minimum detection level after PB treatment. Immunocytochemical analysis of testes from arcoleor induced rat revealed an antibody reaction with a PB-type isozyme. Arocleor induced rat and mouse testis are being compared immunocytochemically. The data indicate that cP450 and cP448 metabolism in the testis is very low and minimally affected by inducing agents.

SUBSTRATE SPECIFICITY AND REGEOSELECTIVITY OF THE CYTOCHROME P450 IN THE C3H/10T1/2 CELL LINE. L H Pottinger and C W Jeffcoat. Environmental Toxicology Center, University of Wisconsin, Madison, WI.

The transformable cell line C3H/10T1/2 contains a P450-inducible P450-metabolizing cytochrome P450. Investigation of its substrate specificity and regioselectivity indicates that this isozyme is distinct from P445. Metabolite profiles for a number of P450 substrates from 10T1/2 microsomes were compared with those for Nipa 1 microsomes which contain P445. Examination of metabolite profiles for the P450 substrates 7,12-dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene (BP) showed that 10T1/2 microsomes produce the proximate carcinogens BP-7,8-diol and DMBA-3,4-diol as major metabolites but no BP-4,5-diol or 7-HOMBA. Parallel studies with Nipa 1 microsomes resulted in completely different metabolite profiles including the production of BP-4,5-diol and 7-HOMBA but no DMBA-3,4-diol. Preliminary results from 10T1/2 microsomal metabolism of aflatoxin B1 (AFB1) showed production of AFB1 and AFB2 in approximately equal proportions while P445 produced only AFB2. Testosterone metabolism by 10T1/2 microsomes also resulted in a non-P4-450 metabolite profile. These results demonstrate that the substrate specificity and regioselectivity of the 10T1/2 P450 is distinct from that of P445.
DIFFERENCES IN CYTOCHROME P-450 MONOOXYGENASE SYSTEM IN STRAINS OF HELIOTHIS VIRESCENS RESISTANT TO ORGANOPHOSPHATE INSECTICIDES AND THE SECONDARY PLANT CHEMICALS, QUERCETIN AND 2-TRIDECANONE. R L Rose, P E Levi, and E Hodgson. Toxicology Program, N.C. State Univ., Raleigh, NC

Differences in P-450 isozymes in strains of the tobacco budworm, Heliotis virescens (F) resistant to organophosphate insecticides and the host plant allelochemicals, quercetin and 2-tridecanone were investigated using a variety of monooxygenase substrates. Relative to the susceptible strain, no differences in specific content of P-450, as determined from the dimethoate CO difference spectra, were noted for the organophosphate or quercetin resistant strains. Organophosphate resistance did not increase enzyme activity toward the substrates examined including p-nitroanisole, methoxyresorufin, phorate, lauric acid, benzphetamine, and benzo(a)pyrene. These results contrasted with those of larvae resistant to quercetin for which a 3-fold increase in activity was observed for hydroxylation of benzo(a)pyrene and 7-methoxycoumarin. Activity toward phorate and O-demethylation of p-nitroanisole and methoxyresorufin. Treatment of H. virescens with 2-tridecanone increases P-450 content and shifts the wavelength of the CO spectrum indicating qualitative changes in P-450 isozymes.

PURIFICATION AND CHARACTERIZATION OF CYTOCHROME P-450 ISOZYMES FROM PHENOBARBITAL-INDUCED CHICKEN LIVER. R P Gupta, D M Ladapula, M B Abou-Doria. Department of Pharmacology, Duke University Medical Center, Durham, NC.

A variety of environmental pollutants and drugs are metabolized by a mixed function oxidase system consisting of cytochrome P-450 and NADPH-cytochrome P-450 reductase. We have purified four cytochrome P-450 isozymes (P-450 PB-B1, PB-B2, PB-D, and PB-K) from chicken liver and characterized them by column chromatography, polyacrylamide gel electrophoresis, spectral properties, Ouchterlony double diffusion analysis, immunoblotting, peptide mapping, fingerprints of [14C]-labeled tryptic peptides and substrate specificity. The oxidized cytochrome P-450s and their ferrous-CO complex exhibited peaks at 412-413 nm and 447-448 nm, respectively. The catalytic activity of P-450 isozymes was examined toward 7-ethoxycoumarin, benzphetamine, aminopyrine, benzo(a)pyrene and 7-methoxycoumarin. The cytochrome P-450 PB-K was catalytically the most active, and immunologically different from the other three isozymes. The later three isozymes could be distinguished from each other on the basis of column chromatographic behaviour during purification, spectral properties and to some extent by peptidylase activity used in studying the metabolism of various toxic chemicals in this species. (Supported in part by NIOSH Grant No. OH00823)

CYTOCHROME P-450 INDUCTION IN CHICKENS EXPOSED SIMULTANEOUSLY TO N-HEXANE AND METHYL ISO-BUTYL KETONE, C Habig, M B Abou-Doria, and D M Ladapula. Department of Pharmacology, Duke University Medical Center, Durham, NC.

The industrial solvent n-hexane produces distal axonopathy in humans and laboratory animals, with the metabolite 2,5-hexadiene (2,5-HD) considered the active agent. Another industrial solvent, methyl isobutyl ketone (MIBK), while not neurotoxic, has been shown to exert a strong synergistic effect on n-hexane induced neuropathy. Chickens (Gallus gallus domesticus) were exposed to 1,000 ppm n-hexane combined with 10, 100, 250, 500, or 1,000 ppm MIBK in inhalation chambers. Other received 1,000 ppm n-hexane, 1,000 ppm MIBK, or air. Following 29 day exposures, animals were assayed for hepatic cytochrome P-450 content, benzphetamine N-demethylase (B2D), ethoxyresoruvin O-deethylation (EROD), cytochrome P-450 reductase, and spinal cord neurofilament cross-linking. Higher dose groups (1,000 ppm n-hexane with 1,000, 500, or 250 ppm MIBK) showed dose-dependent weight loss and clinical signs of neurotoxicity. Significant reductions of P-450 (220-75%) and B2D (190-60%) but not EROD or P-450 reductase were found at all MIBK exposures, increasing in a dose-dependent manner with increased neurofilament cross-linking MIBK’s synergistic effect may result from induction of P-450 type isozymes that may increase oxidative metabolism of n-hexane to 2,5-HD. (Supported by NIOSH Grant No. OH00823)

EFFECTS OF ACRYLAMIDE ON THE HEPATIC MONOOXYGENASE INDUCTIVE RESPONSE OF RAINBOW TROUT. M L Haasch, P J Wejksnora and J J Loeb. Medical College of Wisconsin, Center for Great Lakes Studies, Univ. of Wisconsin-Milwaukee, Milwaukee, WI.

Previous studies have suggested a selective inactivation or inhibition of synthesis of the major 8-naphthoflavone (N-NF)-inducible cytochrome P450 isozyme, as well as other monooxygenase inductive activities, by acrylamide. In the present study the effects of acrylamide on the hepatic MO inductive response of rainbow trout were measured. Rainbow trout were treated by flow-through exposure with 500ppm acrylamide monomer for 14d. Induction of hepatic MO activity was produced by treatment (100mg/kg, i.p., 4d with 4d) with 8-NF. Levels of cytochrome P450 LM4b (P450) were determined by immunoblot using rabbit anti-trout LM4b IgG (gift of D Williams, Oregon State University). Levels of mRNA were determined by nucleic acid hybridization (Northern blot) using pPlf450-3', a 3'-specific, 1.5 kb complementary DNA (cDNA) clone derived from 3-methylcholanthrene-induced rainbow trout liver (gift of D W Nebert, Bethesda). Acrylamide-treated 4d-8-NF- induced animals when compared to non-acrylamide-treated 4d-8-NF-induced animals had decreased (50% and ~71%) levels of hybridized mRNA and cytochrome P450 LM4b isozyme, respectively. The hepatic MO activities of ethoxyresorufin- and ethoxycoumarin-O-deethylation were also decreased (68% and 58%, respectively) in these animals. These data demonstrate that acrylamide effects on MO activity may partly be due to a reduced level of P450 mRNA. (Supported by ES 01089 and ES 014894).
Propanil is an arylamide herbicide that has been reported to be contaminated with 3,3′,4,4′-tetrachloroazobenzene (TCAB) and 3,3′,4,4′-tetrachloroazoxybenzene (TCAOB). TCAB and TCAOB are analogs of the potent cytochrome P-450 inducers 2,3,7,8-tetrachlorodibenzo-p-dioxin. In this study rats were pretreated with propanil, 3,4-dichloroaniline, TCAB and TCAOB, and the effects on hepatic microsomal drug-metabolizing enzymes were determined. Propanil, 3,4-dichloroaniline, TCAB and TCAOB pretreatment produced 1.5-, 2-, 5- and 5-fold increases, respectively, in the 2′-hydroxylation of propanil; however, deacetylation of propanil to 3,4-dichloroaniline was not affected. In addition, these compounds produced 4-, 6.5-, 123- and 103-fold increases, respectively, in the 0-deethylation of ethoxyresorufin, and 3-, 3.5-, 3.5- and 11-fold increases, respectively, in the 0-dealkylation of benzoylresorufin. SDS PAGE suggested induction of cytochromes P-450c and P-450d by TCAB and TCAOB. TCAB was the only compound that induced p-nitrophenol hydroxylase and 1-naphthol UDP-glucuronransferase activities, which suggest there may be differences in the mechanisms of hepatic enzyme induction by TCAB and TCAOB.

**Effect of Dietary Corn Oil on the Induction of Hepatic Microsomal Cytochrome P-450 Isozymes.**

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This study was conducted to determine the influence of corn oil on levels of hepatic microsomal cytochrome P-450 hemoproteins. Male S-D rats were starved 36 hr and then reed a fat-free (FF) diet or a 20% corn oil diet (COD) for 4 days. Some received phenobarbital (Ph) sodium (80 mg/kg, ip daily) for 3 days prior to decapitation. P-450 levels were measured by CO binding spectra, and 5 µg hepatic microsomal protein of each rat was separated by SDS-PAGE, and P-450 isozymes quantitated by gel scanner. P-450 Ph-B (P-450 b) form was quantitated by western blot. Rats fed FF diet and administered Ph had only 21% more P-450 CO binding spectra than noninduced controls, whereas rats fed 20% COD had 58% more P-450, and Ph rats fed 20% COD had 181% more P-450 than FF controls. Quantitative analysis by gel scanner showed 32, 59, and 124% more P-450 protein respectively in FF Ph, COD control or COD Ph rats than in FF controls. P-450 Ph-B forms were not detected in noninduced groups, but were quantitated by western blot as 0.32 n mole or 0.70 n mole/mg protein respectively in FF Ph or COD Ph rats. Our findings suggest that dietary fat influences total amount of P-450 hemoprotein and its inducibility by increasing P-450 hemoprotein synthesis. (Supported by Air Force AFOSR 88-0277)

**Drug-Metabolizing Enzyme Induction by Non-Halogenated N-Substituted Imidazoles.**

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N-benzylimidazole (75mg/kg x 3 days, ig) is a high magnitude inducer of rat hepatic cytochrome P-450 which also induces UDPGluconuronosyltransferase (UDPGT) (morphine and 1-naphthol) and glutathione transferase [GSH(T)](CDNB) activities and depresses sulfate transferase [S](nitronepholine) activity. Similar treatment of rats with imidazoles in which the benzyl substituent has been replaced by an acetyl or phenolic group prevents the changes. Methyl, phenyl, benzyl or 4-acetophenone substitutions induce some enzymes, particularly UDPGT (morphine and 1-naphthol) and GSH, increasing the size of the N-substituent from benzyl to methylenenaphthyl reduces P-450 induction properties, but does not affect the ability to double UDPGT (morphine and 1-naphthol) and GSH activities. A 2′-ketoethenylmethyl substituent (i.e., nalmidone) reduces the induction of all the parameters mentioned above, with the exception of erythromycin demethylase activity. The benzyl and both naphthyl derivatives induce 7-ethoxyresorufin deethylase activity to a similar extent. While the methylenenaphthyl substituent induces pentoxyresorufin dealkylation activity less than the other two, it alone shows high induction of nitronepholine hydroxylase activity. The various microsomal and cytosolic drug metabolizing enzymes are not influenced to the same extent by changes in the structure of the N-substituted imidazoles. A ring substituent separated from the imidazole by at least one carbon appears necessary for cyt. P-450 but not UDPGT induction.

**Time Course for the Induction of Pulmonary Cytochrome P-450 Dependent Monooxygenase Activity by Cigarette Smoke Exposure (Kentucky Reference Cigarette, 1R4F) in Rats.**

K M Chang, P H Ayres, and J D Gehethy. Regulation Tobacco Co., Winston-Salem, NC.

Adult male Sprague-Dawley rats were exposed nose-only to diluted smoke produced from a Kentucky reference cigarette (1R4F) for 60 minutes at a concentration of approximately 30 µg/L nicotine. A time course for the maximum induction of pulmonary aryl hydrocarbon hydroxylase (AH) and ethoxyresorufin O-deethylase (EROD) activities was examined at 2, 4, 6, 8, 16, 30, and 48 hours after the smoke exposure. Rats placed in restraint tubes and exposed to humidified, purified air served as sham controls. Microsomes from sham control rats were prepared at 3 different times (12:00 noon, 6:00 p.m. and 2:00 a.m.) to correct the effect of circadian variation on the enzymatic activity. The inductive effect on both AH and EROD occurred at 2 hour post-exposure, reached a maximum at 6 hour, and returned to the control activity 48 hours after the exposure. The AH activity was 243% and the EROD activity was 529%, respectively, of the control activity at the peak time of induction.

**Supported by USPHS grant GM 39335**
EFFECT OF PREGNENOLONE-16α-CARBONITRILE (PCN) AND DEXAMETHASONE (DEX) ON ACETAMINOPHEN (AA)-INDUCED HEPATOTOXICITY IN MICE. C.D. Klages, C. Madhu, and T.J Maziasz. Univ. of Kansas Medical Center, Kansas City, KS.

Recently, we demonstrated that a microsomal enzyme inducer with a stereoidal structure, PCN, markedly decreased the hepatotoxicity of AA in hamsters. Therefore, it was of interest if PCN, as well as another steroidal microsomal enzyme inducer, DEX, would decrease the toxicity of AA in mice, another species sensitive to AA toxicity. Mice were pretreated with PCN or DEX (100 and 75 mg/kg, ip, for 4 days, respectively) and were orally given AA (300-500 mg/kg, ip). Twenty-four hr after AA administration, liver function was determined by measuring serum activities of sorbitol dehydrogenase (SDH), alanine aminotransfere (ALT), as well as by histopathological examination. Neither PCN or DEX protected against AA hepatotoxicity in mice; PCN had no effect on AA-induced hepatotoxicity, whereas DEX was found to enhance AA-induced hepatotoxicity, and it produced some hepatotoxicity by itself. DEX decreased the glutathione (GSH) concentration (36%) in liver and increased the biliary excretion of AA-GSH, which reflects the activation of AA, whereas PCN produced neither effect. Thus, whereas PCN markedly decreases the hepatotoxicity of AA in hamsters apparently by decreasing the isofrom of P-450 responsible for activating AA to N-acetyl-p-benzoquinoneamine, this does not occur in mice after either induction with PCN or DEX. In contrast, DEX enhances AA hepatotoxicity apparently by decreasing liver GSH levels and increasing the activation of AA. (Supported by USPHS Grant ES-03192 and ES-07079).


Female rats were treated with simvastatin (a novel competitive inhibitor of HMG CoA reductase) to ascertain the possible relationship between changes in hepatic metabolism and the thyroid hypertrophic effect of this agent. The test compound was administered orally at a dose of 50 mg/kg b.i.d. After 5 weeks, simvastatin produced significant increases (35% above control) in serum thyroid stimulating hormone (TSH) and slight decreases in thyroxine levels in female rats. In addition, simvastatin treatment resulted in a significant increase in the systemic clearance of I-135-thyroxine (35% above control). Organ weight changes included increased liver weights and microscopically, thyroid hypertrophy was observed. Simvastatin did not markedly alter liver microsomal enzyme activities with the exception of the anticipated induction of HMG CoA reductase. Thus, the increased clearance of thyroxine in simvastatin treated female rats was not associated with enzyme induction but may have been related to the increase in functional liver mass. The thyroid hypertrophy associated with simvastatin administration in female rats is likely to be the result of increased thyroxine clearance resulting in a feedback stimulation of the thyroid via increased serum TSH.

THE EFFECTS OF PHENOBARBITAL INDUCTION ON THE TOXICITY OF BRODIFACOUM IN THE CANINE. M.J. Murphy, A.C. Reagor, E.M. Bailey. Texas Veterinary Medical Diagnostic Laboratory, College Station, TX. Sponsor: A.C. Ray

A reliable technique for extraction of brodifacoum (BDF) from serum using ether and other:acetonitrile (1:1) was developed. Two HPLC systems (A: 1.5% Acetic acid, pH 4.5:acetonitrile (1:2) with 1% dibutylamine and B: 0.2 M Tris, pH 7.5 acetonitrile (1:3)) were utilized to optimize sensitivity with simultaneous ultraviolet (254 nm & 313 nm) and fluorescent (313 mm excitation with 375 nm emission) detection. The limit of detection is approximately 10 ng/ml from serum with a recovery of 75±4%.

Dogs (N=5) were induced with 35 mg/kg/day phenobarbital. Induction was demonstrated using antipyrine kinetics (Mean Residence Time, Non-Induced Time, Non-Induced = 1.12±1.12, Induced = 6.122 = significant P<0.05). Coagulation parameters were unchanged (Activated Coagulation Time) or prolonged (Prothrombin and Proconvertin Time), and BDF kinetics were unchanged (Area Under the Curve: Non-Induced = 750, 000±440, 000, Induced = 680, 000±250, 000) (Mean Residence Time: Non-Induced = 3,000±210, Induced = 3,800±900) following induction. Thus, no therapeutic benefit from phenobarbital induction to dogs poisoned with BDF was demonstrated by coagulation status or brodifacoum kinetics.

THE RAT AS A MODEL TO EVALUATE THE GASTRIC IRRITATION POTENTIAL OF ALKALINE PRODUCTS. L.J. Sauers, J.K. Maurer, and P.J. Reer. The Procter & Gamble Company, Cincinnati, OH.

Animal models historically used to assess the gastric irritation potential of alkaline products include the dog, pig and cat. In looking at alternative methods that give more pertinent data and are more cost effective, the rat is being evaluated as a potential model. Gastric irritation is known to increase as the residue alkalinity of the product increases. (Reserve alkalinity refers to the buffering capacity of the product), This value is determined by titrating a 1% solution of the product with HCl down to a pH of 9.5 and is expressed as grams of NaOH equivalents/100 cc of product). In our initial experiments to assess the rat as a potential model, we used prototype products differing in their reserve alkalinity. Rats were dosed via oral gavage and necropsy was performed fifteen or sixty minutes after dosing. Evaluation of the stomach mucosal surface via gross morphology, histopathology, stomach/body weights and wet/dry weights revealed differences in the various treatment groups that distinguished products having low reserve alkalinity from those having high reserve alkalinity. This preliminary work suggests that the rat as a potential model to assess the gastric irritation of alkaline products or substances. Future work included determining if the use of anesthetics would affect the response in the rat model.
A number of antiulcerants capable of potent/long-acting suppression of gastric acid secretion have been reported to induce gastric tumors in rats following chronic, high-dose treatment. Although the mechanism is not known, a drug-induced persistent hypergastrinemia may play a role. Fischer-344 rats were given daily oral doses (0, 10, 100, and 1000 mg/kg) of \( \text{LT15461} \), \( \text{2,4-dimethylaminopyrimidine} \), \( \text{5-ethyl-2-thiazolecarboxaldehyde} \), \( \text{2-ethyl-3-pyridinylmethyl} \), and \( \text{3-(4H)-pyridazinone} \), for 3 months. Gastric pH in fasted rats increased in a dose-related manner at 4 hr after dosing on days 1, 45, and 91 but were normal at 24 hr on each occasion. Serum gastrin levels at 8 hr (peak response) also increased in a dose-related manner on days 1, 44, and 90 but returned to control levels (p>0.05) by 24 hr at all dose levels except at the highest dose subsequent to day 1. During the study, the mean (N,F) B sum gastrin:levels were the same number range from 464 to 709 pg/ml vs. 25 to 196 pg/ml in controls. Morphometric analysis showed no increase in the density of enteric-nuclein-like cells in the oxyntic mucosa. Treatment-related histopathologic changes were limited to a slight thickening of the gastric mucosa at the two highest doses.


MDA is a potent hepatocarcinogen in rats and mice and induces positive responses in many in vitro genotoxicity assays; however, the compound is generally nongenotoxic in in vivo assays. We have examined the ability of MDA to induce unscheduled DNA synthesis (UDS), a genotoxic endpoint, and S-phase synthesis (SFS), an indicator of cell proliferation, in rodent hepatocytes. Hepatocytes were isolated from male F-344 rats or B6C3Fl mice following treatment with MDA then incubated with \( \text{H-thymidine} \). UDS and SFS were evaluated by quantitative autoradiography. Control animals yielded 0 net grains/microliter (NG/microliters). Treated animals yielded 2.6 NG (24 ng/ml). In vivo treatment of rats (350 mg/kg) or mice (1000 mg/kg) yielded <4 NG in hepatocytes. Treatment of rats with MDA (300 mg/kg) yielded 2.9% of hepatocytes in S-phase, compared with 0.2% in controls. Significant elevations in serum bilirubin (16-fold over controls), SGOT (5-fold), SGPT (6-fold), and SDH (3-fold) were observed in these animals. These results indicate that MDA is genotoxic in isolated hepatocytes, but not genotoxic following in vivo treatment. MDA does, however, induce significant elevations in cell turnover due to hepatotoxicity-induced regeneration. This increase in cell turnover may be related to MDA’s potent hepatocarcinogenicity.


Increased serum transaminase activities in patients have been reported during clinical trials with tacrine, an anticholinesterase agent being tested for treatment of Alzheimer’s disease. Initial acute and repeated dose toxicity studies in rodents and dogs revealed no clinical biochemical or pathologic evidence of hepatotoxicity. Additional acute studies in rodents at 70 mg/kg revealed elevated plasma transaminases in rats at 1 day post-dose. However, there was no accompanying light or electron microscopic evidence of hepatic injury. Incubation with tacrine at 0.1, 1, 10, 100, and 250 \( \mu \text{g/ml} \) with suspensions of isolated hepatocytes from female rats for 6 hours caused concentration-dependent increases in toxicity at 100 and 250 \( \mu \text{g/ml} \) determined by lactate dehydrogenase latency of cell samples and alanine and aspartate aminotransferase activities in the incubation medium. Hepatocytes from male rats were unaffected. The response of hepatocytes from female rats to tacrine was unaffected by phenobarbital or β-naphthylacetate pretreatment in vivo. The relevance of these findings to elevated transaminases in human beings is uncertain since the tacrine concentrations necessary to demonstrate toxicity in isolated hepatocytes are more than 100 fold greater than the highest levels measured in rats in vivo.

ISONIAZID POTENTIALITY OF A GUINEA PIG MODEL OF HALOTHANE-ASSOCIATED HEPATOTOXICITY. RC Lind and AJ Gandolfi. Dept of Anesthesiology, University of Arizona, Tucson, AZ.

Enhanced oxidative metabolism of halothane (CF, CBrClH; H) via isoniazid (INH) pretreatment has been used to produce a rat model of H-associated hepatic necrosis. Thus INH pretreatment was evaluated in a guinea pig model of halothane hepatotoxicity. Outbred male Hartley guinea pigs (600-800 g) were pretreated with 0, 12.5, 25, or 50 mg/kg INH in saline, ip, x 7 days. After INH, the animals were exposed to 1% \( \text{N}2 \text{O} \) for 4 hr in 40% O2. INH pretreatment did not alter reductive metabolism of H as indicated by plasma fluoride ion levels. INH did cause dose-dependent increases in the oxidative biotransformation of H as indicated by increased plasma trifluoroacetic acid levels of 1.2x, 1.6x, and 1.7x H-only values with 12.5, 25 and 50 mg/kg INH, respectively (N=3-7). This was accompanied by increases in H-induced liver injury as indicated by 84 hr plasma ALT levels and the incidence of centrilobular necrosis (%) (H-only = 31 + 277 [4/7], 12.5 mg/kg INH + H = 898 + 530 [4/5], 25 mg/kg INH + H = 1626 + 605 [3/3], 50 mg/kg INH + H = 2682 + 777 [4/7], untreated = 26 + 6, *p < 0.05 vs H-only). Although INH-pretreated animals demonstrated normal ALT levels at the time of H exposure, further studies will be necessary to determine whether the potentiation of hepatic injury is due to enhanced oxidative metabolism or a co-toxicity. (NIH AM 16715)
A COMPARISON OF SINGLE-DOSE TOXICITY OF CAPTAN, DIFOLATAN AND FOLPET IN RATS.
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Structurally related compounds captan, difolatan and folpet are used widely as broad-spectrum, nonpersistence fungicides for the control of various fungus diseases of seeds, fruits and vegetable crops. The present study was carried out to compare the effect of i.p. administered captan, folpet (20 mg/kg, each) and difolatan (5 mg/kg) on liver and select liver mixed-function oxidases (MFOs) in the rat. Pentobarbital sleeping time test showed that difolatan was most inhibitory to the MFO system followed by captan and folpet. Hepatic cytochrome P-450 and activity of select MFOs were slightly inhibited by all three fungicides 24-hr following the treatment. On the other hand, the activity of SDH and ALT in the serum samples of the treated animals was markedly elevated by folpet and difolatan and slightly by captan. A somewhat similar trend was noted for AST activity. The results indicate that these fungicides are not only inhibitory to MFOs but also toxic to liver.

EVALUATION OF THE EFFECTS IN MICE OF LOVASTATIN AND HEPATOXICANTS. R Rajj and S Bhattacharya.
A. & M. Schwarz College of Pharmacy and Health Sciences, Long Island University, Brooklyn, NY.

Lovastatin, a cholesterol lowering agent, occasionally produces liver dysfunction in human adult patients and animals in serum transaminases. A subchronic toxicity study was undertaken to evaluate the effects in mice of lovastatin in combination with CCl₄, C₆H₄OH & APAP. Thirty male & thirty female B6D2F1 mice, 7 weeks old, weighing 25-30g were divided into 5 equal groups. Animals in the control group received corn oil; in remaining 4 groups received lovastatin 30 mg/kg in corn oil orally by gavage 3 times per week for 13 weeks. All animals in the 1st 2 groups received drinking water ad libitum. In 3rd group, the drinking water containing 3% v/v C₆H₄OH ad libitum; in 4th & 5th, the drinking water containing 0.008% v/v CCl₄ & 0.75% w/v APAP respectively were given ad libitum. All animals received feed ad libitum. After 13 weeks, all animals were sacrificed, blood sample separated and kept frozen. SGPT & ALK-PHOS were determined in each serum sample. Liver tissues were preserved in 10% formalin for histopathology. SGPT was found to be significantly elevated in all experimental animals except the males in C₆H₄OH group. ALK-PHOS was found to be significantly elevated only in the animals of the APAP group as compared to control. The data suggest that some effects on liver are enhanced in mice when they are exposed to lovastatin and known hepatotoxicants together.
EFFECT OF CHLOROTRIFLUOROETHYLENE (CTFE) OLIGOMERS ON LIVERS OF FISCHER-344 RATS. D R Mattie and W R Chase. AAMRL/TRY, Wright-Patterson AFB, OH. Sponsor: M E Andersen.

Male and female Fischer-344 rats were exposed to vapors of CTFE oligomers at 0.25, 0.5, or 1.0 mg/L for 8 h/day for a total of 95 exposures over 90-days. The liver was the target organ at all concentrations. In this study the liver effects of CTFE were compared in male and female rats by electron microscopy. A 4 mm slice of the left lobe of the liver of three rats of each sex and each group was collected at the end of the exposure. Thin sections were cut from the centrolobular and intermediate zones of liver lobules. Repeated exposure to CTFE for 90-days resulted in mild to moderate mitochondrial swelling, a dose-dependent increase in smooth endoplasmic reticulum (ER), and peroxisomal proliferation in both male and female rats. Photographs were scored in order to grade mitochondrial swelling and the relative amount of smooth ER and peroxisomes. In male rats there was disruption of rough ER. Livers of male rats were more sensitive to repeated inhalation exposure to CTFE than female rat livers, consistent with the observed effect of increased liver weights and depressed body weight gain.

THE EFFECTS OF CHINESE HEPATOPROTECTIVE COMPOUNDS ON EXPERIMENTAL LIVER INJURY IN MICE. Y P Liu, J Liu and C D Kleessen. Univ. of Kansas Medical Center, Kansas City, KS.

The purpose of this study was to compare the hepatoprotective effects of 7 compounds extracted from Chinese herbs on 4 known hepatotoxins in mice. These herbal compounds are fulvotestosetins (Ful), oleandric acid (Ola), japonicosinosides (Jgs), notoginosides (Ngs), sweroside (Swe), oxymatrine (Om), and dimethyl dicarboxylate biphenyl (DDB). All have previously been reported to exhibit hepatoprotective effects and are used to treat human hepatitis. Mechanistically different types of acute liver injury were produced in male CF, mice by carbon tetrachloride (CCL4), acetaminophen (APAP), cadmium chloride (Cd) and allyl alcohol (AA). Liver damage was assessed by quantitating serum sorbitol dehydrogenase and alanine aminotransferase activities as well as by histopathological examination. Ful markedly decreased the toxicity produced by all 4 hepatotoxins with the best protection against Cd toxicity; Ola also markedly decreased APAP, CCL4 and Cd-induced hepatotoxicity but had no effect on AA; Jgs and Ngs had moderate hepatoprotective effects on these models except Jgs markedly decreased AA toxicity; Swe decreased Cd and CCL4 toxicity but had no effect on the other two hepatotoxins; Om only decreased APAP toxicity whereas, DDB did not protect against any of the hepatotoxins. The mechanism(s) by which these compounds protect against widely different types of chemical injury requires further investigation. In conclusion, of the 7 compounds examined, Ful and Ola appear to be the most effective in protecting against chemical-induced liver injury. (Supported by USPHS Grants ES-01142).


Trimetrexate is a non-classical antifolate being developed as an anticancer drug and as a treatment for Pneumocystis carinii pneumonia in AIDS patients. Antifungal efficacy may be increased by combining high dose trimetrexate with concurrent leucovorin (LEU) treatment to reduce toxicity in the host. Severe toxicity and mortality produced by five consecutive daily oral doses of 80 mg/kg trimetrexate isethionate was used to assess the suitability of the rat as a protection model for further studies. Groups of 5 male Wistar rats received trimetrexate or trimetrexate plus once (X1) or twice (X2) daily oral administrations of LEU (100 mg/kg/dose) given at the time of trimetrexate dosing (X1 and X2) and 5 hr later (X2 only). Diarrhea and 100% lethality within 8 days produced by trimetrexate was prevented by X1 or X2 LEU treatments. Protection from principal antifolate target organ toxicity was related to LEU dose. Depressed WBC counts, degenerative intestinal lesions, and lymphoid tissue changes seen with LEU X1 are minimal with LEU X2 treatment. This study demonstrates LEU dose-related protection from antifolate target organ toxicity and lethality of trimetrexate in the rat and suggests that LEU administration once or twice daily is an adequate dosing schedule for protection from repeated trimetrexate challenge in this species.

PHARMACOKINETICS OF MOLSIDOMINE IN PATIENTS WITH TOXIC LIVER DEMAGE. J R Welser. Department of Medicine, Medical University of Luebeck, FRG.

The elimination of molsidomine, an antinodal drug that is activated by hepatic ester cleavage to SIN-1, has been evaluated in 14 patients with alcoholic-toxic liver damage. A comparison was made between the elimination half-life time of molsidomine (t 1/2) and two parameters describing the liver function: choline esterase activity (CHE) and the antipyrene clearance (AP) as an index of the microsomal activity of the liver.

In contrast to a former study which included a more limited number of both liver-cirrhotic patients and patients with normal liver function no correlation could be found between CHE and AP. Comparison between t 1/2 and the above mentioned parameters of liver function showed that there was an inverse relationship with CHE, rather than with AP. Independent of the degree of liver demage, SIN-1 plasma levels in the range of 3 to 13 mg/ml could be found in 5 patients.

These results fit well with the respecting data of the former study. The finding was confirmed that only in patients with severe liver demage, characterized by a CHE-activity of about 2 IU/1 and less, the t 1/2 molsidomine is increased considerably (medians: 7.6 h vs. 2.6 h).
PHOSPHOLIPID CHANGES IN RAT AND MOUSE LIVER FOLLOWING Dermal AND DRINKING WATER EXPOSURE TO DIETHANOLAMINE. W L Jenkins and R L Melnick. NTP, NIEHS, RTP, NC. Sponsor: J Bucher.

Diethanolamine (DEA), a chemical widely used in industrial processes and consumer products (e.g., cosmetics), has been reported to alter hepatic phospholipid (PL) composition in rats by competing with choline and ethanolamine incorporation. The present study was initiated to determine if an association exists between hepatic phospholipid changes and toxicity after dermal and drinking water exposure to diethanolamine. F344 rats and B6C3F1 mice were exposed to diethanolamine for 14 or 90 days. Livers were analyzed for phospholipid composition by high performance liquid chromatography after extraction with chloroform/methanol. Phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were decreased approximately 50% in rats treated with 2.5 mg/ml DEA in their drinking water compared with controls. A slight decrease in PC was observed after dermal application of DEA (250 mg/kg). In contrast to rats, the chromatograms of liver PL from mice dosed with DEA either by dermal application (630 mg/kg) or by drinking water exposure (2.5 mg/ml) showed multiple unresolved peaks in the regions of PE and PC elution. These new lipid substances may be involved in the hepatic toxicity of DEA in mice.

HISTOPATHOLOGIC, METABOLIC AND MICROCIRCULATORY CHANGES IN THE ISOLATED PERFUSED CIRRHOTIC RAT LIVER. A H Boyarsky, K H Jung and S Ji. Dept. of Surgery, R W Johnson Medical School/UMDNJ and Dept. of Pharmacol. and Toxicol., Rutgers University, Piscataway, N.J.

Treatment of male Wistar rats with alcohol (A) and CCl4 (C) for 8 weeks produced liver cirrhosis as demonstrated by histopathologic findings. The isolated perfused liver from such rats showed a reduced hepatic O2 uptake as measured with a Clark-type O2 electrode and regional microcirculatory blockade, apparently localized in zone 3 of the liver lobule as evidenced by a lack of staining upon trypan blue (0.1%) infusion. The histologic sections of the cirrhotic liver revealed a dramatic decrease in hepatocyte density, along with the changes characteristic of cirrhosis. These findings in combination with related data from our laboratories suggest a working model of (A + C)-induced liver cirrhosis, which includes: (1) A-induced migration of neutrophils into the liver, (2) activation of C into reactive radical intermediates via the respiratory burst of neutrophils, (3) microcirculatory aberrations caused by these reactive molecular species, and (4) increased shunting of flow away from tissue with high cellularity to metabolically non-functioning tissue. Supported in part by R W Johnson Medical School Grs Grant No.481271552.

IMMUNOTOXICITY OF 2,4-DIAMINOTOLUENE IN FEMALE B6C3F1 MICE. K L White Jr, J A McCay, D L Musgrove, R D Brown, M L Stern, M P Holisapple and A E Munson. Medical College of VA/VCU, Richmond, VA.

2,4-Diaminotoluene (DAT) is an amino substituted aromatic which is used in the production of tolune disocyanate, dyes, resins, hydraulic fluids, urethane foams, and fungicide stabilizers. The objective of these studies was to evaluate if the immune system was a target for DAT induced toxicity. Mice were exposed by oral gavage for 14 days daily to vehicle (distilled water) or DAT at doses of 25, 50, and 100 mg/kg. DAT produced a dose dependent increase in liver weight (42%). Treated mice had decreased IgM (46%) and IgG (56%) responses to SRBC, expressed on a whole spleen basis, however, there was an increase in B cell number (18%). Proliferative capacity was not decreased in response to mitogens (concanaavalin A, phytohemagglutinin, and lipopolysaccharide) or allogeneic cells (MLR). The delayed hypersensitivity response to keyhole limpet hemocyanin (KLH) was increased (123%) while Natural Killer Cell activity was decreased (99%). Phagocytosis of peritoneal macrophages was unaffected by DAT exposure, but the phagocytic ability of splenic macrophages was suppressed (45%). Host resistance to the bacterial challenge, Streplococcus pneumoniae and Listeria monocytogenes was decreased, possible as a result of decreased complement component C3 and reduced phagocytic activity, respectively. No decreased was observed with either of the two tumor models PYB6 fibrosarcoma and B16F10 melanoma. (Supported by NIH Contract No. ES 55094).
SUPPRESSION OF HUMORAL IMMUNITY FOLLOWING DERMAL EXPOSURE TO BENZ(a)PYRENE IN B6C3F1 MICE. M C Parrott, T T Kawabata, and K L White, Jr. Medical College of Virginia/VCU, Richmond, VA.

Benz(a)pyrene, B(a)P, has been shown to be immuno-suppressive when administered parenterally. The objective of this study was to determine if dermally administered B(a)P is capable of suppressing the antibody forming cell response (AFC) to the T-dependent antigen sheep erythrocytes. Shaved abdominal areas of female B6C3F1 mice were exposed for 14 days daily to vehicle (acetone:olive oil) or B(a)P at doses of 5, 20, and 40 mg/kg. A dose dependent suppression was observed. AFC response of mice treated with 40 mg/kg was 49% of vehicle controls. B(a)P was not immunosuppressive when administered by the dermal route. Immunosuppression occurred without changes in body, liver, spleen, or thymus weights. Hematological parameters were unaffected. In recovery studies, the AFC responses of B(a)P (40 mg/kg) exposed-mice by the dermal route returned to control values by 45 days after cessation of treatment. The in vitro T-dependent AFC response of splenocytes from dermally exposed mice was similarly suppressed one day after final treatment and returned to control levels after a 30-day recovery period. The in vivo AFC response of animals exposed by subcutaneous injection of B(a)P in corn oil failed to recover, even when evaluated after a 90-day recovery period. These studies suggest that the persistent suppression observed following corn oil exposure results from a depot effect of B(a)P being released from the corn oil. (Supported by PHS grant ES03434)

EFFECTS OF BENZ(o) PYRENE [B(a)P] AND ITS METABOLITES ON THE ANTIBODY RESPONSE IN VITRO. R S Toman, S Lim, and S S Gill. Department of Entomology, University of California, Riverside, CA.

The effects of B(a)P and its metabolites: 4, 5-, 7,8-oxide, 5-, 7-, 9-hydroxy, and 1,6-dione on antibody producing ability of mouse spleen cells were studied in Mishell-Dutton, in vitro, culture system. B(a)P inhibited the plaque forming cell (PFC) response of SRBCs, T-lymphocyte and macrophage dependent response, in a dose dependent manner, but had no effect on the TNP-LPS response, a T-lymphocyte and macrophage independent response. B(a)P 4,5- and 7,8-oxide inhibited the PFC response to SRBCs at concentrations ≥ 1 X 10^-6M. This was accompanied by cytotoxicity as evidenced by reduced cell viability at the end of the culture period. B(a)P 7-hydroxy, 9-hydroxy, and 1,6-dione (1 X 10^-5.1 X 10^-9M) neither reduced the PFC response nor the cell viability. 5-Hydroxy B(a)P (1 X 10^-5M) increased the PFC response to SRBCs and TNP-LPS response and reduced the number of viable cells recovered at the end of the culture period. These results suggest that the B(a)P metabolites do not contribute towards the inhibition of PFC response observed following B(a)P addition in vitro.

ENHANCED INFLUENZA VIRUS REPLICATION IN FISCHER-344 RAT LUNG FOLLOWING PHOSGENE INHALATION. J P Uhrich,1,2 and G R Burleson3. 1NSI-ES,RTP,NC; 2NYU Med Center; 3 Inhalation Toxicology Division, Health Effects Research Laboratory, US EPA, RTP, NC.

Animal infectivity models have been important in the demonstration of enhanced susceptibility to infection following low level toxican exposure. Infectivity models have historically focused on bacterial and viral infection in the mouse system utilizing mortality as the endpoint of toxicity. This study demonstrated enhanced susceptibility to viral infection using a rat influenza virus-infectivity model following challenge with the toxicant gas phosgene. Phosgene-exposed influenza virus-infected rats demonstrated peak titers 1 day post infection similar to those of the air exposed rats. However, a prolonged and significantly enhanced pulmonary influenza virus infection was detected through day 4 post infection in phosgene exposed rats. No virus was detected by day 5 post infection in either exposure group. Therefore, inhalation of phosgene gas resulted in increased susceptibility to pulmonary influenza virus infection. In addition, this study demonstrated the effective use of a viral infectivity model able to detect more subtle and descriptive mechanistic endpoints of toxicity than are typically measured. (This abstract does not necessarily reflect EPA policy.)
SUPPRESSION OF PULMONARY NATURAL KILLER ACTIVITY BY CONTINUOUS OZONE EXPOSURE. J D Sutzman, L L Keyes, and G R Burdess. NSI-ES and 2 Inhalation Toxicology Division, Health Effects Research Laboratory, US EPA, RTP, NC.

Ozone is an oxidant gas and an ubiquitous air pollutant that causes an increased susceptibility to infectious diseases. Ozone has also been reported to exert deleterious health effects on pulmonary physiology, biochemistry, and immunology. Natural killer (NK) activity is an important nonspecific first line of defense against viral and neoplastic disease. The present study evaluated the effect of continuous ozone exposure on pulmonary NK activity. Fischer-344 male rats were exposed to either clean filtered air or to 1.0 ppm ozone for 23.5 hr per day in Rochester exposure chambers. NK activity was quantified by a 4 hr 51chromium release assay. Effector cells were prepared by finely mincing whole lung followed by collagenase treatment. Effector:target cell ratios of 100:1, 50:1, and 25:1 were quantified. Pulmonary NK activity was suppressed in Fischer-344 rats following exposure to 1.0 ppm ozone for 1, 5, or 7 days. This suppressed pulmonary NK activity, as a result of continuous ozone exposure, may have serious consequences for susceptibility to viral and neoplastic diseases. (This abstract does not necessarily reflect EPA policy.)

CHARACTERIZATION OF IMMUNE ALTERATIONS RESULTING FROM ACUTE EXPOSURE OF RATS TO TRIBUTYLIN OXIDE. R J Smialowicz, M M Riddle, R R Rogers, R W Luebbe, C S Copeland and G G Ernst. U.S. EPA, Research Triangle Park, NC.

Male Fischer 344 rats were dosed by oral gavage with bis(tri-n-butyltin) oxide (TBTU) in peanut oil on ten consecutive days, at dosages ranging from 1.25 to 10 mg/kg/day. Thymic involution was observed at a dosage of 2.5 mg/kg/day and Con A and PHA re-ponses were reduced at 5 mg/kg/day. No alterations in the lymphoproliferative responses to pokeweed mitogen, St. Typhimurium mitogen or in the one-way mixed lymphocyte reaction were observed. Furthermore, NK cell activity, the in vitro generation of CTL, and the production of IL-2 by splenic lymphocytes were unaffected by exposure of rats to TBTU. In vivo, the delayed-type hypersensitivity response to DNP-serum albumin was not affected by TBTU exposure. On the other hand, the plaque forming cell (PFC) response to sheep red blood cells (SRBC) was enhanced in rats dosed at 5 mg/kg/day, while the PFC response to TNP-LPS was unaffected in TBTU-exposed rats. Enumeration of splenic lymphocyte populations from TBTU-exposed rats revealed a reduction in 3X5, but not W3/25 or Ig6, positive cells. These results indicate that T lymphocytes are a primary target for TBTU-induced immune alteration and that the enhancement of the PFC response to SRBCs in TBTU-exposed rats may be mediated by alterations in the suppressor T lymphocyte population.

CYTOSINE ARABINOSIDE (ARA-C) INHIBITS PRE-B CELL MATURATION. K S Cocke and D Wiersa. Lilly Research Laboratories, Toxicology Division, Lilly Research Laboratories, Eli Lilly & Company, Greenfield, IN.

ARA-C presumably alters cellular differentiation by hypermethylation of DNA. The present studies were undertaken to determine if ARA-C alters differentiation of mouse bone marrow pre-B cells in culture. B-cell depleted bone marrow cell suspensions were pulsed with ara-c for 4 hours and were then cultured for 3 days. B cell maturation was analyzed phenotypically by immunofluorescence. Exposure to 10^-9 M ara-c decreased pre-B cells and B cells by >50%. This reduction was shown to be dose-dependent between 5 x 10^-6 M and 5 x 10^-5 M ara-c. Continuous exposure to 5 x 10^-5 M ara-c during the 3 day culture period reduced B cell generation to less than 35% of control cultures. Because B cell development is dependent on accessory cell function, we examined whether ara-c exposure (1 or 4 hours) altered bone marrow stromal cell proliferation as determined by adherent colony formation. No decrease in stromal cell proliferation or cytotoxic effects were observed. Only continuous exposure to 5 x 10^-5 M ara-c inhibited stromal cell proliferation to less than 25% of control. These results demonstrate that ara-c inhibits B cell maturation at early stages of differentiation in vitro. It is interesting to speculate that ara-c may alter B-lymphopoiesis at maturation stages preceding the pre-B cell stage.

THE ROLE OF METABOLIC BIOACTIVATION IN IMMUNOSUPPRESSION BY CCl4. N E Kaminski, D W Barnes, S D Jordan, and M P Holappa. *Dept. of Pharmacol. & Toxicol. Medical College of VA/VCU, Richmond, VA. *Dept. of Pharmacol., School of Medicine, East Carolina University, Greenville, NC.

The role of metabolic bioactivation in carbon tetrachloride (CCl4)-mediated immunosuppression was investigated in B6C3F1 mice. We have previously reported that exposure to CCl4 results in marked immunosuppression. To investigate the role of metabolic activation in immunosuppression by CCl4, mice were pretreated with either the cyto-P-450 inhibitor, aminooacetocitrile (AAN), or inducer, ethanol (EtOH), in an attempt to modulate the immunosuppressive effects of CCl4. Treatment of mice for 7 consecutive days, i.p., with CCl4 at doses of 250, 500 and 1000mg/kg resulted in a marked suppression of the SRBC antibody response, 47, and 50%, respectively. Treatment of mice with the inhibitor, AAN, 1hr prior and 6 hrs post CCl4 treatment resulted in complete abrogation of the immunosuppression by CCl4. Oral pre-treatment of mice with ethanol, 4mg/kg, for 3 days prior to treatment with CCl4, 50, 100 and 300mg/kg, resulted in a marked potentiation of the immunosuppression by CCl4. To characterize the role of CCl4 metabolic activation on the SRBC response, in vivo, CCl4 (0.3-3.0 mM) was incubated with naive spleen cells and metabolically active hepatocytes for 3hr. Spleen cells co-incubated with hepatocytes and CCl4 demonstrated a marked inhibition of the SRBC response, whereas, CCl4 had no effect on splenocytes alone. Studies are presently underway to determine the effects of CCl4 on total cytochrome P-450 content and specific substrate activities. (Supported by NIH ES03564, ACS IN-105L and NIOSA ES20415.)
809 PIMOZIDE DOES NOT ALTER 2,3,7,8-TCDD-INDUCED IMMUNOTOXICITY. N K Snyder, B A Fuchs, and M P Holapple. Department of Pharmacology and Toxicology, Medical College of Virginia/VCU, Richmond, VA.

Recent reports suggest that a hypothalamic site of action may mediate endocrinological effects associated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure. A study was undertaken to determine if TCDD-mediated immunosuppression could be antagonized by treatment with pimozide, a dopamine receptor antagonist shown to reverse TCDD-mediated hypoprolactinemia. Female B6C3F1 mice received either a single oral dose of 0.3, 3 or 30 μg/kg TCDD or corn oil (CO), were injected i.p. with 0.6 μg/kg pimozide or 10% ethanol in saline twice per day, or received both pimozide and TCDD. All mice were administered 4 X 10^8 sRBC i.v. on Day 3, and were sacrificed by exsanguination (cardiac puncture) under CO2 anesthesia on Day 7. Serum was collected; body, liver, spleen and thymus weights were recorded; and splenic antibody response to sRBC challenge was evaluated. TCDD produced a marked dose-related suppression of antibody response, dose-related increases in thymus and spleen weights and dose-related increases in liver weight. Coincident treatment with pimozide did not alter the immunotoxicity induced by TCDD. [Supported by NIH grant ES03520 and Training grant ES07087].

811 IMMUNOTOXICITY OF 2,2'-DICHLOREDIETHYL SULFIDE. J A Blank, R L Joiner, D P Houchens, G S Dill, and D W Hobson, Battelle Columbus Division, Columbus, OH. Sponsor: C T Olson.

2,2'-Dichloroethyl sulfide (sulfur mustard, HD) is an alkylating agent that has vesicant effects on the skin, eyes, and respiratory tract. Serious systemic effects such as leukopenia and bone marrow aplasia have also been observed after HD exposure. The purpose of this study was to examine the immunotoxic properties of HD in mice and to compare the effects observed following HD exposure with those of another alkylating agent, cyclophosphamide (CP), a known immunosuppressant. Following a determination of the approximate LD50 for HD (LD50 = 22 mg/kg), host susceptibility to a L1210 tumor challenge, delayed type hypersensitivity (DTH) response to keyhole limpet hemocyanin and humoral immune (HI) response to sheep red blood cells were examined in mice exposed subcutaneously to a single 2.5, 11.25, or 20.0 mg/kg CP dose. Splenic cellularity, spleen, and thymus weight was decreased in HD and CP treated mice 4 days after exposure. While CP inhibited the HI response and increased tumor susceptibility, HD appeared to only affect the HI response at the high dose level. [Supported by USAMRDC DAMID-83-C-3129.]

810 EVALUATION OF THE HOST RESPONSE TO T. SPIRALIS INFECTION IN CYCLOPHOSPHAMIDE-TREATED RODENTS R H Luebke, C B Copeland, M M Riddle, R R Rogers, and R J Smailowicz. U.S. EPA, and G Ernst, NSI, Inc., Research Triangle Park, NC.

Host responses to T. spiralis infection were examined in C57BL/6J mice and F-344 rats exposed to cyclophosphamide (CY). Host resistance was assessed at 14 d post-infection by counting adult worms remaining in the small intestine. Exulsion of adult worms was similar (>99.5%) in treated and control rats. In contrast, control mice retained 3% of their original worm burden whereas CY-treated mice harbored 16% of the original number of worms. These results show that adult T. spiralis expulsion by rats was less sensitive to CY administration than it was in mice. In rats, the in vito cellular response to infection was assessed by measuring the proliferative response of mesenteric lymph node cells (MLNC) to an extract of T. spiralis muscle larvae (TeE), 7 days after infection. Control rat MLNC were markedly stimulated by culture with TeE; however, the response of MLNC obtained from rats dosed orally at 6 mg CY/kg/d for 10 d prior to infection was reduced to approximately 30% of control levels. That the proliferative response was suppressed while adult worm expulsion remained intact suggests that the in vitro assay is more sensitive to CY treatment than adult worm expulsion.

812 EFFECTS OF CARYABRYL (CA) AND ITS METABOLITES [ALPHA-NAPHTHYL (αN) AND ALPH A-NAPHTHYL BETA-GLUCURONIDE (αNG), AND ALPH A-NAPHTHYL SULFATE (αNS)] ON CELL CYCLE TRAVERSE BY GT-2 CELLS. J S Bavari, ZG Kueyymski, and CL P Casale, Univ. of Nebraska Medical Center, College of Pharmacy and College of Medicine, Omaha, NE.

Since the cytoplasmic transducer for the IL-2 receptor signal appears to be an estrogen, we assessed the effects of CA, an anti-esteratic insecticide, and its metabolites on IL-2 driven proliferation of GT-2 (a cloned cytolytic T-cell; mouse). Cycling cells were stopped by a 6 h incubation without IL-2, then exposed to test chemical and restimulated with IL-2. Flow cytometry revealed a biphasic distribution of treated (CA, 5X10^-5M) and control cells, at 6 hrs. Cells were distributed evenly between Go/G1 and G2/M of the cell cycle, with few cells in S phase. By 12 hrs, many control cells had exited Go/G1 and G2/M, producing a population of largely G0/G1 and S phase cells. Treated cultures remained biphasic (Go/G1 and G2/M), at 18 hrs. An intermediate pattern was seen at 5X10^-4M CA. Cell viability was high (>99%) in all cultures. At 5X10^-7 and 5X10^-5M, CA reduced cell number (at 66 hrs) by 8% and 50%, respectively. An increased (14-18 hrs) doubling time (30-24 hrs for controls) was seen at 5X10^-5M CA. [3H-thymidine uptake (at 22 hrs) was reduced 12%, 25, and 90% by CA and 1,10, and 29% by αN at 5X10^-5], 5X10^-6, and 5X10^-5M, respectively, while αNG and αNS had no effect. ES/USDA supported.
EFFECTS OF MACROCYCLIC TRICHOTHECENE CONGENERS ON THE VIABILITY AND MITOGENESIS OF MURINE SPLENIC LYMPHOCYTES. B J Hughes*, B B Jarvis*, and R R Sharma*. *Center for Environmental Toxicology, Utah State University, Logan, UT. †Department of Chemistry and Biochemistry, University of Maryland, College Park, MD. Sponsor: P B McCay.

The effects of several congeners of the macrocyclic class of trichothecene mycotoxins on murine splenic cells in vitro were investigated. The mycotoxins were roritoxin B, mycotoxin B, roritins A, B, and D, echinocandin B12, B5, and B4, 16-hydroxycytoxycerriform A, and verrucarins A and J. Lymphocytes from CD-1 mice were cultured with each of the mycotoxins for 48 h to assess cytotoxicity. The maximum effect of various trichothecenes produced on cells occurred at concentrations ranging from 10^-6 to 10^-4 M. Mycotoxins had no effect at concentrations ranging from 10^-12 to 10^-10 M. The mitogenic stimulants concanavalin A, lipopolysaccharide, phytohemagglutinin, and pokeweed mitogen were added to splenic lymphocyte cultures along with varying concentrations of selected mycotoxins. Blastogenesis was inhibited at concentrations two to five orders of magnitude lower than those which produced lethality in resting lymphocytes.


PD 118332 is an orally effective inhibitor of allergic mediator release, as measured by in vitro and animal models. To assess the compound's effects on immune function, male Fischer 344 rats were evaluated for splenic T and B lymphocyte percentages, antibody-forming cell (AFC) response to sheep red blood cells (sRBC), Concanavalin A and Pokeweed Mitogen induced lymphocyte proliferation, Natural Killer cell activity, and ability of the reticuloendothelial system (RES) to clear sRBC. The drug was administered orally to rats at 25, 50, and 100 mg/kg/day for 14 consecutive days. A vehicle control and two positive controls (cyclosporine A and cyclophosphamide) were run concurrently. A dose of 100 mg/kg/day PD 118332 decreased average body weight gain and was lethal to five of the 40 rats. However, even this overtly toxic dose did not alter splenic cellularity, relative percentages of T and B lymphocytes, lymphocyte proliferation, or clearance of sRBC by the RES. AFC response showed a dose-related increase in the number of IgM secreting cells. Based on these assays, the immune system does not appear to be a specific or significant target of PD 118332 toxicity.

EFFECTS OF REPEATED EXPOSURE TO COMBINATIONS OF BENZENE AND TOLUENE ON IMMUNOLOGIC RESPONSES IN MICE. G C Hsieh, R P Sharma, and R D R Parker, Toxicology Program, Utah State University, Logan, UT.

Benzene and toluene are known contaminants of groundwater. Groups of CD-1 male mice were continuously exposed to benzene (166 mg/L), toluene (80 and 325 mg/L), and combinations of benzene (166 mg/L) + toluene (80 or 325 mg/L) in drinking water for 4 weeks. Benzene-induced anemia was alleviated by simultaneous toluene treatment. Leukopenia and lymphopenia were observed in benzene only and benzene + toluene (80 mg/L)-treated mice. The cytopenia, however, was less severe in the benzene + toluene (325 mg/L)-treated group. Immunotoxicity induced by benzene was characterized by involution of thymic mass and suppression of both B- and T-cell mitogenes, mixed lymphocyte culture response, the ability of cytotoxic lymphocytes to lyse tumor cells, antibody production response to sheep red blood cells, and the activity of interleukin-2. Toluene, 325 mg/L, completely inhibited these adverse effects when it was coadministered with benzene, while the low dose of toluene (80 mg/L) did not protect against benzene-induced depression of immune function. Toluene showed no obvious immunotoxic effects at the levels used. Results indicate that toluene, in appropriate combination, has an antagonistic effect on benzene immunotoxicity. (Supported in part by USGS G-1255)

3-METHYLCOLANTHRENE INDUCED TUMORS MAY ESCAPE IMMUNE SURVEILLANCE BY PGE2 MEDIATED IMMUNOSUPPRESSION. G C Mather, W H Exon and J L Bussiere. Dept. of Vet. Sci., University of Idaho, Moscow, ID.

Altered immune status among 3-methylicholanthrene (3MC) exposed animals has been hypothesized to allow cells with malignant potential to escape immune surveillance. Experiments were designed to delineate the effects of 3MC on specific portions of a complex immunoregulatory circuit known to regulate immune surveillance as characterized by nonspecific cell-mediated immunity. 3MC dissolved in 0.2% DMSO media was incubated in vitro with spleen cells or resident peritoneal macrophages from male Sprague Dawley rats. These cells were then assayed for NK cytolytic activity or for their capacity to produce IL2, interferon, or PGE2. Similar assays were performed following in vivo exposure to 3MC dissolved in tricaprylin. Finally, tumors induced by 3MC injection were established as cell lines and their capacity to inhibit NK cytolysis and produce PGE2 determined. Both in vitro and in vivo exposure to 3MC suppressed splenic NK activity. PGE2 production was reduced in vitro but enhanced following in vivo exposure. Likewise, 3MC induced tumor cell lines were found to produce PGE2 in quantities sufficient to suppress NK activity. These data suggest that the failure of immune surveillance following exposure to 3MC, as characterized by nonspecific cell-mediated immunity, may be mediated by increased PGE2 production by macrophages and by cells within the tumor.
A one-way mixed lymphocyte culture assay was evaluated as a biomarker of T lymphocyte dysfunction following exposure of normal human PBMLs to xenobiotics suspected of producing immune injury in man. These included TCDD, PCDF, and BaP. The cells were preincubated with 4-5 concentrations of each xenobiotic prior to culture in vitro for 6 days at a 1:1:1 stimulator to responder ratio. Stimulator cells were derived from a cryopreserved pool containing the most relevant HLA-DR antigens. A dose-dependent inhibition of proliferation, independent of changes in cell viability, was seen for all three compounds. Regression analysis provided ID50 values of 0.9, 0.7, and 3.2 μM for TCDD, PCDF, and BaP, respectively. Published pharmacokinetic data for rodents were used for human risk assessment modeling in order to determine the dose required to produce a similar T-cell dysfunction in a 70 kg man. The values were 262 μg TCDD, 1125 μg PCDF, and 51 μg BaP. The quantity of TCDD was significantly greater than any level of dioxin previously measured in an exposed population. This study has shown T-cell dysfunction in PBMLs may be used to evaluate the immunotoxic potential of selected xenobiotics and represents an important adjunct for assisting with risk extrapolation from immunotoxicity testing data obtained in rodents.

Traditionally, immunotoxicology studies have been conducted in rodent models, however, a need exists for a non-rodent model of human relevance. Studies were undertaken to establish immunotoxicology assays in cynomolgus monkeys. Peripheral blood mononuclear cells were obtained and evaluated in a lymphocyte proliferative response, a mixed leukocyte response (MLR) and T & B cell enumeration. A range of concentrations were evaluated for T cell mitogens Concanavalin A (0.1μg/ml-50μg/ml) and Phytohaemagglutinin (0.05 μg/ml-20μg/ml). Pokeweed (1/15-1/60 dilutions) was used to assess B cell proliferation. Peak response on day 3 for Con A was 5 μg/ml (121,000 cpm), for PHA was 1μg/ml (88,000 cpm) and for Pokeweed was 1:60 dilution (25,000 cpm). For the MLR, day 6 was determined to be peak day of response. Pooled cynomolgus leukocytes were used as stimulator cells at a responder:stimulator ratio of 1:2 (6200 cpm (R); 20,000 cpm (S)). T cell (52%) and B cell (18%) enumeration was performed using a direct fluorescent staining method. Both sexes responded comparably in each assay, with exception of the MLR; females had a higher response. Even though studies were conducted on wild caught animals, inter-animal variation is such that the cynomolgus monkey can be used as a non-rodent species for immunotoxicological evaluation.
A review of our approach for immunoassay indicated 3 areas for possible refinement. These included the addition of a 2-cell functional assay to our primary assessment, modification of timing between immunization and dosing regimens, and evaluation of rat strains commonly used in routine toxicology studies for primary immunoactivity testing. The mixed lymphocyte response (MLR), measured T cell function, was compared with the antibody plaque response (APR) to sheep erythrocytes (T-dependent, B-cell function) by identifying desensitization (De) mediated immunoactivity in rats and mice. Both the MLR and APR were suppressed (p < .05) in animals treated for 10 days with De. The relative sensitivity of the assays in identifying this suppression varied with species. Optimum timing for immunization and dosing protocols was based on the APR in mice immunized on days 4, 0 or +3 relative to the final day of De dosing. The APR indicated the greatest sensitivity in identifying De mediated suppression resulting when dosing and immunization protocols completely overlapped. Applicability of the APR assay to rat strains commonly used in toxicology studies was also evaluated. Sprague-Dawley rats gave higher (p < .05) APRs than Fischer or Wistar rats (2130, 920, 690 plating/10⁶ spleen cells, respectively). All responses were sufficient magnitude for use in immunotoxicology studies. The data suggest that limited refinement of our approach may enhance its utility in immunotoxicity assessment.

A rapid screening protocol was used to evaluate the potential immunoactivity of a large number of flavoring ingredients. This protocol incorporated key elements of the NTP's tier testing strategy, including body weight, lymphoid organ weight and cellularity, as well as functional tests of both humoral (antibody plaque-forming cell (PFC) response to sheep erythrocytes) and cell-mediated (L. monocytogenes bacteria challenge) immunity. The test compounds were administered orally to groups of 10-20, 6-8 week old female mice daily for 5 days at 3 dose levels. Infectious challenge or immunization took place on days 3 and 5 of dosing, respectively. The antibody PFC response was measured 4 days after immunization, and mortality and survival were monitored for 10 days post-infection. Cyclophosphamide, 80mg/kg, served as a positive immunoactive control. Positive and vehicle control data collected over a 3-year period in 2 strains of mice (B6C3F1 and 129) demonstrated good reproducibility in the measured parameters. Approximately 100 compounds representing various chemical classes were tested, and fewer than 10% were found to modulate the immune response. This rapid, economical screening battery for potential immunotoxicity means to evaluate a large number of structurally-diverse compounds and mixtures to prioritize them for more definitive testing.
AZIDINE AND CYCLOPROPYL SUBSTITUTED CARBON-7 ACTINOMYCIN D ANALOGS (AZMA AND CPMA): IN VITRO CYTOTOXICITY AND BIOCHEMICAL MODES OF ACTION. D P Rosenbaum and S K Sengupta, Department of Pharmacology, Boston University School of Medicine, Boston, MA. Sponsor: C T Walsh

Two new C-7 substituted analogs of Actinomycin D (AMD), AZMA and CPMA, demonstrate DNA intercalative (agarose gel electrophoresis) and alkylating (DNA broad hyperchromicity) properties. CPMA appears to be more potent than AMD but less potent than AMD in vitro (IC50 mM: CPMA AZMA & AMD: 1110, 90.7, 660; 60.3; B16, 33.9, 87.7, 3.0). In vivo, CPMA is more active than AMD and AZMA against both leukemia and solid tumors (% Increase in life-span: CPMA, AZMA & AMD L1210, 100, 30, 55; B16, 74, 21, 52).

Recently, we have tried to correlate cytotoxicity to DNA double-strand breaks (DSB). DSB are believed to be the major cause of DNA breaks (SSB). In L1210 cells, DSB/SSB rad-equivalent ratios for AMD, AZMA and CPMA are 3.13, 6.67, and 3.85, respectively. The data for AMD did not fit our model until we found that it was very unstable in our in vitro and in vivo test systems (TL and HPLC). Further results on protein-associated strand breaks (PASB) of DNA in L1210 cells and isolated nuclei, and the relative uptake and disposition of these drugs and metabolites in L1210 cells will also be presented.

(Supported by NCT CA 26281-06)

DNA REPAIR IN HUMAN LUNG CELLS EXPOSED TO CHROMATE. H S Park and C M Witmer, Joint Graduate Program in Toxicology, Rutgers University and Robert Wood Johnson Medical School, Piscataway, N.J.

It is known that exposure to hexavalent chromium greatly increases the risk of lung cancer. Previous studies from our laboratory demonstrated that three-hour exposure of A549 human lung cancer cells, which have the properties of type II alveolar cells, to a fifth of a mM caused dose-related single strand breaks in DNA as well as DNA-protein crosslinks. In order to determine whether or not these types of DNA lesions play a critical role in hexavalent chromium induced carcinogenicity, studies of repair of single strand breaks in DNA and DNA-protein crosslinks were carried out by the alkaline elution technique. A549 cells were exposed to 20 mM potassium dichromate (50% inhibitory concentration) for 3 hrs. Eight hours after removal of the potassium dichromate, single strand breaks were completely repaired. In contrast, DNA-protein crosslinks were persistent and had even increased approximately two fold at 10 hr after removal of the chromium. The crosslinks appear to have reached a plateau value at 10 hr which had not changed at 20 hrs. This lack of repair suggests that these lesions may cause permanent DNA damage.

IN VITRO TOXICITY OF TRISODIUM NITROTRIACETATE (Na Nitrite) AND ZINC NITROTRIACETATE (Zn-NTA) IN RAT PROXIMAL TUBULE EPITHELIAL (PTE) CELLS. K A Becher, B L Anderson, A A LeBeouf and B F Trump. Dep't of Path., Univ. of MD Sch. of Med. and MIESS, Balto., MD, and Human and Environmental Safety Division, Proctor & Gamble Co., Cincinnati, OH. Sponsor: T M Jones

In vivo doses of Na Nitrite lead to acute renal cell injury characterized by cytoplasmic vacuolization and hyperplasia. Long-term studies at high doses of Na Nitrite result in renal necrosis. One hypothesis is that these effects are secondary to delivery of znic to PTE cells, which absorbs zinc but not NTA. Therefore, we studied the in vitro effects of NaNitrate and ZnNaNTA on rat PTE cells as a model for mitogenicity studies. Exposing PTE cells to NaNitrate and ZnNaNTA causes a time- and dose-dependent drop in viability. Cells were treated with each compound (10, 20, 50, 100 M), 4 and 24 h. Viability was measured by uptake of neutral red. Viability after 4 h exposure to 100 M NaNitrate and ZnNitrate was 93% and 92%, respectively. After 24 h continuous exposure, viability was 45% and 43%, respectively. Lower doses (10, 20, 50 M) of both compounds were not toxic to rat PTE cells by this assay. These results indicate that neither form of NTA induces cell death at doses that can be obtained in vivo when ingested at cardiogenic doses in rats. Na Nitrate in the diet produces maximal blood NTA of 2.2 M.

CELL REPLICATION IN MALE AND FEMALE MOUSE LIVER AFTER INHALATION EXPOSURE TO UNLEADED GASOLINE. T L Goldsworthy, C S Sprankle, J Slasser, and B E Butcher. CIIT, Research Triangle Park, NC. Sponsor: H Heck.

Chronic inhalation exposure to unleaded gasoline (UG) induced an increase in liver tumors in female but not male mice. Nongenotoxic mechanisms such as hyperplasia may play a role in this carcinogenic process. Unexpectedly, gavage administration of UG showed an increase in the labeling index (LI) at 24 hr in male but not female mice (Tox. Appl. Pharmacol. 85, 11 (1986)). To investigate cell replication under conditions that produced tumors, B6C3F1 mice were exposed to 2000 ppm PS-6 blend of UG vapors 6 hr per day, 5 days a week, for 6 weeks. No changes in day 4 serum enzymes or liver palmitoyl CoA oxidation activity were observed. Histocitotoxicidogenic LI after a single pulse of H-thymidine in the mice were 0.06, 0.2, 0.2 and 0.5% for control, day 1, 4, and 39, respectively. LI for females were 0.1, 1.2, and 0.8%. LI results using 7-day minipumps in males were 1.4 and 7% for control, week 1 and 6. LI for females were 0.4 and 20% after 1 week. One week after ending UG exposure LI returned to control levels. While the fold increase of LI over controls was similar in both sexes, the absolute LI was up to ten times greater in the treated females. These data illustrate the importance of measuring LI under conditions of the bioassay and suggest that UG-induced cell replication may be related to the sex-specific pattern of tumor production.
829 DIETARY LIPID MODULATION OF XENOBOTIC METABOLIZING ENZYMES IN MICE TREATED WITH DIMETHYLBENZ(A)ANTHRACENE. M H Silva, L A Brody, D Mitchell, L J Faulkin, and R D Hammock. Departments of Entomology & Environmental Toxicology, University of California, Davis, CA. Sponsor: C N Allday.

N-3 fatty acids such as linoleate have a promotional effect on incidence of some types of cancer and certain marine fish oils such as Menhaden oil appear to decrease tumor incidence/increase tumor latency. To elucidate the effects of n-3 and n-6 polyunsaturated fatty acids on the xenobiotic metabolism in Lewis, BALB/c mice fed defined diets for 40 weeks, after an initial treatment with DMBA. The diets were: chow; 1, 10 and 20% corn oil (CO); 10% Menhaden oil (MO); 10% hydrogenated cottonseed oil (HCO); 1%CO+9%MCO; 1%CO+9%HCO; 10%CO+0.0027 indomethacin (IN). Results showed no diet-related effects on body weight, liver weight, cytosolic or microsomal protein. Specific activities of cytosolic epoxide hydrolase (EH), catalase and glutathione peroxidase were increased in animals fed diets with MO. Glutathione-S-transferase specific activities were highest in groups fed diets containing primarily HCO. P<0.0150 activity was decreased and microsomal EH was increased for all synthetic diet groups compared with chow. Cholesterol EH activity showed no difference from chow and DT-diaphorase activity was elevated in some groups with no dietary pattern.


Multiple forms of glutathione (GSH) S-transferase (GST, EC 2.5.1.18) are present in human tissues. The placental form of GST (GST π) is reported to be elevated in some tumors and has been suggested to be a marker of carcinogenesis. In present studies we have compared the levels of GST and the related enzymes in five human lung tumors and the adjacent normal tissue from the same subject. As compared to the normal tissue, GST activity was elevated in two tumors and suppressed in one tumor. In the other two tumors, GST activity was similar to that in the adjacent normal tissue. Metchnikoff temperature using the antibodies against GST μ of placenta indicated that GST μ type isoenzyme(s) were induced in two, suppressed in one, and were unaltered in the remaining two tumors. GSH reductase activity was found to be elevated in all five tumors whereas GSH levels were elevated in all but one tumor. These results were also consistent with the results of studies with three small cell lung cancer cell lines suggesting GSH and the related enzymes are differentially affected in lung tumors. Particularly, GST μ type isoenzyme(s) are not uniformly elevated in all tumors and therefore, may not be ideal as a marker of carcinogenesis. (CA 27967 and GM 32304).


Previous epidemiologic studies have indicated that patients with bladder cancer known to have been exposed to arylamines in the workplace exhibit an unusually high proportion of the slow arylamine acetylator phenotype. We compared the capacities of human liver postmitochondrial preparations (S9) from 9 organ transplant donors to N-acetylate SMZ and BZD to test if BZD is acetylated in parallel with SMZ, a substrate for polymorphic acetylation. The S9 and arylamines were incubated with a 10-fold excess of acetyl coenzyme A. Parent substrates and N-acetyl metabolites were measured after microbore liquid chromatography by UV absorption maxima for quantitation. We observed a range of acetylation of SMZ from 7.6 to 39%, with an antimode at 24%, yielding 5 slow and 4 rapid acetylators. The range for BZD was 56 to 100%. Linear regression analysis demonstrated a significant direct correlation (r = 0.718; P <0.02) between the acetylation of SMZ and BZD. These results support the epidemiologic association of bladder cancer with slow acetylator phenotype and justify the assessment of arylamine exposure with SMZ as an estimate of risk from arylamine exposure (Supported in part by NIEHS Contract ES-55109 and SRI Project 392D32-57).

832 EFFECTS OF BUTYLATED HYDROXYANISOLE (BHA) ON MOUSE LIVER GLUTATHIONE S-TRANSFERASE (GST) ISOENZYME ACTIVITY TOWARD AFLATOXIN B1-8,9-EPOXIDE (AFBO) AND BENZO[α]PYRENE-7,8-DIHYDRODIOXIDE-9,10-EPoxide (BPDE). H S Ramsdell and D L Eaton. Department of Environmental Health, University of Washington, Seattle, WA.

We have studied the effects of BHA, a dietary antioxidant which induces GSTs and inhibits chemical carcinogenesis, on levels of hepatic GST isoenzymes and their conjugating activity with environmental carcinogens. Mice were fed either chow with or without 0.75% BHA. Liver cytosol was prepared, GSTs were isolated by affinity chromatography and isoenzymes purified by chromatofocusing. GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB), AFBO and BPDE. Three major GST isoenzyme peaks were observed in control samples (MI, MII and MIII) and these accounted for about 5, 50 and 20% of the total GST, respectively. Four minor peaks were also present. With BHA treatment MII was the predominant isoenzyme, such that MI,II and III constituted 5, 15 and 60%, respectively. MIII had the highest specific activity with CDNB but relatively little activity with AFBO. MI accounted for the majority of the AFBO conjugating activity but had low specific activity with CDNB and was not increased by BHA treatment. MI and MII were low with BPDE, both about 10% of that of MIII. Thus, BHA treatment of mice induces a GST isoenzyme that does not appear to contribute substantially to the detoxification of AFBO or BPDE but has high specific activity with CDNB. These results illustrate the limitations of reliance on surrogate substrates to assess GST activities in liver cytosol fractions. (Supported by NIH grants T32 ES-07032, ES-03935 and CA-47561.)
ACTIVATION OF 1,6-DINITROPYRENE BY AN OXYGEN-INSENSITIVE NITROREDUCTASE. P L McCuneagle and E Djuric. Dept. Gynecology-Obstetrics, Wayne State University, Detroit, MI.

1,6-Dinitropyrene is a carcinogenic pollutant that is activated via reductive metabolism. We have examined the properties of rat liver cytosolic nitroreductase. The cytosol-catalyzed DNA- and protein-binding of 1,6-dinitropyrene was the same in aerobic and anaerobic incubations. This indicates that the reactive N-hydroxylamine derivative can be formed under aerobic conditions. Based on inhibitor studies, 1,6-dinitropyrene is not appreciably metabolized by known reductive enzymes in rat liver cytosol. The cytosolic nitroreductase was partially purified and has a molecular weight of about 500,000. In incubations with the crude enzyme fraction, amine and nitroso metabolites were formed. Addition of oxygen to these incubations had no effect on metabolite formation. In cytosolic incubations, however, formation of the amine metabolite was decreased by the presence of oxygen while formation of the nitroso metabolite was increased. It therefore appears that the partially purified nitroreductase, unlike whole rat liver cytosol, does not produce metabolites that react with oxygen. The ability of the nitroreductase to metabolize 1,6-dinitropyrene in aerobic cells may be an important factor contributing to the potent carcinogenicity of 1,6-dinitropyrene in laboratory animals.

Carcinogenicity of Iron with Hexachlorobenzene in C57Bl/10ScSn Mice. A G Smith, J E Francis, P Curthow, M M Manson and J R P Cabral*. Toxicology Unit, Medical Research Council, Carshalton, Surrey, UK, and *International Agency for Research on Cancer, Lyon, France.

Studies on the mechanism of hepatotoxicity of polychlorinated aromatic hydrocarbons implicate a role for iron especially in the ability of these chemicals to induce uroporphyrin. Iron overload greatly sensitises C57Bl/10ScSn mice to the porphyrinogenic action of hexachlorobenzene (HCB) which also causes hepatic tumours. The influence of iron on the carcinogenicity of HCB was determined. Male C57Bl/10ScSn mice received s.c. 12 ml/kg Inferon (iron-dextran; 600 mg Fe/kg) and after one week were fed control diet or one containing 0.1% HCB for up to 18 months. No hepatic hyperplastic nodules were observed in iron-loaded mice. HCB gave a 5% incidence of nodules in combined 12 and 18 month groups which did not receive iron. In contrast, treatment of iron-loaded mice with HCB caused iron-excluding foci of hepatocytes at 3-6 months. After 12 and 18 months 55% and 100% of mice respectively had advanced hyperplastic nodules which were iron-excluding. In the 18 month group 90% also had hepatocellular carcinoma. Another finding was that iron alone induced porphyria after 3-6 months. Thus the combination of a single iron dose and dietary HCB is synergistic in causing liver cancer.

The increased formation of altered foci in the livers of aflatoxin B1 treated partially hepatectomized rats resulting from ethoxyquin administration. G E Neal1, H G Mandel1, M M Manson1 and J R P Cabral1. Toxicology Unit, MRC Laboratories, Carshalton, UK, 1Dept of Pharmacology, George Washington University, Washington DC, USA, and 2International Agency for Research on Cancer, Lyon, France.

The dietary administration of ethoxyquin (EQ) to rats protects against the carcinogenic action of aflatoxin B1 (AFB1) due to the induction of detoxifying phase II metabolism by EQ. GST is induced with a zonal distribution. The early stages in chemical hepatocarcinogenesis, however, also involve the induction of cell populations resistant to the cytotoxic action of the carcinogen (with a focal distribution). The possibility that a proliferative response provoked by 1/4 partial heptectomy (PH) in the livers of EQ-treated rats, might potentiate the carcinogenic action of AFB1 has been examined. Results of experiments using F344 rats demonstrated that the number of altered cell foci (52 weeks later) was increased by 82% in rats fed a diet containing 0.5% EQ and 4 ppm AFB1 for two weeks following PH compared with PH rats fed AFB1 diet alone, and increased 30% in rats given EQ for two weeks prior to PH followed by two weeks feeding with EQ and AFB1. The significance of these results will be discussed in relation to parameters believed to be involved in hepatocarcinogenesis.

Importance of immune surveillance in chemical-induced carcinogenesis. J L Bussiere, J H Exon, and G G Mather. Dept. of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Univ. of Idaho, Moscow, ID.

The importance of immune surveillance was tested in a 3-methylcholanthrene-induced tumor model. Specifically, this study investigated the role of non-specific cell-mediated immunity in carcinogenesis, through natural killer (NK) and macrophage effector cells. Sprague-Dawley rats were treated with specific antibody to NK or macrophages during specific periods of carcinogenesis: 0-2 weeks (initiation, early promotion), 5-7 weeks (pre- early tumor formation), 10-12 weeks (after tumor formation), and all 3 time periods. Tumor incidence and latency, as well as the effect on specific immune parameters, were measured following each treatment period to determine the efficiency of the Ab treatment. Measurement of immune function included NK activity, and production of IL-2, PGE2, and IFN. There was an increase in tumor incidence in NK-depleted rats, while rats treated with anti-Mac Ab showed a decrease in tumor incidence. This may have been due to an increase in NK activity seen in this group. Timing of treatment was also important, with the initiation, early promotion (0-2 weeks), and all 3 time period treatment having the greatest effect. This study indicates the importance of non-specific cell-mediated immune surveillance in chemical-induced carcinogenesis.

Incidence of hepatocellular carcinomas and development of glutathione S-transferase placental form (GST-P) positive foci were assessed in rats administered phenobarbital (PB) or 2-acetylamino-fluorene (2-AAF) after initial treatment with clofibrate (CP). Male, 7-week-old F344 rats were divided into 5 groups: groups 1 and 2 were fed 0.3% CP containing diet for 30 weeks, and then 0.05% PB or 0.01% 2-AAF diet up to week 78. Group 3 was given 0.3% CP only for 30 weeks. Groups 4 and 5 were given 0.05% PB or 0.01% 2-AAF diets starting at week 30. All rats were subjected to two-thirds partial hepatectomy (PH) at week 3. The area of GST-P-positive lesions was markedly decreased in group 2 as compared to group 5 at the 48 and 78 week time points. Furthermore, the respective incidence of hepatocellular carcinomas were 1/17 (CP-2-AAF) and 7/7 (2-AAF). No significant differences between group 1 and 4 were evident with regard to either GST-P positive foci or tumors. From these results, treatment with CP appeared to inhibit generation GST-P-positive foci and hepatocellular carcinomas by 2-AAF administration. GST-P-negative foci or tumors were not observed in any of the groups, and therefore under the present experimental conditions, CP did not itself appear to act as an initiator.

B-CELL LYMPHOMA/LEUKEMIA (BCL) IN RATS AND MICE TREATED WITH 2-HYDROXYETHYL-NITROSUREA (HENU) AND EFFECTS OF COADMINISTRATION OF 2,4,5-TRICHLOROPHENOXYCETIC ACID (245T) AND PENTACHLORPHENOL (PCP). S S Mirvish, J Nickols, D D Wiener, S J Temp, H H, and Eppley Inst Res Cancer, Univ Neb Med Ctr, Omaha, NE

We found BCL induction in MRC-Wistar rats treated chronically with HENU (JNCI 78:287). Since BCL is common in Midwest farmers, we repeated the test with cotreatment by 2-pesticides linked with BCL in Sweden. GC-MS analysis showed 0.8 and 2.7 ppb tetrachlorodibenzo-p-dioxin and 1.5 and 670 ppb tetrachlorodibenzofuran in 99% pure 245T and 86% pure PCP (Aldrich Chem). MRC-Wistar rats received chronically 75 mg HENU/liter as before and/or 600 mg 245T or 500 mg PCP/kg diet. Tumor incidence: Acute myelocytic leukemia, 10% of HENU-treated rats vs 6% of rats treated with HENU + PCP. BCL: 21% in HENU group, not affected by pesticides. Liver tumors, mostly adenomas: 16% of HENU group but 40% (6/15) of females given HENU + PCP and 67% (6/9) of females given PCP alone. HENU induced BCL in 77% and 52% of Swiss mice given 75 or 38 mg HENU/liter water (controls, 4%), with much higher incidence in females. BCL was confirmed by phenotyping surface antigens and by gene rearrangements. Hence in rats PCP induced liver tumors and perhaps enhanced AML induced by HENU. Grants: Neb Health Dept 65-38, 89-39; NIH CA36727, ACS SIG-16.

NO SYSTEMIC CARCINOGENIC POTENTIAL FROM SKIN APPLICATION OF PETROLEUM HYDROCARBONS (PHC). J J Freeman, S C Lewis, R H McKee, and R D Phillips. Exxon Biomedical Sciences, Inc., East Millstone, NJ

Certain petroleum-derived materials particularly those rich in polycyclic aromatic hydrocarbons, are carcinogenic to mouse skin. However, the carcinogenic activity to internal organs of petroleum liquids following dermal exposure has been largely unexplored. Thus, Exxon data from skin painting studies on 131 materials was evaluated for associations (if any) between the chronic dermal application of PHC and the development of primary systemic tumors. The laboratory reports were reviewed for nonmetastatic systemic tumor incidences initially judged to be significant (statistically or biologically). Incidences of the following classes of tumors initially appeared to be elevated in one or more treatment groups: spindle cell sarcomas of the skin, myelogenous leukemias, lymphoid neoplasms, hepatocellular tumors, hemangiosarcomas of the liver, pulmonary adenocarcinomas, mesotheliomas and astrocytomas. However, closer examination of classes of oils tested and the database as a whole indicated no relationship between treatment and the development of nondermal tumors. Rather, the tumors were judged to be spontaneous in origin and fortuitous in distribution. Thus, there was no clear evidence that dermal application of PHC distillates elicited tumors other than squamous cell tumors of the skin.

CARCINOGENIC POTENTIAL OF GASOLINE AND DIESEL ENGINE OILS. R T Plutnick, and R H McKee. Exxon Biomedical Sciences, Inc., East Millstone, NJ

The present studies used the mouse skin model to assess the dermal carcinogenic potential of a series of fresh and used engine oils from both gasoline and diesel engines. The objective of this study was to assess the potential carcinogenic hazards associated with exposure to these materials.

The fresh gasoline engine oils were noncarcinogenic and were low in polycyclic aromatic hydrocarbon (PAH) content. The majority of the used gasoline engine oils were dermal carcinogens, and PAH levels were elevated. Both the fresh and used diesel engine oil samples were noncarcinogenic, and PAH levels in the used samples were not significantly different from the fresh oils. The carcinogenic potency of used gasoline engine oils was related to drainage interval, but other factors including contribution of the fuel due to blowby and driving cycle may also have been important. The used diesel engine oils were not carcinogenic even after extended use.
CARCINOGENICITY STUDY OF THE PESTICIDE PENVALERATE IN MICE. J R P Calvili and D Galendo. International Agency for Research on Cancer (IARC), Lyon, France.

Penvalerate is a pesticide widely used in agriculture. Recent mutagenicity studies on a series of synthetic pyrethroids provided no evidence of the mutagenicity of Penvalerate. Penvalerate was studied in a long-term experiment for carcinogenicity in mice. Inbred C57BL/6 mice were given Penvalerate by gavage at three dose levels (0, 40, and 80 mg/kg bw) five days a week for 104 weeks. All survivors were killed at 120 weeks. Survival rates were slightly affected by deaths due to toxic manifestations of Penvalerate in the females receiving 60 mg/kg bw. In all experimental groups various types of tumours were observed. An increased incidence of liver-cell tumours was observed in male mice receiving 80 mg/kg bw Penvalerate. No significant difference in the incidence of other types of tumours was observed in treated groups when compared with controls. Penvalerate-induced granulomas occurred concomitantly in the liver, spleen and lymph nodes of male and female mice. The overall incidence of these granulomas did not increase with dose. However, in the present experiment, the no-effect level for these lesions could not be established.

PREVENTION OF NICKEL SUBSULFIDE CARCINOGENESIS BY LOCAL ADMINISTRATION OF M. BOVIS ANTIGEN. K S Kaspierzak and J M Ward, Laboratory of Comparative Carcinogenesis, National Cancer Institute, FCH, Frederick, MD.

This study was designed to determine the effect of local inflammation on nickel subsulfide (NiSulf) carcinogenesis. Male, F344/WCr rats, 60 - 100 g, 20 rats/group, received single intraperitoneal injections into both hind limbs of 2.5 mg of NiSulf alone or with 0.5 mg of M. Bovis lyophilized cell walls (MB), 1 mg Cortisol (CORT), or 1 mg Indomethacin (IND). Two groups of rats received NiSulf combined with sc (neck area) injection of 1 mg MB, or 2 mg IND. At 60 wk after the injections, the yield of muscle tumors was 90% in the NiSulf group. 86% in NiSulf+CORT, and 75% in NiSulf+IND (not different statistically). No injection site tumors were found in rats given NiSulf+MB. However, the sc MB injection enhanced carcinogenicity of NiSulf by shortening the latency and increasing the yield of tumors to 100% at wk 39. The sc treatment with IND gave similar result: 95% tumor yield was reached in 41 wk. Although local treatment with CORT or IND had no effect on the final tumor incidence, it shortened the latency of tumors to 17 wk compared with 23 wk for NiSulf only. MB, CORT, IND, or the injection vehicle (water) alone did not produce tumors. The prevention of NiSulf tumors by local MB may result from attraction of numerous macrophages and formation of giant cells which could both immobilize the carcinogen and destroy NiSulf-transformed cells.


3,2-Dimethyl-4-aminobiphenyl (DMAB) is a carcinogenic aromatic amine with a wide carcinogenic activity. It induces carcinomas in the colon, small intestine, urinary bladder, mammary gland, preputial glands and subcutis in rats. However, no pancreatic tumor has been reported. We recently found the development of pancreatic adenocarcinoma in male F344 rats treated with DMAB ranging from 50 to 167 mg/kg bw. In two 60-week experiments originally designed for the study of prostate carcinogenesis. In one experiment, in which rats aged 3 weeks were given 3 consecutive weekly s.c. injections of DMAB at a dose of 150 mg/kg and were killed 57 weeks later, the incidences of foci, nodules, adenomas and carcinomas in situ were 17/17 (100%), 17/17 (100%), 8/17 (47%) and 1/17 (6%), respectively. In this experiment, no apparent effects of subsequent treatment with high fat or beta carotene after DMAB treatment on pancreatic lesions were observed. Tumor incidence for the pancreas was more effective when the dose of DMAB was higher and necrogenic to the pancreas. Induced pancreatic lesions were essentially the same as those reported in rats treated with asenure and 4-hydroxyaminoquinolino-1-oxido.

CHRONIC TOXICITY STUDIES OF o-BENZYL-p-Chlorophenol in F344 Rats and B6C3F1 Mice. M Heinmanton, M Ryan, A Peters and I Glenn. Battelle Columbus, Ohio and NIEHS, Research Triangle Park, NC.

o-Benzyl-p-chlorophenol (CAS No. 120-32-1) is an aryl halide biocide with widespread occupational and industrial exposure. Gavage studies were conducted to assess chronic toxicity. Rats were administered 0, 10, 50, 120, and 240 mg/kg bw body weight of test chemical in corn oil 5 times weekly for 103 weeks; mice were given 0, 120, 240, and 480 mg/kg. In female rats, treatment produced a reddish-yellow urogenital staining that was dose and time dependent. High dose rats showed alterations in the excretion of urinary enzymes that included an increase in alkaline phosphatase and a decrease in galactosidase and in N-acetyl-B-glucosaminidase. In mice, treatment produced decreased survival and clinical signs of toxicity (rough haircoat and thin appearance) in high dose mice with a dose-related depression in body weight gain, efficiency of food utilization, and kidney weight. Gross renal lesions were observed in high dose mice at necropsy. The kidney appears to be a target organ in both rats and mice which is in agreement with predictions made from the results of prechronic and chemical disposition studies. (Supported by NTP Contract N01-ES-45042).
HEPATOTOXIC EFFECTS OF DICHLOROACETATE (DCA) AND TRICHLOROACETATE (TCA) IN B6C3F1 MICE. I M Sanchez and R J Bull. Pharmacology/Toxicology Graduate Program, College of Pharmacy, Washington State University, Pullman, WA.

DCA and TCA induce hepatocellular tumors in B6C3F1 mice. DCA tumor induction is closely associated with increases in liver weight caused by the chemical, while this is less apparent with TCA. Our purpose was to relate differential liver pathology of DCA and TCA to their tumorigenicity. Mice were given DCA or TCA at doses of 300, 1000 and 2000 mg/L in drinking water for 14 days. Two hours before sacrifice animals received an injection of 3H-thymidine. Liver sections were stained with H&E or PAS and contiguous sections were taken for autoradiography. DCA-treated animals displayed dose-related hypertrophy, accumulation of glycogen, areas of focal necrosis, and the number of cells labeled with 3H-thymidine increased by 10-fold at 1000 and 2000 mg/L relative to 300 mg/L and control, whereas TCA treatment caused only a two-fold increase at 2000 mg/L. TCA-treated animals had little evidence of necrosis, glycogen accumulation or increased cell size. These results show that the hepatotoxic effects of DCA probably account for an unusually steep dose-response curve relative to TCA. (Supported by U.S. Air Force Grant #AFOSR-86-0284).


Unrefined lubricating oils contain relatively high levels of poly cyclic aromatic hydrocarbons (PAH) and have been shown to induce tumors in mouse skin. Exxon has developed a method of refining these materials, a proprietary severe hydrotreatment process which is optimized for removal of PAH, sulfur and nitrogen. The specific objectives of the current study were to assess PAH reduction and to then directly evaluate the dermal carcinogenic potential of materials produced by this method. The test samples consisted of unrefined light and heavy vacuum distillates from a naphthenic crude oil as well as the corresponding hydrotreated products. Positive, negative, and vehicle control groups were also included. Each sample was applied in twice-weekly aliquots to the back of 40 male C3H/HeJ mice for two years. Analytical studies demonstrated significant reductions in the levels of several specific PAH, as well as product sulfur and nitrogen. In the dermal carcinogenesis studies the unrefined oils and the positive control sample induced tumors whereas oils produced by the proprietary severe hydrotreatment process and negative controls did not. Thus the data demonstrated that this process was an effective means of converting carcinogenic feedstocks to noncarcinogenic products.

EVALUATION OF CHRONIC AND REPRODUCTIVE EFFECTS OF p-NITROCHLOROBENZENE (PNCB) IN RATS. R S Nair, P R Johannsen, Monsanto Company, St. Louis, MO, R E Schroeder and C S Auletta. Bio/Dynamics Inc., East Millstone, NJ.

PNCB dissolved in corn oil was given by gavage to SD® rats (60/sex/group) at dosages of 0, 0.1, 0.7 or 5.0 mg/kg/day for 2 yrs. Body weights, food consumption, blood (hematology and clinical chemistry) and urine were examined periodically. All gross lesions and over 40 tissues were examined microscopically. In this study, PNCB was not carcinogenic. Significant increases in methemoglobin levels were noted in the mid- & high-dose group; slight anemia was noted throughout the study in the 5 mg/kg group. Spleens showed brown pigment in reticuloendothelial cells. Separate groups of 15 male and 30 female rats were given the same dosages of PNCB as in the chronic study for about 14 weeks prior to mating, during mating, gestation and lactation. F₁ parental rats were similarly dosed from prior to mating through lactation. Gross observations, body weights and reproductive parameters were recorded. Other than a slight decrease in pregnancy rate and male fertility index in the 5.0 mg/kg group in the F₂ generation, no reproductive effects were observed in F₀ or F₁ generation.

CARCINOGENICITY STUDIES OF WOLLASTONITE IN RATS. B Adkins, Jr, E E McConnell*, and L Hall*. NSI-Environmental Sciences and National Toxicology Program/NIEHS, Research Triangle Park, NC.

Wollastonite is a naturally occurring calcium metasilicate acicular mineral which is used in a variety of commercial applications and has been proposed as an asbestos substitute. Groups of male Fischer 344 rats were exposed in Binnner's type inhalation chambers to 0.0 to 360 fibers/cc of wollastonite (WA-10) for 6 hours/day, 5 days/week for 12 or 24 months and were then held for lifetime observation (until 102% survival). They were compared to nonexposed chamber controls and positive controls (UTCC chrysotile asbestos). Six rats from each group were sacrificed at 3,12 and 24 months and the lungs were examined histopathologically. The incidence and severity of pulmonary fibrosis of wollastonite exposed rats was comparable to the control group while asbestos was moderately fibrogenic. The incidence of pulmonary neoplasms (benign and malignant combined) was 1/56 (controls), 0/57 (12 month wollastonite exposure), 1/60 (24 month wollastonite exposure) and 20/52 (12 month chrysotile asbestos exposure). The results of this study show that wollastonite under the conditions of this study was not fibrogenic or carcinogenic in male Fischer 344 rats. (Supported by NIEHS Contract NO1-ES-4-5044).
Data from a 21 month oncogenic bioassay in CD-1 mice (Charles River, Wilmington, Mass.) were analyzed to see if the staggered placement onto and removal from it were associated with large variations in control data. All naive control (NC; \( n = 57/sex \)) and 67% of vehicle control (VC; \( n = 62/sex \)) mice were begun in groups (\( n = 13 \)) from 7/9-9/19/77. VC mice were fed Charles River Powdered Rat/Mouse Food #3200 with pure corn oil as vehicle. An USEPA GLP audit found the study in compliance. Data were analyzed by t-test, ANOVA, chi-square, Fisher's Exact Test and product limit life tables (P.S0.05). Comparisons of NC to VC groups for each sex were rare statistically, but not biologically, significant for: weekly body weights and food consumption; hematology; clinical chemistry; organ weights and organ/body ratios; survival; incidences of tumor and non-tumor lesions. Preliminary analyses indicate a similar lack of significant differences when the 4 or 5 subgroups in each group were compared.

Thus placement of groups of mice onto a chronic bioassay over several weeks did not introduce large variations into the data while making terminal sacrifices and autopsies easier. The incidences obtained from the subgroups provide a range of values which may more accurately reflect the true biological response of the strain than the single incidence value obtained if each group is treated as a single unit.

The chronic toxicity and oncogenicity of levobunolol, a nonselective beta adrenoceptor antagonist, was evaluated in Swiss mice and Wistar rats. The drug was administered in the diet to mice at 0, 12, 50, and 200 mg/kg/day for 80 weeks and to rats at 0, 0.5, 2, 5, 30, and 180 mg/kg/day for 2 years. In mice, a dose-related suppression of body weight gain was apparent at Week 52 but not at Week 80. Uterine leiomyomas were present in 4 of 50 females at 200 mg/kg but not in any other group. The incidence of all other tumor types, as well as gross and microscopic findings, was similar among dose groups. In rats, significant body weight gain suppression occurred at 5, 30, and 180 mg/kg. Brown discoloration of perianal fur and steel-blue discoloration of hairless skin were evident in high-dose animals. No other differences between treated and control groups were evident. The relevance of the increased incidence of uterine leiomyoma in mice to man is questionable because it occurred only in one species at more than 200 times the projected therapeutic dose.

Ingestion of opium pyrolysates, containing a novel class of highly genotoxic compounds, has been associated with a high incidence of oesophageal cancer in north-east Iran (Friese et al., Carcinogenesis, 8, 1423-1432, 1987). The pyrolysate of morphine, a major opium alkaloid, was therefore studied for carcinogenicity in mice and rats.

Groups of 27-30 male and female C57 BL/6 mice, six weeks old, were fed by gavage 0, 2.5, 5 or 10 mg/kg bw MD once a week for 114 weeks. The treatment had no effect on body growth or survival rates. No significant difference in the incidence of tumours was observed in MD-treated groups compared to controls.

MD was also administered by gavage at the dose of 10 mg/kg bw to BDF1 female rats on days 15-19 of pregnancy. Survival rates both before and after weaning were similar in the progeny of the treated and control animals. MD increased the incidence of pituitary tumours in the female progeny and produced two neurogenic tumours in the male progeny. These results, together with previous studies, reveal the potential carcinogenicity of opium pyrolysates.

High body weight gain in PB-treated mice was correlated with formation of multiple hepato-cellular adenomas, while no or only single lesions occurred in those animals with low body weight gain (Wolff et al., Carcinogenesis 7: 1935, 1986). To assess possible differences in the PB induction of P450, yellow A\(^{T}/A\) (C3H x C3H) P-1 hybrid male mice were fed 0.05% sodium PB in the NIH-31 diet for 7 months. Livers from the heaviest and the lightest mice in the untreated and PB groups were assayed for total cytochrome P450 content, 7-ethoxycoumarin-O-deethylase (EROD) and pentoxycoumarin-O-deethylase (PROD) activities. Total P450 content was induced 61% in the PB-treated light mice but only 32% in the comparable heavy mice. In contrast, P450IIB1-specific PROD activity was increased by PB 5-fold in the heavy mice but only 3-fold in the light mice. P450IIB1-specific ER activity was increased 84% and 70% in the light and heavy mice, respectively. These data suggest that the regulation of inducibility of the mouse P450IIB1 gene expression differs between the two phenotypic subpopulations and is correlated with promotion of liver tumors.
853 INHIBITION OF LIVER TUMOR FORMATION BY ALPHA-
HEXACHLOROCYCLOHEXANE (a-HCH) IN DIETHYL-
NITROSAMINE (DEN) INITIATED B6C3F1 MICE.
J C Siglin, 1, 2 C M Weghorst, 3 D B Rodwell 4 and
J E Klaunig 5. Springborn Life Sciences, Inc.,
Spencerville, OH and 2Dept. of Pathology,
Medical College of Ohio, Toledo, OH.

A-HCH is an isomer of Lindane, a commercial insecticide. Studies with a-HCH have shown this isomer to be a potent liver tumor promoter in chemically initiated rod-
ents. The present study examined the effects of a-HCH in B6C3F1 mice initiated with DENA. The study consisted of 4 groups. Each group contained 15 male and 15 female mice. At 15 days of age, mice in groups 2 and 4 received a single ip dose of DENA (5 μg/gbw); mice in groups 1 and 3 received saline. Beginning at 4 weeks of age, the mice were fed either basal diet (groups 1 and 2) or a diet containing 250 ppm a-HCH (groups 3 and 4). After 24 weeks of exposure, mice from each group were sacrificed and necropsied. All males treated with DENA exhibited hepatic tumors. In DENA treated male mice that received a-HCH, the mean number of hepatic tumors was significantly decreased suggesting an inhibitory effect due to a-HCH. In initiated females receiving a-HCH, an increased incidence of liver tumors was observed suggesting promotion.

855 FREZING INITIATION OF BLADDER CARCINOGENESIS
PROMOTED BY SODIUM O-PHENYLPHENATE (OPP- Na) OF
RAT URINARY BLADDER. N Shinojo, R Hasegawa,
K Imaida, M Takahashi, N Ito, and
K Hayashi. Division of Pathology, National
Institute of Hygienic Sciences, 1-18-1 Kamiyoga,
Setagaya, Tokyo, Japan; 2First Department of
Pathology, Nagoya City University Medical
School, 1-Kawasumi, Mizuho Nagoya, Japan.

Urinary bladder tumor initiating activity of in
situ freezing was investigated on two-stage carcinogenesis in male F344 rats. OPP- Na was used as a promoter in this experiment. At the start of the experiment, freezing was performed by touching the serosal surface of the bladder with a frozen steel rod. 2% or 0% OPP- Na feeding was started 6 weeks after freezing and continued until the end of experiment. Animals were sacrificed at week 36, and all of urinary bladder were examined histopathologically. As a result, 19 out of 25 rats (76%) developed bladder tumors (carcinomas in 12 rats and papillomas in 7 rats) treated with freezing and 25 OPP- Na feeding, whereas only one animal (5%) demonstrated a bladder tumor (carcinoma) in the group given 25 OPP- Na without prior freezing. No tumors were induced by freezing followed by 13 OPP- Na feeding and freezing alone. The result thus confirms the promoting effect of OPP- Na and suggests that in situ freezing of the bladder exerts tumor-initiating activity in two-stage rat urinary bladder carcinogenesis.

854 TEST OF ALKYL CARBAMATES FOR TUMOR INI-
TIATION AND FOR TUMOR PROMOTION IN RAT
LIVER. M A Pereira, H P Glauert,
M M Khoury, P Barnwell, M Hensley and
A Davis. Environmental Health Re-
search and Testing, Inc., Cincinnati, OH; 2University of Kentucky, Lexington, KY; 2American Cyanamid Company, Wayne, NJ.

Five alkyl carbamates (C), methyl C (MeC),
ethyl C (EtC), propyl C (PrC), hydroxypropyl C (HPC), and ethynylphenyl C (EHC), were tested for tumor initiating and pro-
moting activity in rat liver. The test for tumor initiating activity consisted of administer ing by gavage to male S-D rats received 500 ppm sodium phenobarbi-
tal in their drinking water until sacri-
ficed ten weeks later. None of the C at
1/5 the LD50 induced gamma-glutamyltrans-
peptidase (GPT)-positive foci, indicating lack of tumor initiating activity. The tumor promoting activity test consisted of initiation with 80 mmole/kg diethyl-
nitrosamine administered 18 hrs. after a
PH. One week later, the rats started to receive five days a week the C at either
1/10 or 1/20 the LD50 until sacrificed
ten weeks later. The alkyl C increased
GPT mean and total foci volume, the
number of focci/cm³ (not EHC), and the
number of foci/liver (not EHC). These results suggest that the alkyl C are tumor promoters and did not demonstrate tumor initiating activity.

856 HEPATOCYTE PROLIFERATION IN THE MOUSE
AND RAT FOLLOWING ACUTE TREATMENT WITH
TRICHLOROETHYLENE. K L Fry, C M
Weghorst, and J E Klaunig. Department of
Pathology, Medical College of Ohio,
Toledo, OH.

Trichloroethylene (TCE) and tetrachloro-
ethylene (PER) are hepatocarcinogens in
B6C3F1 mice, but not Fisher 344 rats. These compounds may be functioning through nongenotoxic carcinogenic mechanisms. In the present study we investigated whether the hepatic pro-
liferative effects of TCE, PER and trichloroacetic acid (TCA) in mouse and rat liver following acute exposure correlated with carcinogenicity. Pheno-
obarbital (PB) was used as a positive control. Rodents were gavaged daily with TCA (500 mg/kg), TCE or PER (1000 mg/kg). PB (500 ppm) treatment was via drinking water. Solvent treated controls were also performed. Immedi-
ately prior to treatment, osmotic mini-
pumps containing 4-thymidine were implanted subcutaneously. Animals were killed at 3, 7, and 14 days post-
treatment, necropsied, and livers were processed for autoradiography. Hyper-
plastic and hypertrophic effects of the compounds were examined. In the mouse, TCE, TCA, PER, and PB induced increased DNA synthesis, but not 7 and 14 days of treatment. Only PB increased increased DNA synthesis in the rat.

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A sentinel species is an organism particularly suited as a sentry warning of danger from environmental contamination. To this concept we add sentinel bioassays as a biological endpoint to serve the same function. The two can be applied to short term and long term monitoring of hazardous waste sites, site evaluation and assessing clean up procedures. Examples of candidate sentinel species are: Peromyscus leucopus/maniculatus (white footed/ deer mouse) a native rodent found in most habitats over 80% of the U.S. and maintained as breeding colonies. Litalurus Nebuloso (brown bullhead catfish) common in bodies of water in the eastern half of the U.S., maintained as laboratory breeding colonies and used as monitor of carcinogens. Tradescantia (spiderwort, a plant) used for both in situ field and laboratory assessment, easily maintained as a stock in the greenhouse/laboratory, suitable for monitoring air, soil and water with a number of mutagenic and somatic assays. Examples of candidate sentinel bioassays are: DNA adduct formation; electron transport system of photosynthesis; tumor formation.

Small mammals trapped at sites contaminated with mercury, strontium-90, and benzo(a)pyrene were evaluated for their ability to serve as indicators of exposure to these chemicals. Residue analyses and a hemoglobin-adduct assay were performed on several species including the short-tailed shrew (Blarina brevicauda), the white-footed mouse (Peromyscus leucopus), and the cotton rat (Sigmodon hispidus). Accumulation of mercury in kidney tissue and strontium-90 in bone was correlated with the degree of contamination of the environment. There was a positive relationship between contaminant uptake and tropic level of the species. Carnivores had the highest levels of contaminants, followed by omnivores with intermediate levels, and herbivores with the lowest levels. Small mammals were not good indicators of the presence of benzo(a)pyrene in the environment. (Research supported by the Office of Defense Waste and Transportation Management.)

862 THE USE OF ALKOXYRESORUFIN O-DEALKYLATION AS A
POSSIBLE INDICATOR OF EXPOSURE TO ENVIRONMENTAL
CONTAMINANTS. R.W. Nims and R.A. Lubet,
NCC, National Cancer Institute, Frederick, MD.

Biochemical monitoring of individual animals for exposure to a variety of environmental contaminants has substantial appeal. Employing assays for pentoxysorufin (PTR) or benzoxysorufin (BZR) O-dealkylation catalyzed by a specific form of cytochrome P450 (IIIB), we observed 30-fold induction in rat liver following exposure to DDT, α-hexachlorocyclohexane, 2,4,5,2′,4′,5′-hexabromobiphenyl or Aroclor-1254. Combined with immunohistochemistry techniques, we could determine induction in rats exposed for only 3 days to DDT (<12 ppm) or α-hexachlorocyclohexane (<40 ppm) in the diet. Ethoxysorufin (ETR) O-dealkylation, catalyzed by P450IAl, was induced 50-fold in the rat by 5,6-benzo-flavone, 3,4,5,3′,4′,5′-hexabromobiphenyl or Aroclor-1254. ETR O-dealkylation is induced in most tissues in most animal species, including man. In contrast, the O-dealkylation of BZR and P450IIB1 is inducible in some species but not in others. In the mouse, rat, rabbit and patas monkey, this activity is induced 20-fold by phenobarbital, while in the hamster, gerbil, trout and quail, minimal induction is observed. Measurement of these O-dealkylations in liver S9 samples may afford a relatively simple assay for animal exposure to environmental contaminants.

863 CHEMICAL STABILITY AND BIOLOGICAL SUITABILITY OF
A 25-CHEMICAL MIXTURE OF GROUNDWATER
CONTAMINANTS FOR ANIMAL TOXICOLOGY STUDIES.
H. C. Yang, T. Gooch, C. W. Jameson, D. Lemole, M.
J. Luster, R. Chapin, R. E. Morrissey, B. A. Schatz,
R. Harris, J. A. Chatman, D. D. Morse, R. Moseman, N.
Collingworth, D. C. Low, Triangle Park, NC. Midwest Research
Triangle Park, NC. Radiac Corporation, Research
Triangle Park, NC.

Animal experiments were conducted on a mixture of 25 frequently detected groundwater contaminants. P344 rats and B6CF1 mice were given this chemical mixture in drinking water, at various concentrations, for 14 days and the mortality, body weight changes, water and food consumption rates were assessed. The technically achievable stock was unsuitable for animal studies because of marked toxicity and unpalatability. Diluted (5X or more) solutions, though caused lower water and food consumption rates and reduced body weight gain, appeared to be suitable for animal studies. Thus, the 5X and 10X dilutions of the stock were later used as the high dose level for 14-day and 13-week studies, respectively. So far, no developmental or reproductive toxicity has been observed. Immunotoxic effects and enhancement of C57 hepatotoxicity by the chemical mixture were seen and they are reported separately in this meeting. Extensive chemical analyses during these studies revealed that estimated intake of the components of the mixture may be achieved.

864 STUDIES ON IRRADIATED GRANARY WEEVIL WITH
REFERENCE TO HUMIDITY AND TEMPERATURE.
S. Sriramarao, R. S. Saini and D. Romanovits, Z.
Selma University, Selma, AL. Tuskegee University, Tuskegee, AL. West Georgia College, Carrollton, GA. Sponsor: S. Reddy.

Adult granary weevils Sitophilus granarius (L.) were exposed to gamma radiation (0.60 to 0.15 GY) and maintained at different temperatures (25°, 30° and 35°C) and relative humidity (R.H.) ranges (17%, 65% and 95%). A significant decrease in the moisture content (M.C.) of the whole insect was observed. With raise in temperature, further decrease in M.C. was noted. Similar changes were also noticed when the weevils were exposed to different humidities. Analysis of epicuticular hydrocarbons (C23-C35) did not show any significant change. However, variations in quantitative distribution of alkanes were observed. The SEM examination of the epicuticle indicated no apparent changes in the Chitin exoskeleton of the irradiated insects. The results suggest that variation in humidity and temperature decreased M.C. without any influence on epicuticle of granary weevil. (Supported by DOE #DE-FG02-86CH10288. AU003).

865 THE EFFECT OF ZEARALENONE ON REPRODUCTIVE
PARAMETERS OF FEMALE MINK. J. K. Cameron, S. J.
Bursian, and R. J. Aulerich, Department of Animal
Science and Center for Environmental Toxicology, Michigan State University, East Lansing, MI.

Two studies were conducted to determine the sensitivity of female mink to the mycotoxin zearalenone (Z). Nine yearling females were ovariectomized and then administered Z via the diet at 0, 10, and 20 ppm for 21 days. Uteri from the Z-treated mink had increased vascularization and were heavier than uterine control females (1.73 g and 0.78 g). There was also a dose-related increase in vulva size. In a second trial, 24 mature pastel females were fed 0, 10, and 20 ppm Z, beginning 4 weeks prior to breeding and continuing until 3 weeks after whelping. Mink fed 20 ppm failed to reproduce. Uterus from these animals were highly vascularized and one female had evidence of implantation sites. The gestation period averaged 54 days for the control group and 62 days for the 10 ppm group. Litter size of control females averaged 4.6 kits and the average litter size of the 10 ppm group was 3.8 kits. Seventy-eight percent of 10 ppm kits were female. Mortality of kits to 3 weeks of age was higher in the 10 ppm group compared to controls, but kit birth weight to 3 weeks of age was similar in the two groups. There was no adverse effect on survivability of adult females and no visible signs of toxicity were noted.
Tracheal explant cultures from species with varying numbers of P-450-containing nonciliated epithelial cells in the upper airways were used to study comparative AFB; metabolism, DNA binding and adduct repair. Explants were cultured in media containing 0.5 μM 14C-AFB1. The level of conversion of AFB1 to metabolites AFL, AFM1 and AFQ1; AFB1-DNA adduct formation and rate of adduct repair was highest in explants from rabbit, followed by the hamster, with the rat showing little metabolism, DNA binding or repair. Repair was completely inhibited in hamster tracheal explant cultures treated with the repair inhibitor novobiocin demonstrating removal of AFB1-DNA adducts is an enzymatic process. Ultrastructural alterations were most severe in explants derived from rabbit and hamster, while explants from the rat were relatively unaffected by AFB1. These results demonstrate that the carcinogenic activation and repair capabilities of tracheal epithelium vary among species and that these processes likely relate to the presence of SER-containing nonciliated tracheal epithelial cells in these species. (Supported in part by USPHS ES 04813)
870 SUBCHRONIC TOXICITY OF MERCURY ON FEEDING AND GROWTH IN TWO SPECIES OF CATFISHES, S Ravindra Reddy and Geetha Belliappa Department of Zoology, Bangalore University, Bangalore, India. Sponsor: D Desaiab

Investigations into the effects of mercurial compounds on fish are generally restricted to the uptake, bioaccumulation and elimination of the heavy metal and to its effect on histology, physiology and biochemistry of various organ systems of fish. This paper details the impact of subchronic toxicity of mercury on feeding, food partitioning and growth of the catfishes Heteropneustes fossilis and Mystus vittatus. In toxicant free water, the maintenance, optimum and maximum feeding rates amounted to 5.83, 25.24 and 122.23 mg/g fish/day for H. fossilis and 13.11, 29.13 and 136.89 mg/g fish/day for M. vittatus respectively. H. fossilis at sublethal concentrations of 0.084 and 0.168 mg HgCl₂/l exhibited a higher maintenance level of 10.19 and 14.56 mg/g fish/day, and M. vittatus at concentrations of 0.054 and 0.150 mg HgCl₂/l indicated a maintenance level of 14.36 and 17.48 mg/g fish/day respectively. The heavy metal had an inhibitory effect on the growth of the two species of fish. Increase in 'specific dynamic action' at maximum feeding level in either species of fish exposed to sublethal levels of mercury suggests that, the presence of the metallic ion in water effects the energy cost of converting food into body substance.

871 ACUTE AND CHRONIC TOXICITY OF ALUMINUM TO FISH AND INVERTEBRATES. W J Birge, J A Black, T M Short, A G Westerman, S B Taylor, and E M Silverhorn, Graduate Center for Toxicology, University of Kentucky, Lexington, KY and *Kentucky DEP, Frankfort, KY. Sponsor: L W Robertson

Due to its abundance in soils, surface and domestic waters, fossil fuels and its many industrial uses, aluminum is of considerable environmental health concern. To examine the aquatic toxicity of aluminum, 22 acute toxicity tests were performed with eight fish and invertebrate species exposed in natural and reconstituted waters at three hardness ranges (i.e., 50, 100, 200 mg/L CaCO₃). The LC₅₀ values determined with the fathead minnow were 6.01, 9.93, and 21.14 mg Al/L for soft, moderately hard, and hard reconstituted water, respectively. Those for Daphnia, the most sensitive species, were 4.36, 7.78, and 25.64 mg Al/L at the same water hardness. LC₅₀ values in moderately hard reconstituted water for six additional species ranged from 11.07 mg Al/L with the bluegill sunfish to 125.3 mg Al/L with the isopod Lirceus. Chronic values for aluminum (100 mg/L CaCO₃) were 0.34 mg/L in a 33-d early-life stage test with the fathead minnow and 0.15 mg/L in a 21-d test with Daphnia pulex. Chronic values were hardness related and the Criterion Continuous Concentrations in mg Al/L were calculated to be 0.08, 0.12, 0.18 and 0.23 at water hardnesses of 50, 100, 200, and 300 mg/L CaCO₃, respectively. High aluminum concentrations significantly altered test conditions (e.g. pH).

872 ROLE OF RAINBOW TROUT FLAVIN-CONTAINING MONOOXYGENASE IN THE IN VITRO BIOTRANSFORMATION OF ALCIDICARB. K Schlenk and D R Buhler, Toxicology Program, Oregon State Univ., Corvallis, OR.

Flavin-containing monooxygenase (FMO) is a xenobiotic enzyme primarily localized in the endoplasmic reticulum which may affect the toxicity of nitrogen and sulfur-containing chemicals, such as organophosphate and carbamate pesticides, encountered in the environment by aquatic organisms. Alicant (Temik), a sulfur-containing pesticide was found to be a substrate for FMO in rainbow trout (Salmo gairdneri). Rainbow trout microsomal in vitro biotransformation of aclidicarb was examined in liver, kidney, and gill. In all tissues the major metabolite was aclidicarb sulfoxide. Additions of antibodies raised to NADPH-cytochrome P-450 reductase led to 50% and 30% reductions in sulfoxide formation in liver and kidney microsomes. However, liver and gill microsomal incubations containing N,N-dimethylaniline (DMA) and methimazole, also showed reduced sulfoxide productions suggestive of both FMO and cytochrome P-450 to aclidicarb biotransformation. Tissue distributions of FMO as determined by western blotting using antibodies raised against rabbit lung FMO were correlated with the activities of DMA N-oxidase and thionitrobenzoate-enhanced methimazole-oxidation. Supported by NIEHS grants ES00210, ES03850 and ES07060.

873 PHARMACOKINETICS OF ORMETOPRIM IN RAINBOW TROUT. B F Droy,*M Goodrich,*J J Leon, and K M Kleinow. Dept. of Veterinary Physiology, Pharmacology and Toxicology, LSU, Baton Rouge, LA and *Dept. of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI.

Ormetoprim (OMP) is a broad spectrum antimicrobial drug used in aquaculture in combination with sulfadimethoxine to control bacterial outbreaks. Uptake, bioavailability, disposition, and elimination were examined in trout after intravenous and oral dosing (8 mg/kg) of OMP. Plasma clearance was rapid following single (T₁/₂ α = 0.54 hr, T₁/₂ β = 17.54 hr) and multiple (T₁/₂ α = 0.67 hr, T₁/₂ β = 35.67 hr) iv dosing with an apparent volume of distribution of 4483.7 ml/kg (single dose). Plasma protein binding was low (≤3%) and nonspecific. Dispositional data 96 hr post steady state (iv) revealed highest concentrations in bile, liver, spleen, kidney, intestines and egg with significant residues in skin and muscle. Peak absorption time of orally administered OMP was 12 hrs with a bioavailability of 97.1%. Oral dispositional data revealed the highest concentration in bile, kidney, and liver suggesting renal and first pass excretion. Significant OMP residues were seen in skin (0.9 ppm) and muscle (0.15 ppm) at 38 days. This information will be integral in designing therapeutic dosing regimens and drug withdrawal protocols in trout. (Supported by FDA grant No. FD-0-U-000158-02).
Tissue Distribution of Dietarily Administered Aldicarb in Rainbow Trout. D A Erickson, M S Goodrich, and J L Lesh. Medical College of Wisconsin and Center for Great Lakes Studies, Milwaukee, WI.

The distribution of 14C-aldicarb residues in the tissues of rainbow trout were determined by monitoring radioactivity at various periods up to 96 hrs following treatment. Fish used in the study were fasted for 3 days prior to dosing (54 μg/kg) by gavage. They were maintained at 12° C and were unanesthetized during treatment. Aldicarb residues were initially present in the greatest amounts (% dose/g tissue) in gill, heart, pyloric caeca, stomach, anterior intestine, liver, bile, and perivisceral fat. These residues were rapidly eliminated from gill tissue and fat but were more slowly eliminated from the other tissues. Concentrations of aldicarb residues in the bile continued to increase over time. Although the concentration of aldicarb residues in the tissues not mentioned above tended to parallel the concentration in whole blood, the aldicarb residues present in muscle were significant when considered in terms of total tissue mass. Total muscle contained approximately 21, 12, and 8% of the total dietary dose of aldicarb at 24, 48, and 96 hrs, respectively, following gavage treatment. The data indicate that significant amounts of aldicarb are rapidly taken up by rainbow trout consuming contaminated food and remain for at least 96 hrs after feeding. (Supported by grants ES01080 and ES04184).

Primary Culture of Trout Hepatocytes on an Extracellular Matrix. M M Lipsky, M W Kahng, T R Sherman, R Reischlussel, R O Bennett, and E B May. Department of Pathology, University of Maryland School of Medicine, Baltimore, MD. Sponsor: T W Jones

Non-mammalian species, particularly teleosts, have received increasing attention as models for toxicologic studies. In preliminary studies, it was noted that rainbow trout (Salmo gairdneri) hepatocytes readily attached to extracellular matrix-coated culture dishes (ExtraCell, but not to plastic or collagen-coated culture surfaces. Attachment efficiency generally exceeded 98%. The unique property of hepatocytes not attaching to plastic culture surfaces was used to remove non-hepatocyte components, resulting in a highly enriched hepatocyte suspension. Attached hepatocytes maintained high viability (> 75%) for the 21 days of the study. Electron microscopic observations indicated that the hepatocytes maintained a well-differentiated phenotype. Variability among fish was apparent in NADP+ - cytochrome P450 reductase and NADH-cytochrome b5 reductase activity. Tyrosine aminotransferase inducibility also varied, but in general the hepatocytes maintained differentiated function. This study demonstrates a usefulness of trout hepatocytes as a model for comparative research.

Pharmacokinetics and Metabolism of Triclopyr BEE in Coho Salmon. M G Barron, M A Mayes, P G Murphy, R J Nolan. Mammalian and Environmental Toxicology Research Laboratory, The Dow Chemical Company, Midland, MI.

The pharmacokinetics and metabolism of triclopyr butoxyethyl ester (BEE) was studied in yolk sac fry of the coho salmon. Triclopyr BEE was rapidly absorbed by the fish, then rapidly deesterified. Triclopyr acid was the principle metabolite observed in fish and exposure water, and was the principle residue in the fish at all times. A compartmental model describing the dynamics of triclopyr BEE and acid in fish and water was developed which allowed prediction of ester accumulation under various exposure regimes. The model indicated the rate limiting step for triclopyr BEE elimination from fish was transfer from the peripheral compartment to the metabolic compartment, rather than slow metabolism. Exposure to triclopyr BEE in the presence of a carboxylesterase inhibitor had no effect on toxicity or total residue levels in the fish, but ester concentrations in the fish increased by seven times. The observed lack of change in triclopyr acid observed during esterase inhibition was predicted by the pharmacokinetic model, and suggested that mortality resulted from lethal tissue concentrations of triclopyr acid or total residues.


Lipid peroxidation (LP) is thought to occur by free radical attack on membrane polyunsaturated fatty acids (PUFAs). Previous investigators have reported that PUFAs levels are higher in cold acclimated fish than in mammals. Therefore, the present studies have compared LP in liver microsomal membranes obtained from the rat and from old and young, 16°C-acclimated, rainbow trout. Malondialdehyde (MDA), an indicator of LP, was measured by HPLC as the MDA-thiobarbituric acid adduct. LP was induced by incubation of microsomes with ascorbic acid (1 mM), FeCl3 (10 μM) and ADP (1 mM) for 10 min. Rat liver microsomes were extensively peroxidized (30.7μM MDA/mg protein). In contrast, very low levels of MDA were found in liver microsomes from young trout (1.09 nmol MDA/mg protein). HPLC analysis of microsomal vitamin E indicated that the trout had markedly higher levels than the rat (9.86 vs. 0.23 nmol/mg protein). Of interest, microsomes from old trout produced MDA levels approximately 1/2 those of the rat despite high vitamin E (10.13 nmol/mg protein). GC analysis of microsomal fatty acids revealed marked differences between the rat and young and old fish. The relationship between susceptibility to LP, fatty acid content and anti-oxidants is being further explored.
CARbamates have been widely used as insecticides since 1959. These insecticides are effective due to a their inhibition of acetylcholinesterase; however, recent evidence has indicated that long-term exposure may have significant neurological sequelae separate from acetylcholinesterase effects. Both carbaryl and aldicarb have potential for long-term human exposure due to high usage and contamination of ground water. We examined the toxic effects of aldicarb and carbaryl at doses of 10 to 80 ppm in astroglia and glioma cells. In immature astroglia, carbaryl (20 to 80 ppm) significantly decreased cell number and viability on days 1 and 3 of treatment. Gliosis was evident by day 3 at doses of 40 to 80 ppm. In glioma cells the effects on cell number and viability were even more profound, and there was a substantial decrease in radiolabeled amino acid incorporation by 3 days of treatment. Aldicarb, without activation, increased cell numbers in the 20 ppm group on days 1 and 3 of treatment, but none of the concentrations thus far tested, affected viability in immature astroglia. Glioma cells treated with low doses of aldicarb had greater incorporation of radiolabeled amino acid than did either the carbaryl-treated or the control groups. Subsequent studies are underway using S-9 activation. Funded by Creative Match Grant, Virginia Tech.

Young chickens were administered oral doses of either aldicarb (0.2 mg/kg body weight/day) or carbaryl (100 mg/kg body weight/day) for seven days as this regimen had been demonstrated, in previous studies, to alter locomotor activities. In addition to histological examination, activities of brain acetylcholinesterase (ACHE), and neurotoxic esterase (NTE), liver and plasma cholinesterase (ChE), and carboxylesterase were measured at different intervals after dosing to determine if these parameters could be used to indicate, predict or monitor changes in locomotor activity. Effects on enzyme activities following administration of these carbamates were compared in young and adult chickens. Activities of brain ACHE, plasma ChE, plasma carboxylesterase and liver ChE were inhibited in young chicks by both carbaryl and aldicarb. Enzyme activities were not, however, inhibited in adult chickens. Carbamate treatment did not cause histological damage nor did it inhibit activities of NTE and liver carboxylesterase activities either in young or adult chickens. In this study, esterase determinations and histological studies could not be used to predict or support carbamate-induced functional deficits.

FUNCTIONAL ANALYSES OF CELL-MEDIATED IMMUNITY AND QUANTITATION OF LYMPHOCYTE SUBPOPULATIONS IN NICE EXPOSED TO THE CARBAMATE PESTICIDE ALDICARB. P. T. Thomas, H. V. Ratejczak, D. Demetral, K. Hagen, and K. Baren. ITI Research Institute, Chicago, IL and Rhone-Poulenc AG Company, Research Triangle Park, NC.

Adult female B6C3F1 mice received distilled water only or water containing 1.0, 10, or 100 ppb of aldicarb daily for 34 days. The target concentration of aldicarb present in the 100 ppb dosing solution was analytically verified. To further develop an immune profile of this compound, following aldicarb exposure, the ability of splenic natural killer cells as well as specifically sensitized cytotoxic T-lymphocytes to lyse YAC-1 lymphoma and P815 tumor cells, respectively, was evaluated. To supplement the functional assays, the impact of aldicarb exposure on the percentages and absolute numbers of total T-cells, T-suppressor, T-helper and B-cells was evaluated. The absence of statistically significant effects on any of these parameters supports earlier reports that aldicarb does not pose an immunologic health threat to humans.


Mini-osmotic pumps prefilled with physostigmine (0.12 mg/kg/hr), scopoline (0.1 mg/kg/day), or trihexyphenidyl (1 mg/kg/day) were implanted s.c. in guinea pigs. Soman (60 µg/kg, s.c.) was administered 4 or 7 days after the pump implantations. Toxicities and mortalities of animals occurring after the soman injection were monitored. The latency of tremors, convulsions and loss of righting reflex (LRR) in drug-pretreated groups were significantly increased as compared with the vehicle-pretreated group. The incidence of LRR was markedly reduced in the groups pretreated with either physostigmine alone or in combination with muscarinic blockers. The mortality in the physostigmine-pretreated group was markedly reduced. The mortality was further reduced in the groups infused concomitantly with a muscarinic blocker. These results suggest that pretreatment of subjects with physostigmine and muscarinic receptor blockers may constitute an effective protection against soman-induced toxicities. (Supported by DAM 17-85-C-5036.)
CLINICAL CHEMISTRY AND HEMATOLOGY VALUES IN CD RATS DURING SUBCUTANEOUS EXPOSURE TO SARIN AND SOMAN. JA Crowell, EM Parker, TJ Bucci, *JC Dacre, Pathology Associates Inc., NCTR, Jefferson, AR and US Army Biomedical R&D Laboratory, Fort Detrick, MD.

Sarin, Type I (isopropyl methylphosphono-fluoridate) and Soman (pinacolyl methylphosphono-fluoridate) were administered by gavage to groups of male and female CD rats for 13 weeks. Doses of 300 (MTD), 150, 75, and 0 ug/Kg Sarin were given, based upon a range-finding determination of the maximum tolerated dose without lethality (MTD). Orbital sinus blood was collected from 6/sex/dose prior to treatment (baseline), during weeks 1, 3, 5, 7, and at necropsy. Serum was analyzed for alanine aminotransferase, aspartate aminotransferase, urea nitrogen, creatinine, and creatine kinase. Acetylthiocholine hydrolysis was measured in plasma and in erythrocyte lysates. A complete blood count was performed. Neurapraxia target esterase was measured in brain at necropsy. Plasma cholinesterase was depressed according to dose to as low as 22% of control; erythrocyte acetylcholinesterase was also depressed but less consistently than plasma cholinesterase. No other clinical chemistry or hematology parameters were dramatically altered. (Supported by US Army Medical R&D Command, contract #DAMP3688).

ORGANOPHOSPHATE-SENSITIVE ESTERASES IN THE LIVER AND PLASMA OF THE CHICKEN EMBRYO. S J Smucker and B W Wilson, University of California, Davis, CA.

Liver and plasma esterases are important in the metabolism of xenobiotics and the detoxification of organophosphates (OPs). This research combines sedimentation analysis and OP inhibitors to characterize OP sensitive esterases during development. Chicken embryo liver and plasma esterases were separated on sucrose gradients and quantified with automated microplate reader assays for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) using acetylthiocholine and the BChE inhibitor iso-OMPA. Carboxylesterases (CaE) were measured with nitrophenylacetate (NPA) and distinguished using paraxon (PO) and DFP. There were four main esterases in the liver, a monomer (G1) and tetramer (G4) BChE and two CaE peaks, a DFP-sensitive (IC50 = 5.0 X 10^-8M) and a DFP-resistant form (IC50 = 7.9 X 10^-3M) extremely sensitive to PO (IC50 = 2.0 X 10^-9M). There were G1, G2 (dimer) and G4 BChEs, G2 and G4 AChEs, and a small but detectable NPA esterase in plasma. The roles of these enzymes in OP toxicity and detoxification deserve further study. Supported in part by NIH ES 00202.

PHYSOSTIGMINE AS AN ADJUNCT TO PYRIDOSTIGMINE PRETREATMENT IN PAROXAN TREATED LONG-EVANS RATS. S S Singh, W O Cook, J A Drellinger Southwest Research Institute, San Antonio, Texas.

The efficacy of physostigmine as a post exposure therapeutic drug to treat nerve agent toxicity was studied using a battery of neurobehavioral (NB) tests. Twenty four male 300-500 g Long-Evans rats were administered one of the following regimens: vehicle control (Group 1); pyridostigmine, paroxan, atropine (Group 2); pyridostigmine, paroxan, atropine, physostigmine (Group 3); or pyridostigmine, atropine, physostigmine (Group 4). The doses of pyridostigmine, paroxan, atropine, and physostigmine were 50ug, 1.0xLD50, 25mg, and 75ug/kg respectively. The animals were tested 48 hours prior to drug administration, immediately following drug administration, and 48 hours post drug administration. NB endpoints included: Figure 8 Maze (FMB) activity (counts/5 minutes) at 0 minute, Grip Strength (GS) (kg) at 65 minutes, Accelerating Rotarod (AR) (sec) at 70 minutes, and Startle Response (SR) (maximum amplitude and latency) at 60 minutes. The blood, plasma, RBC, and brain cholinesterases (CHE) activity were determined for 12 rats at the end of NB testing. The remaining rats were used for pathologic evaluation of brain and spinal cord. There was a significant decrement in performance in the paroxan-treated rats when tested immediately after drug administration, at 48 hours post drug administration. Group 3 rats performed better on the AR but were weaker as compared to Group 2 rats on the GS. Blood, RBC, and brain Che activity were reduced by 48%, 57%, and 47% respectively in Group 2 rats, while blood and RBC Che activity were reduced by 38% and 48% in Group 3 rats.

JOINT ACTION OF ORGANOPHOSPHATES ON ACETYLCOLINESTERASE IN VITRO. J Li and J D Murphy, Department of Environmental Health, University of Washington, Seattle, WA.

It has been proposed that standards for mixtures of chemicals with the same site and mechanism of action can be derived with the assumption that their joint toxic action is additive. We initiated a study to systematically test this assumption by determining the additivity of anti-cholinesterase action of direct inhibitors of acetylcholinesterase in vitro. Seven pairs of organophosphates were tested with purified bovine erythrocyte acetylcholinesterase over a range of concentrations of each which alone produced 20-80% inhibition. The results indicate that the joint anticholinesterase action can be predicted by a dose additive model. For example when the pairs were tested at approximately the 25% inhibition concentration for each, the experimental joint % inhibition, the joint % inhibition predicted by a close-additive model, and the joint % inhibition predicted by an effect- additive model were respectively: ethyl paraxon/methyl paraxon 51.5, 48.6, 60.0; methyl azinphos-oxon/ethyl azinphos-oxon 45.0, 44.4, 52.5; methyl paraxon/methyl azinphos-oxon 42.6, 42.5, 51.5; ethyl paraxon/ethyl azinphos-oxon 48.9, 49.2, 55.8; and ethyl paraxon/disopropyl fluorophosphate 43.5, 45.9, 56.9. Other experiments showed that the presence of the sulfur analogues, ethyl parathion or methyl azinphos did not alter the in vitro inhibition by their oxons. The results indicate the joint action of organophosphate insecticides at the acetylcholinesterase target site is dose-additive, and less than effect-additive, when tested in a simple target enzyme-inhibitor system (supported in part by research grant ES03424 from NIEHS).
886 CHLORPYRIFOS: INHIBITION OF HEN BRAIN ACETYLCHOLINESTERASE (ACHE) AND NEUROTOXIC ESTERASE (NTE) IN VIVO AND KINETICS OF NTE INHIBITION IN VITRO. T. Moore, US Kayyal, JH Fowke, and RJ Richardson. Toxicology Program, The University of Michigan, Ann Arbor, MI.

Chlorpyrifos (diethyl 3,5,6-trichloro-2-pyridyl phosphorothionate; CPS) is reported to cause delayed neuropathy at doses > LD50 after a long delay. CPS dosed at 75, 150, and 300 mg/kg (po, corn oil) produced inhibitions of AChE of 58, 75, and 86%, and of NTE of 21, 39, and 78%, respectively, in brains of atropinized hens 4 days after dosing. A 16-day time-course found maximal inhibition of AChE (86%) on day 1 and NTE (38%) on day 4 post dose (150 mg/kg); no signs of delayed neuropathy were observed. The difference in time-course of inhibition in vivo prompted further study of inhibition kinetics in vitro. CPS-oxon (CPO) titration curves were first-order, with apparent 150s (Km) of 2.3 (ACHE) and 193 (NTE), but the time-course of NTE inhibition with CPO indicated the presence of a Michaelis-like complex (rate constants were not linearly related to [CPO] and there was zero-time inhibition by CPO, which was reversed by increasing [substrate]). Further kinetic studies are needed to determine if a significant amount of a reversible CPO-NTE complex could exist in vivo and if non-neurotoxic NTE inhibitors could be used to dissociate it in order to intervene in delayed neuropathy. (Supported, in part, by a gift from the Dow Chemical Company).

888 TOLERANCE TO AN ORGANOPHOSPHORUS INSECTICIDE, CHLORFENPVINPHOS, IN RATS. S. Tsuchiya, Y. Majima, Y. M. Yoshida, Y. Takashima, T. Shirasu. Institute of Environmental Toxicology, Nisshoku, Ibaraki, Japan.

Tolerances to organophosphate cholinesterase inhibitors have generally been ascribed to cellular type tolerances, such as a down regulation of the cholinergic receptors. The first oral acute administration of sublethal dose of chlorfenvinphos (CVP), organophosphate cholinesterase inhibitor, reduced the toxicity of the second dosage in male Fischer rats. The purpose of this study was to determine the nature of this tolerance. LD50 found after the second administration was increased about 3 fold by a 24-hr pretreatment of a half LD50 dose (15 mg/kg) of CVP. The pretreatment slightly increased the iv LD50 of CVP to a 1.4 fold level. The iv LD50s of carbaryl and oxotremorine were not substantially changed by the pretreatment. The pretreatment persistently inhibited the cholinesterase (CHE) activities of the brain, erythrocytes and plasma. The pretreatment, however, greatly reduced these CHE inhibitions caused by the second dosage, so that the brain and erythrocyte CHE activities after the second dosage were higher than the controls. The treatment reduced the plasma CVP concentration of the second dosage one fourth as expressed by the area under the curve. In conclusion, the CVP tolerance might be due to the reduced bioavailability of oral CVP, suggesting a metabolic tolerance.

887 ENHANCEMENT OF PROPOXUR TOXICITY BY ISO-OMPA PRETREATMENT: EVIDENCE OF POTENTIATION. R C Gupta and W L Kadel. Breathitt Veterinary Center, Murray State University, Hopkinsville, KY.

iso-OMPA (1 mg/Kg, sc) pretreated rats receiving a non-toxic dose of Propoxur (5 mg/Kg, ip) showed the toxic signs of parasympathetic (anti-cholinesterase) preponderance, comparable to those elicited by its sublethal acute dose (15 mg/Kg, ip), indicating at least 3-fold potentiation. A single ip dose of 25 mg/Kg Propoxur was minimal lethal dose. Each drug when given alone in low doses (1 mg/Kg iso-OMPA, 5 mg/Kg Propoxur) did not alter the activity of acetylcholinesterase (AChE), whereas the activity of carboxylesterase (CarbE) was significantly (P < 0.01) reduced in plasma, liver, hemidiaphragm, and discrete brain regions (cortex, stem, striatum, and hippocampus), suggesting tremendous nonspecific binding of Propoxur. Their combined administration in low doses, however, significantly (P < 0.01) down regulated the AChE activity in all brain regions and hemidiaphragm. It is evident from in vivo as well as in vitro experiments that the observed potentiation of Propoxur toxicity by iso-OMPA pretreatment could be due either to irreversible inhibition of nonspecific binding sites, such as CarbE and butyrylcholinesterase (BuChE) and/or interference in the metabolic detoxification of Propoxur, leaving its higher free concentration for AChE inhibition.

889 IN VIVO PENETRATION AND METABOLISM OF METHYL PARATHION IN LARVAE OF THE TOBACCO BUDWORM, HELIOTHIS VIRESCENS (P.) FED DIFFERENT HOST PLANTS. S F Abd-Eghafar, W C Daumeran and E Hodgdon. Toxicology Program, North Carolina State Univ. Raleigh, NC.

The effect of wild tomato, and peppermint plants on the toxicity, penetration, and metabolism of methyl parathion was investigated in larvae of the tobacco budworm. Third and fifth-instar larvae were more tolerant (3.3, 2.7 and 2.2, 1.7 fold), respectively, than those fed an artificial diets. Penetration rate was the same in all larvae fed different diets. Metabolites detected were three chloroform-soluble and five water-soluble metabolites. P-nitrophenol was the major metabolite. The data suggested that the two plants induced enzymes responsible for detoxifying methyl parathion.
EVALUATION OF THE POTENTIAL DEVELOPMENTAL EFFECTS OF TRICLOPYR IN THE NEW ZEALAND WHITE RABBIT. H D Kirk, T H Hanley, D I Eisenbrandt and J F Quast, Mammalian and Environmental Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI 48674.

Triclopyr (3,5,6-trichloro-2-pyridinloyxycetic acid) was administered to pregnant New Zealand White rabbits to evaluate embryonal/fetal toxicity and teratogenic potential. Triclopyr was administered as a single bolus dose in corn oil suspension at dose levels of 0, 10, 25, 75 mg/kg/day on days 6-18 of gestation. A slight increase in maternal mortality was observed at 75 mg/kg/day, consistent with the results of preliminary studies in which excessive mortality was observed at dose levels of 100 mg/kg/day and above. Other maternal parameters were not affected by gavage administration of triclopyr to pregnant rabbits. No treatment-related effects were observed on any developmental parameters in any of the treatment groups. Based on these results, 75 mg/kg/day was considered the no-observed-effect level (NOEL) for developmental toxicity in rabbits.

INHIBITION OF RAT HEPATIC LOW KM ALDEHYDE DEHYDROGENASE (L KM ALD) BY MOLINATE: POTENTIAL IMPLICATIONS.

N D Faiman, E M Finckhiner, M B Bauer, and B W Hart. Department of Pharmacology and Toxicology, University of Kansas, Lawrence, KS.

Molinate (5-ethyl hexahydro-1H-azepine carbonate) is a thiocarbonate herbicide. Its chemical structure exhibits a similarity to 5methyl, N,N-diethylthiocarbamate (DDT), which we have recently identified as a metabolite of disulfiram. The basis for disulfiram's use in the treatment of alcoholism is its inhibition of hepatic L Km ALDH, leading to the subsequent increase in acetaldehyde after ethanol ingestion. The resulting adverse reaction known as the disulfiram-ethanol reaction or DER. Since DETC is an effective hepatic L Km ALDH inhibitor in rats, similar inhibition would be expected with molinate. Male rats were treated with a series of doses of molinate, and liver L Km ALDH determined. At a pretreatment time of 3 hr, approximately 50% inhibition of L Km ALDH was found at a dose of 2.96 mg/kg ip of molinate compared to approximately 10 mg/kg ip for DETC. Molinate-induced enzyme inhibition was fairly rapid with approximately 20% occurring within one hr. These findings have considerable significance, since accidental absorption (inhalation, dermal) of molinate could produce a severe DER in unsuspecting individuals consuming ethanol. (Supported by a grant from NTIAA, grant no. AA 03577).

PRECHRONIC (14-DAY) TOXICITY OF METHYLENE BIS (THIOCYANATE) ORALLY ADMINISTERED TO FISCHER 344 RATS AND B6C3F1 MICE. B. Myers, L Billups, R. Irwin*, L. Burka* and G. Wolfe. *NIEHS/NTP, RTP, NC and HLA, Rockville, MD.

Methylene bis (thiocyanate) (MBI), an antifouling agent, was examined for toxic effects in F344 rats and B6C3F1 mice by 14-day prechronic testing. MBI, suspended in 0.6% methyl cellulose, was administered orally at 0, 10, 20, 40, 80 and 160 mg/kg/day (12 dose-days) to both sexes and species. Extensive mortality occurred at MBI dosages > 20 mg/kg in rats and 40 mg/kg in mice. Dose-related clinical signs included turgidity, prostration and low body temperature in both sexes and species; tremors in mice; wheezing, dyspnea and ataxia in rats. Additional effects observed in the 20 mg/kg dosage groups increased (p<0.05) liver/body wt ratios in both sexes and species, decreased (p<0.05) body wts in rats, decreased (p<0.05) thymus wts in male rats and decreased (p<0.05) testicle wts in male mice. Gross and microscopic lesions were noted in both stomach regions of mice dosed with 20-40 mg/kg and rats dosed with 10-80 mg/kg (both sexes). The nonglandular stomachs of these animals were characterized by ulceration, granulation tissue, hyperkeratosis and anacanthosis. Lesions of the glandular stomach included inflammation and granulation tissue. Thirteen-week subchronic studies are being initiated. (Supported by Contract No. N01-ES-85191).

CLOTTING EFFECTS AND PLASMA DRUG CONCENTRATIONS AFTER SINGLE ORAL DOSES OF BRODIFACOUM OR WARFARIN IN THE DOG. D F Gerken, R A Sams, S Ashcraft, and K Lee. The Ohio State University, Columbus, OH. Sponsor: V L Carter, Jr.

Accidental poisoning from warfarin (Warf) and brodifacoum (BDF) anticoagulant rodenticides occurs frequently in the dog. Comparative BDF and Warf studies of the clotting response and plasma anticoagulant concentrations in the dog have not been performed. The objective of this research were to determine the onset and duration of anticoagulant activity and to compare the clotting effects with their respective plasma concentrations.

After an overnight fast, six dogs were given 2 mg BD/kg body weight (BW) orally, four dogs were administered 10 mg Warf/kg BW, and four control dogs were given empty gelatin capsules on the Study day 1. No abnormal clinical signs were observed throughout the study although significant changes were seen in the blood clotting parameters. Evidence of anticoagulation was noted in the Warf-treated dogs for seven days and in the BDF-treated dogs for 15 days after dose administration. Peak plasma Warf concentrations ranged from 45 μg/ml to 61 μg/ml plasma and peak plasma BDF concentrations ranged from 39 ng/ml to 520 ng/ml plasma. In this study a direct relationship was observed between the length and severity of anticoagulation, the peak plasma anticoagulant concentration and the length of time the anticoagulant was detected in the blood.
A study to measure potential dermal and inhalation exposure to a chlorpyrifos/allethrin home fogger was conducted with five human volunteers. Following label indicated fogging treatment and resting at identical carpeted hotel rooms, human subjects in dosimeter clothing were led through a routine of Jazzercise™ exercises. Dosimeter clothing consisted of cotton gloves, socks, tights, and shirts. Following exercise in each room the subjects removed the contaminated dosimeter clothing and put on fresh clothes. In addition to clothing, floor deposition was monitored with gauze and aluminum foil corner pads. Clothing and corner pads were extracted with ethyl acetate and extracts analyzed by electron capture gas chromatography. A majority (55-61%) of the fogger insecticides were deposited on the floor. The coefficients for transfer of chlorpyrifos and allethrin from floor to clothing were virtually identical ranging from 5% on shirts to 29% on socks. By summing the area under the curve with respect to time it was possible to calculate upper limit cumulative dermal exposure as if contact had been continual. Calculated absorbed dosages were 6.8 µg/kg for adults with the assumption that no clothes were worn.

Pesticides are used almost universally; many of them have high acute and chronic toxicities. These two facts have led to a growing number of poisoning incidents. Such incidents may occur at the individual or group level and may be voluntary or accidental. From the point of view of public health, accidental epidemics occurring periodically are of most interest. Contamination of food is a typical source of chemical accidents. We reviewed the literature for reports of epidemics due to contamination of foods with pesticides. Clinical and epidemiological aspects of these epidemics were studied. The 62 cases chosen are divided into four groups on the basis of implications for prevention: (a) food contamination during transport or storage - 16 cases; (b) consumption of seed dressed for sowing - 10 cases; (c) accidental addition of pesticides to food - 20 cases; and (d) food contamination due to bad agricultural practices - 16 cases. Specific regulatory measures can be taken to prevent each type of incident. In addition, structures should be set up in advance so that such episodes can be quickly and efficiently detected and stopped.

Trichloroethylene is an extensively used industrial solvent and an hospital anesthetic (Trilene) used outside the United States. Using the spin-trapping agent a-phenyl-N-tert-butylnitronine (PBN) and electron spin resonance (EPR) techniques we found 10-30 femtomoles trapped radicals per gram liver in rat liver lipid extracts after inhalation of 1% trichloroethylene under hypoxic conditions (7% O2) for 2 hr. At O2 concentrations of 10% or 20% the EPR signal intensity was reduced. When rats were exposed to 1% trichloroethylene by inhalation for 20 min, then killed by 100% N2 and allowed to metabolize the absorbed trichloroethylene for 1 hr., anaerobic liver metabolism caused a 100% increase in trapped radicals. Metryrapone, an inhibitor of the 52 KD form of cytochrome P-450, administered 1 hr before PBN and trichloroethylene exposure, inhibited 80% of free radical formation. These results indicate that trichloroethylene is reductively metabolized to free radical products by the 52 KD form of cytochrome P-450 in the endoplasmic reticulum of the rat liver. Similar results were obtained by administering tetrachloroethylene i.g. with the PBN. Supported by NIH Grant No. ES03067.

EFFECT OF TOXAPHENE ON 32P INCORPORATION IN RAT MYOCARDIAL SARCOPLASMATIC RETICULUM. C H Trotmann, C Showers, J A Cameron and U Desai, Deps. of Chem and Biol, Jackson State Univ and Dept Neuro, Univ Miss Med Ctr, Jackson, MS.

Activation of Phospholamban by kinases dependent on either c-AMP or calmodulin results in phosphorylation of Ca++ ATPase in myocardial sarcoplasmatic reticulum (SR). Since toxaphene (T) inhibited calmodulin activity in heart and brain tissues we determined the T effects both in vitro and in vivo on 32P incorporation in SR. For in vivo studies rats fed P.O with 0, 25, 50, 75 and 100 mg/kg for 3 days and SR from hearts was prepared. 32P incorporation was studied by incubating the SR with 32P (NEN) in control and T treated rat SR. For in vitro, different concentrations of T, were added to the SR before the addition of 32P. After 5 min incubation the reaction was stopped by filtering followed by washing. The filters were counted for radioactivity. The results show that T in vitro reduced 32P incorporation in a concentration dependent manner reaching a 50-60% inhibition at 50 nM. Rats treated with T also showed a concentration dependent decrease of 32P incorporation at 10, 25 and 50 mg/kg doses. These data suggest that T may alter phosphorylation of SR proteins. (Supported by NIH/MBRS Grant # 08047 and MIRF #5T32HL0 7635).


Male Sprague-Dawley rats, fed a diet containing 0.04, 0.2, or 1.0 ppm selenium (Se) for 2 weeks, were treated with perfluorodecanoic acid (PFDA: 0 or 35 mg/kg in corn oil, single I.P. injection) and killed 2 weeks later. Control animals were pair-fed. PFDA significantly increased Se-dependent glutathione peroxidase (Se-GSHper) activity and Se content in liver cytosol of rats on 0.04 ppm Se diet, but not rats on 0.2 and 1.0 ppm diets. However, ELISA determination of Se-GSHper protein showed that PFDA caused a decrease in rats on 0.2 and 1.0 ppm Se diets, but nont rats on 0.04 ppm diet. Yet the ratio of Se-GSHper activity to antibody-reactive protein was increased by PFDA in all 3 dietary groups. The addition of PFDA to liver cytosol from rats with adequate Se status did not increase Se-GSHper activity. The results suggest that PFDA modulates Se-GSHper activity possibly at the protein level by a mechanism that has yet to be elucidated. (Supported by NIH grant CA43719).

SUBCHRONIC TOXICITY OF BETA-CHLORONAPHTHALENE IN MICE. B Sonawane, R Rubenstein, C DeRosa, A Bathija, H Choudhary and S Irene, U.S. Environmental Protection Agency, Washington, D.C.

B-chloronaphthalene was evaluated for potential toxicity in an oral 90-day subchronic study. Groups of CD-1 mice (20 animals/sex/group) were administered 0, 100, 250 and 600 mg/kg/day of B-chloronaphthalene by gavage in corn oil (Groups 1 thru 4, respectively). In males, mortality pattern showed a significant positive trend and the high dose group was significantly different from the control group. Clinical signs such as languid, hunched posture, dyspnea, thin and rough hair coat were more frequently observed in the high dose group of females than in males. No significant differences were observed in either sex in total body weight gain; however, total food consumption was significantly increased in the high dose group males. Hematology and clinical chemistry data showed no apparent compound-related effects. The liver and gall bladder weights, both absolute and relative, were significantly increased in Group 4 of both sexes. Increased liver weights in Group 4 correlated with the histopathological observations of centrilobular hepatocellular enlargement. A variety of spontaneously occurring incidental lesions was observed in all the groups but these were not treatment related.

1,2-Dibromo-3-chloropropane (DBCP) is a potent renal DNA damaging and necrogenic agent in the rat. Studies with various methylated and deuterated DBCP analogs have demonstrated a relationship between these two toxic phenomena. A series of halogenated propanes were used to further examine structure-activity properties. 1,2,3-Trichloropropane was at least as effective as DBCP in causing tubular necrosis, whereas 1,2,3-trichloroacetonitrile was much less potent. A similar rank order was observed with respect to renal DNA damage. Of the dihalogenated propanes, 1,2-dibromoacetonitrile was approximately ten times less potent than DBCP with respect to renal necrosis and DNA damage, whereas 1,3-dibromoacetonitrile and 1-bromo-3-chloropropane did not cause appreciable effects. The observed differences in toxic potency could not be explained by differences in tissue distribution. The demonstrated correlations further substantiate the hypothesis that DNA damage may be an initial event in the development of organ cell death by halogenated propanes.


Since 1981 several studies have been performed in order to assess the magnitude of Hexachlorobenzene (HCB) pollution in Spain. Different areas of the country have been chosen and HCB accumulation in human adipose tissue and excretion by maternal milk have been monitored. In a selected area the distribution of the chemical in the body, the blood transport patterns and the relationship among the blood levels of HCB and the urinary concentration of pentachlorophenol (PCP) have also been assessed. The results found in the different populations show that HCB concentrations in the adipose tissue and maternal milk range from 0.0 to 3.7 mg/kg (lipid basis) with no obvious differences between industrial and agricultural areas. Adipose tissue levels show sex-related differences and correlation with age. HCB blood transport and tissue distribution pattern differs from that of other organochlorine compounds. Serum HCB levels do not correlate with urinary levels of PCP suggesting an exogenous origin for the later.


CTFE oil is a mixture of halogenated hydrocarbons formed by chlorotrifluoroethylene polymerization. Different chain length ranges are used as oils and greases. The fluid under study contained eleven major oligomers. Gas chromatography separated CTFE into two peak groups. Upon atomization, small oligomers preferentially vaporized from the droplets. F-344 rats were exposed to CTFE at total concentrations of 0.25, 0.48, and 0.98 mg/L. The CTFE remaining aerosol was 5.3, 5.8 and 8.9 percent, respectively, resulting in aerosol and vapor exposures of differing composition. Aerosol mass median aero-dynamic diameters (geometric standard deviations) were 1.2 (2.6), 0.97 (2.2), and 1.14 (2.2), respectively. All eleven major constituents were quantified by capillary column chromatography for both aerosol and vapor components of the atmosphere. Gas chromatography/mass spectroscopy were used to identify the molecular configuration of all oligomers. Five, six, seven, and eight carbon oligomers were present. At various times after exposure, animals were sacrificed and tissue collected for analysis. The analytical results were used in toxicity evaluations and pharmacokinetic modeling described elsewhere. (Supported by DOD Contract No. F33615-85-C-0532.)

TOXICOLOGICAL ASSESSMENT OF CHLORINATED DIPHENYL ETHERS IN THE RAT. D. C. Villetenuve, I. Chu, Y. E. Secours and V. E. Valli. Environmental and Occupational Toxicology Division, Environmental Health Directorate, Ottawa and Biopath Analyst, Guelph, Canada.

Chlorinated diphenyl ethers are environmental contaminants that have been found in Great Lakes fish and birds. Because of their presence in the food chain, and potential for human exposure, the present short-term study was conducted to assess their toxicities. Groups of 10 male and 10 female rats were each given by gavage 2,2',4,4',6-pentachlorodiphenyl ether (CDE1), 2,2',4,4',5,6-hexachlorodiphenyl ether (CDE2) or 2,2',3,3',4,4',5,6-hexachlorodiphenyl ether (CDE3) at dose levels of 0.04, 0.4, 4.0 or 40 mg/kg b.w./day for a period of 28 days. Increased liver weights were observed in the highest dose groups of all 3 congeners, but only CDE2 caused a significant increase in hepatic microsomal aminopyrine and antiline hydroxylase activities. No hematological changes were observed. Histological examination revealed that the liver and thyroid were the target organs of CDE treatment but the changes were mild and adaptive even at the highest dose levels. It was concluded that CDEs caused no overt toxicity at the dose levels studied.
CHANGES IN \( ^{3} \)-MUSCIMOL BINDING TO BRAIN MEMBRANES IN RATS TREATED WITH DIELDIN. B.J. Mehrotra, M. Veerapalli and J. Desaih. Dept Chem, Tougaloo College and Dept Neuro, Univ Miss Med Ctr, Jackson, MS.

Cyclodiene and organophosphate compounds have been shown to alter GABA mediated Cl- channel activity. The present studies were conducted to determine the effect of dieldrin, a cyclodiene compound, on \( ^{3} \)-Muscimol binding to rat brain membranes. Rats were treated F.O with dieldrin at 0, 10, 20, 40 mg/kg and these treatments were started the rats were sacrificed. The plasma membranes were prepared from brain tissue. \( ^{3} \)-Muscimol binding was reduced as well as dieldrin treated rat brain membranes was determined by filtration method. For in vitro effect the membranes were preincubated with dieldrin at 1 to 20 nM concentrations before the addition of \( ^{3} \)-Muscimol. The data show that dieldrin reduced Muscimol binding both in vitro and in vivo. The reduction in Muscimol binding was significant and concentration dependent in vitro whereas the reduction was not dose dependent in vivo. The results suggest that dieldrin may not be a potent modulator of GABA receptor itself but an effective modulator of Cl- channel activity. (Supported by NIEHS/MBRS Grant #BB 08110).


Male Fischer 344 rats were dosed daily 1G for 5, 10 or 15 weeks with 0, 0.1, 1, 10 or 25 mg/kg body weight. Urinary alkaline phosphatase and lactate dehydrogenase activities were elevated at 5, 10 and 15 weeks of PCB exposure and the kidney-to-body weight ratios were elevated at 10 and 25 mg/kg dose levels after 10 and 15 weeks of exposure indicating nephrotoxicity. Hypercalcemia was present at the highest dose level after 5 and 10 weeks of exposure but serum calcium concentration was normal at 15 weeks. Femur density was increased at the 10 mg/kg dose level after 5 weeks, at all dose levels after 10 weeks and at all dose levels which did not demonstrate overt toxicity after 15 weeks of PCB exposure. Cross-sectional, medullary and cortical areas of the femur were significantly decreased at the higher dose levels after 10 and 15 weeks of exposure. The percent medullary area though was significantly decreased after 10 and 15 weeks of PCB exposure indicating that not only was there a decrease in medullary size but a decrease relative to the cortical bone area. These results demonstrate that PCB exposure affects calcium metabolism and bone morphometry and is nephrotoxic. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

DOES ENDOSOMOSIS OF LDL PLAY A ROLE IN THE MOVEMENT OF HEXABROMOHEXNYL (HBB) INTO PERITELIAL CELLS? S J Jong and J A Bernstein. Department of Environmental and Industrial Health Toxicology Program, University of Michigan, Ann Arbor, MI.

The pathway by which HBB, given systemically to the rat, moves from the blood into the intestine is unknown but is not via the bile duct (Matthews et al., Toxicol. Appl. Pharmacol., 16, 1970). The finding (Kaufman and Bernstein, J. Toxicol. Env. Health, in press) that low density lipoprotein (LDL) facilitates the movement of the bromine across the cellular membrane of 3T3L1 adipocytes and the demonstration (Malik and Gothrie, J. Lipid Res., 23, 476, 1982) that hexachlorobiphenyl associates with LDL suggest that LDL might carry HBB from the blood into intestinal cells when LDL is internalized by endocytosis. To test this hypothesis, the rate of entry for LDL-associated \( ^{14} \)C HBB into wild type Chinese hamster ovary (CHO) fibroblasts was compared with the rate of entry into CHO mutants lacking functional LDL receptors. Although LDL does not enter the mutant by endocytosis, LDL does enter both cell types by diffusion alone. HBB. Both cell types were equilibrated in a medium containing LDL-associated "cold" HBB and free "cold" HBB for 24 hours and then exposed to LDL-associated [\( ^{14} \)C] HBB plus free "cold" HBB. The radioactivity in the cells was determined at various times after exposure. The amount of HBB found to be 2.5-5 X greater in the wild type cell at 1 minute post exposure than in the mutant. By 5 minutes the rates of entry into both cell types were equivalent. It does appear that LDL facilitates the entry of HBB into the cell by mechanisms dependent on the presence of LDL receptors. The relative importance of LDL mediated diffusion in the entry of HBB into the cell must now be evaluated.


To assess the potential of 100 ppm epichlorohydrin (ECH) EI to induce hepatic and renal toxicity, 60-day-old male F344 rats were exposed to either 0 or 100 ppm ECH via inhalation. The dosing regimen was either a 4 hour exposure with sacrifice on days 0-3 after treatment or a 4-day exposure of 4 hours per day with sacrifice 0.1 and 3 days after treatment. Endpoints assayed were liver and kidney weights, histopathological evaluation of liver and kidney, urine and serum chemistries, hepatic cytochrome P-450 and hepatic glutathione. The hepatic endpoints did not appear to be affected by exposure to ECH. A small but statistically significant increase in relative kidney weight occurred on day one post-exposure. However, neither serum nor urine chemistries appeared to be affected by ECH and histological reports have not, as yet, verified any level of kidney damage. The biological significance of this small increase in relative kidney weight, in the absence of other indicators of renal toxicity, is unknown. (This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.)
EFFECT OF MULTIPLE DOSES OF PERFLUORODECANIC ACID (PFDA) ON GROWTH AND LIPID METABOLISM IN FEMALE SPRAUGE-DAWLEY RATS. T Borges, H P Glauert, R E Peterson, L W Robertson.  

Dose-related effects of PFDA were studied in female rats (0.0, 0.3, 1.0, 3.0, 10.0, or 30.0 mg/kg, four i.p. injections/rat at two week intervals). Rats were killed one week after the last injection. Rats receiving cumulative doses < 10 mg/kg showed normal weight gain and feed intake; rats receiving cumulative doses > 10.0 mg/kg increased their feed intake but gained weight normally; rats receiving > 40 mg/kg decreased feed intake and lost weight. Although peroxisomal acyl-CoA oxidase was increased in a dose-dependent manner, peroxisomal β-oxidation as measured by flux through the pathway was decreased. Plasma triglycerides, as well as HDL, VLDL, and LDL when expressed as percent of the total cholesterol, were not significantly different than control animals. A sharp band migrating in the HDL region in the 110protein patterns of two higher dose groups was noted. These results show that 1) PFDA decreases peroxisomal β-oxidation even though the activity of fatty acyl-CoA oxidase, the first enzyme in the pathway, is increased; and 2) PFDA decreases feed efficiency. (Supported by NIH grant CA43719)

EFFECTS OF HALOACETONITRILES (HAN) ON CYTOCHROME P-450 AND MONO-OXGENASES IN VITRO AND IN VIVO. E L Lin, J K Matox, and B H McFarland, USEPA, HEDL, Cincinnati, OH.  

Incubation of microsomes with monochloroacetanitriile (MCAN), dichloroacetanitriile (DCAN), trichloroacetanitriile (TCAN), bromochloroacetanitriile (BCAN) and dibromoacetanitriile (DBAN) in the absence of NADPH resulted in the decrease of cytochrome P-450 (P-450) content. In the presence of NADPH, the extent of destruction of P-450 was either increased or decreased, depending on both the HAN and the source of microsomes (from uninduced, 3-methylcholanthrene- or phenobarbital-induced rats). The loss of P-450 was partly due to the conversion of P-450 to cytochrome P-420. All HAN inhibited aminopyrine-N-demethylase (AmN), aniline hydroxylase (AnHx), and 7-ethoxycoumarin-O-deethylase (ECo) in vitro. With the exception of DCAN, all the other HAN also inhibited 7-ethoxycoumarin-O-deethylase (ECo). Generally, the reactivity of the HAN were DBAN > BCAN > TCAN > DCAN > MCAN. Following oral dosing, MCAN had no significant effect on these parameters and the di- and trihalogenated HAN decreased P-450 concentration at 1, 3, and 18 hr. They also inhibited AmN at 18 hr, AnHx at 3 and 18 hr, and ECo at all three time points. In contrast to the in vitro study, ECo was increased at 1 hr. However, the enzyme activity fell to the control level by 3 hr and became depressed at 18 hr. (Abstract does not necessarily reflect EPA policy).

GASTRIC, RENAL AND HEPATIC TOXICITY OF CHLOROPROPANONES (CP). C L Smallwood, L W Condie, B A Merrick. USEPA, HEDL, Cincinnati, OH.  

CPs are chlorination by-products of pulp mill effluents or of humic acids in drinking water. Prior work has shown that MonoCP, 1,1-diCP and 1,3-diCP react directly with sulfhydryls and are cytotoxic in hepatocyte suspensions. The present study examines in vitro chemical reactivity and systemic toxicity of 8 CP isomers in mice after oral gavage in corn oil. Serum chemistries and histopathology were performed 24 hr after treatment and glutathione (GSH) levels were measured after 2 hr exposure. All isomers except 1,1,1-TrICP, reacted at equimolar concentrations of 5 mM directly with thiophenol in vitro. In vivo, gastric necrosis and edema occurred with all CP isomers except 1,1,1-TrICP. MonoCP, 1,1-diCP and 1,3-diCP most effectively depleted gastric GSH levels. 1,1-diCP decreased hepatic GSH and produced diffuse hepatic necrosis, radiating from periporal to midzonal areas with increasing dose. PentaCP and HexaCP resulted in mild hepato-cellular degeneration but also produced moderate to severe renal proximal tubular necrosis. Kidney GSH was greatly reduced by HexaCP. Liver damage was accompanied by increased serum transaminase activities and renal toxicity was evidenced by elevated blood urea nitrogen levels. Thus, chemical reactivity of CP isomers with sulfhydryls is associated with in vivo toxicity. (Abstract does not necessarily reflect EPA policy).

SUBCHRONIC TOXICITY STUDIES OF PENTACHLOROBENZENE (PeCB) IN F344 RATS AND B6C3F1 MICE. L S Sondelbach, ASK Hurley, HJ Esber, and RB VanRyzee, NRC/IDP. Research Triangle Park, NC and EG&G Mason Research Institute, Worcester, MA.  

To assess the subchronic toxicity of PeCB, male and female F344 rats and B6C3F1 mice were studied for PeCB for 14 days (0, 100, 330, 1000, 3300, and 10000 ppm) and 13 weeks (0, 33, 100, 330, 1000, and 2000 ppm). Toxicity in both species at 14 days included mortality, body and organ weight changes. By 13 weeks, reduction of body weights and food consumption were seen only in rats. In both species, liver and kidney weights (absolute and relative) were increased, and in rats the thymus weight decreased. Central and midzonal hepatocellular hypertrophy, and focal necrosis appeared correlated with increased serum LDL and GGT. Protein casts, renal tubule degeneration, and mineralization of renal papilla were associated with proteinuria and glucosuria. Decreased thyroid hormones and increased TSH suggested hypothyroidism. Increased liver porphyrin was more pronounced in mice than rats. Under conditions of this study, primary target organs for PeCB appeared to be liver, kidney, lungs and thyroid in rats, and liver in mice.
1,2,3 TCB was evaluated for oral subchronic toxicity in a 13 week study using Crl:CD BR rats. Male and female rats (30/sex/dose) were treated with 0, 50, 250 or 500 mg/kg/day of the test material by corn oil gavage. Decreased body weight in spite of normal to increased food consumption, and increased relative and absolute liver and kidney weights were observed in males and females of the mid- and high-dose groups. Alterations in clinical chemistry parameters indicated the presence of kidney damage, and gross examination revealed mottled kidneys and/or kidneys with rough surfaces in males and females in the 250 and 500 mg/kg/dose groups. Histopathological examination demonstrated a corresponding chronic nephritis characterized by necrotic cellular casts, purulent casts, protein casts and/or tubular regeneration which is indicative of an ongoing degeneration of tubular epithelial cells. No significant toxicological effects were observed at 50 mg/kg/day in either sex, which is thus identified as the No Observed Adverse Effect Level. The toxicity of TCB will be compared to the known toxic effects of other chlorinated benzenes to assess structure/activity relationships.

In order to evaluate the influence of route of exposure on target organ toxicity and PK of halocarbons, we subjected male and female rats to equivalent inhalation and oral CCl₄ exposures. 350-450 g rats with an indwelling arterial cannula inhaled 100 or 1000 ppm CCl₄ for 2 hr through a 1-way valve. Based on the following equation: 0.5 x minute volume x time x inhaled conc., an equivalent oral dose of 20 or 200 mg/kg was given in an aqueous emulsion as a bolus. Serial blood samples were taken and analyzed for CCl₄ in order to obtain blood concentration versus time profiles. Blood, lung and liver samples were taken 24 hr after dosing for measurement of serum and microsomal enzymes. Tung and liver microsomal proteins were separated by SDS-PAGE and 400 enzymes quantitated by a gel scanner. Rats inhaling CCl₄ attained near steady-state blood levels during the 2-hr exposure, which were 3 times lower than peak levels achieved after an equivalent oral dose. AUC and toxicity indices were higher for the ingestion than for the corresponding inhalation groups. Our findings indicate that route of exposure can significantly influence the PK and target organ toxicity of CCl₄ in rats. (Supported by EPA CR812267 & Air Force AFOSR 07248)

DISTRIBUTION AND EXCRETION OF PERFLUORO-N-DECANOCIC ACID (PFDA) IN RATS. W. E. GEORGE, G. L. PILCER, and M. L. ANDERSEN. Harry G. Armstrong Aerospace Medical Research Laboratory, Toxic Hazards Division, Wright-Patterson AFB, OH.

PFDA, a straight chain 10 carbon fluorinated carboxylic acid, causes anorexia, body weight loss, peroxisome proliferation, and extreme hepatomegaly in rats. The liver is the main target organ with interference in lipid metabolism and utilization a major toxicity effect. In this study, the tissue distribution and excretion of 14C-labeled PFDA was determined 7 and 30 days after a single i.p. injection at three dose levels, 50, 20, and 2 mg/kg. Body weights, food and water intake, fecal and urine output were measured daily and the total carbon-14 measured in urine and feces. Blood and tissue levels were measured at 7 and 30 days. There was a dose dependent weight loss and recovery. The principal route of excretion was in the feces, 0.5-1.5% daily with a small amount excreted in urine and no 14CO₂. Although carbon-14 was found in all tissues, the major portion was in the liver with significant amounts in muscle, skin and blood. A comparison of tissue levels indicates PFDA is found in all tissues decrease over time, appear to be carried to the liver and slowly excreted in the feces.

INFLUENCE OF THE PATTERN OF INGESTION ON THE PHARMACOKINETICS OF PERCHLOROETHYLENE (PER) IN RATS. R. RAMANATHAN, S. MURALIDHARA, C. R. DALLAS, J. M. GALLO, AND J. V. BRUCKNER. Deps. Pharmacology & Toxicology and Pharmaceutics, College of Pharmacy, University of Georgia, Athens, GA.

The objective of this study was to investigate the influence of different oral administration regimens on the systemic uptake, disposition, and elimination of PER, a common contaminant of drinking water supplies. PER was given to anesthetized male Sprague-Dawley rats as an aqueous Emulphor emulsion, either orally as a single bolus or infused through a surgically implanted gastric catheter over 2 hr in doses of 10, 25, 50, and 100 mg/kg. Blood samples were collected from an indwelling carotid arterial cannula during and post infusion from 0 to 540 min. The blood samples were analyzed for PER using a GC-EC head space technique. Cmax, AUC, elimination half-life and other PK parameters for the two patterns of ingestion were determined and contrasted. AUC and Cmax were lower and the terminal elimination half-life (t½) was longer when PER was infused than when given as a single oral bolus. The AUC and Cmax values were proportional to dose, though the increase in Cmax was nonlinear at the 100 mg/kg dose. These findings indicate that the PK of PER can be significantly influenced by the pattern of ingestion. (Supported by U.S. EPA Cooperative Agreement CR812267 and U.S. Air Force AFOSR 872428 & 880277)
PHYSIOLOGICAL PHARMACOKINETIC MODELS FOR 1,1,1-TRICHLOROETHANE (TCA) AND 1,1,1-TRICHLOROETHYLENE (TCE) IN RATS FOLLOWING INHALATION AND ORAL EXPOSURES. J M Gallo, C E Dallas and J W Bruckner, Deps. of Pharmaceutics and *Pharmacology & Toxicology, College of Pharmacy, University of Georgia, Athens, GA.

Accurate prediction of blood and tissue concentrations of volatile organic compounds (VOCs) for different exposure routes would be an important contribution to risk assessment of VOCs. Physiological pharmacokinetic (PBPK) models were developed for TRI and TCE in rats to predict blood and tissue concentrations, and will be used to scale model predictions to humans. In the present investigation, TRI and TCE were administered to rats by inhalation and by oral gavels at two doses. Blood and exhaled breath TRI and TCE concentrations were monitored during and following the inhalation exposure while blood concentrations were measured following the oral doses. Blood flow-limited PBPK models were developed for each compound, based on both literature and experimentally-determined values for the model parameters. The PBPK model for TRI was characterized by both a pulmonary and a linear metabolic elimination pathway, while the elimination of TCE required a Michaelis-Menten metabolic component. Overall, model predictions of blood and exhaled breath TRI and TCE concentration agreed with observed values. (Supported by U.S. Air Force AFOSR 870246 and U.S. EPA CH 812267)

PHYSIOLOGICALLY-BASED COMPUTER SIMULATION OF INHALATION EXPOSURES OF MALE P-344 RATS TO CHLOROTRIFLUOROETHYLENE Oligomer. A Vinegar, D L Pollard, E R Kinkead, and R B Conolly. NSI Technology Services Corp., Dayton, OH.

CITE, a candidate hydraulic fluid, consists of chlorotri fluorooethylene oligomers. A physiologically-based pharmacokinetic model for CITE was developed with pharmacokinetic data collected after exposure of male P-344 rats to 0.5 mg/L for 6 hr or for 6 hr/day, 5 days/wk; 90 days. Gas chromatograms of CITE showed two groups of peaks. Model development focused on the first group of lighter molecular weight oligomers. Partition coefficients were estimated from tissue/blood concentration ratios obtained at the end of the 90 day exposure. The model indicated that CITE pharmacokinetics are sensitive to the f: b blood and blood-to-air partition coefficients and to the rate of CITE diffusion in fat. Moreover, the model showed that although the 90 day exposure was intermittent, fat concentrations of CITE increased over time to the point where fat storage of CITE drove continuous blood and tissue exposure which was modulated upward during the daily inhalation exposures. This observation is probably relevant to hepatic lesions which developed during the 90 day study and which are described elsewhere. This study provides insights of the pharmacokinetic behavior of CITE and illustrates PBPK modeling of mixtures of structurally similar materials. (Supported by DOD Contract No. F33615-85-C-0532)

PROTECTION AGAINST CARBON TETRACHLORIDE HEPATOTOXICITY WITH ALPHA TOCOPHERYL SUCINNATE ADMINISTRATION. M K Fariss, E E Hylton, C H Stubin, K L Foster and G E Magee. Environmental and Molecular Toxicology, Department of Pathology, Medical College of Virginia/Virginia Commonwealth University, Richmond, VA.

We have previously demonstrated that alpha tocopheryl succinate (TS) protects rat hepatocytes from chemically-induced toxicity. To test the cytoprotective nature of TS in vivo, we examined the ability of vitamin E congeners to protect rats from the hepatotoxic and lethal effect of carbon tetrachloride (CCL4) administration. Rats were given a single 100 mg/kg intraperitoneal dose of TS, alpha tocopherol (T), beta tocopherol (B) and vehicle control, then fasted for 24 hours prior to the oral administration of CCL4. Following a toxic dose of CCL4, rats were monitored for weight loss, survival time, plasma ALT and AST levels, liver tocopherol congener concentrations and liver histopathology. Our results showed that only rats pretreated with TS were protected from the hepatotoxic and lethal effects of CCL4. The administration of TS dramatically increased the LD50 for CCL4 from 2.5 (vehicle control) to 4.4 g CCL4/kg. Pretreatment with TS also significantly reduced plasma liver specific enzyme levels in rats 48 hrs following a 2.9 g/kg dose of CCL4. These data clearly demonstrate that TS is a unique and potent cytoprotective agent in vivo.

TRICHLOROETHYLENE (TCE) AND TRICHLOROACETIC ACID (TCA) INDUCTION OF MICROSONAL CYTOCHROME P-450 AND PEROXISOME PROLIFERATION IN RATS AND MICE. M E Knuckles, University of Alabama at Birmingham, Birmingham, AL. Sponsor: R E Meeks

Various structurally diverse compounds induce peroxisome proliferation and cytochrome p-450dependent fatty acid hydroxylase. In this study, TCE and its major metabolite TCA were examined for their ability to induce cytochrome p-450 and peroxisome enzymes in F-344 rats and B6C3F1 mouse livers. Rats and mice were administered TCE or TCA for 10 consecutive days by gavage in corn oil. Induction of cytochrome p-450 was determined by measuring differential hydroxylation of the omega- and omega-1 positions of 1-2-lauryl-3-11-carboxylic acid. Separation of omega- and omega-1 hydroxylates was accomplished by HPLC using an isocratic system of methanol/30% acetic acid (62:37:3.8:0.2%). Peroxisome proliferation was determined by the increased beta-oxidation of palmitoyl-CoA. TCE induced peroxisome proliferation up to 4-fold in mice, but induction in rats was not significant. TCA induced peroxisome proliferation up to 3-fold in both rats and mice. Induction of omega-hydroxylation up to 7-fold was observed for both species treated with TCE whereas TCA treatment caused a 9-fold increase. Neither compound caused a significant increase in omega-1 hydroxylation. These data suggest that induction of omega-hydroxylation may be independent of peroxisome proliferation.
It is well established that ozone (O₃) exposure causes adverse biochemical and cellular processes in lung tissue. However, the genetic effects of O₃ are still uncertain. This study was conducted to evaluate potential genetic effects of O₃ by measuring body cell proliferation and the induction of chromosome aberrations (CAs) in pulmonary alveolar macrophages (PAMs). Four groups of female P344/M rats (5 rats/group) were exposed by inhalation for 6 hr to air only, 0.12, 0.27, or 0.8 ppm O₃. Four hr before sacrifice, rats were injected i.p. with colchicine (6 mg/kg body weight) to arrest metaphase cells for CA analysis. PAMs were recovered from each rat by lung lavage 24 hr after exposure. The mitotic index (MI) of the PAMs was also determined. A statistically significant increase in the frequency of CAs and a decrease in the mitotic index were detected after exposure to 0.27 ppm O₃. Inhalation of 0.8 ppm O₃ induced a 4-fold increase in MI. Results from this study indicate that exposure to low doses of O₃ can cause changes in cell cycle kinetics and induce genetic damage in respiratory tract cells. (Research sponsored by the U.S. DOS/ONR under Contract No. DE-AC04-76EV03013 and by NIH Grant ES04162.)

**Effects of Particle Adsorption on Benzo(a)Pyrene-Induced Sister Chromatid Exchange.**

Benz[a]pyrene (B[a]P), a combustion by-product and a ubiquitous toxic contaminant, is frequently found adsorbed to airborne particles. At present, solvent extracts are most commonly utilized in the measure of genetic damage from such particulates. Evidence in this study, however, strongly suggests that whole particle bioassays more accurately reflect the genotoxicity and the overall biological activity of adsorbed pollutants on cells. Specifically, Syrian hamster embryo cells were exposed for 24 hours to whole particles: wood charcoal; coal fly ash (CFA); and titanium dioxide each with adsorbed B[a]P and, for comparison, to equivalent doses of B[a]P in solution. Results demonstrate that particle adsorption significantly decreased the number of B[a]P induced SCEs/cell as compared to those induced by equivalent doses of B[a]P in solution. SCEs induced by B[a]P adsorbed to wood charcoal were 3-6 fold lower than those induced by corresponding doses of B[a]P in solution. SCEs induced by B[a]P adsorbed to either CFA or titanium dioxide were ~2 fold lower than those induced by equivalent doses of B[a]P in solution. This study demonstrates that particle adsorption can affect the genotoxicity of B[a]P and is highly relevant because, unlike most investigations, it reflects the effects from actual cellular exposure to ambient pollutants.
ALERTATIONS IN ETHYLATING EFFECTS OF ETHYLENE OXIDE (ETO) USING DIFFERENT TREATMENT REGIMENS. D G Elford, T Swearengen, K Begley, E R Savage, Jr, W Moorman, and J McLaurin. NIOSH, DBS, ETB, THS, Cincinnati, OH.

Several concentration x time dosing regimens of ETO were investigated to determine the degree of ethylation in F344 rats. Exposures were for 5 days/wk for 6 wks. at 1) filtered clean air-control, 2) 100 ppm x 6 hrs., 3) 40 ppm x 1.5 hrs., and 4) 600 ppm x 1 hr. Sixty days after inhalation exposures were ended, a single ip injection of 25 ug/kg bw of 14C-ETO was administered to each animal. Bound radioactivity was determined in brain, kidney, liver, lung, lymphocytes, spleen, and testes. A significant increase in radioactivity over controls was observed in lung, spleen, and lymphocytes at all ETO treatment regimens; however, such increases in brain, kidney, and testes were seen only at the 100 ppm x 6 hrs dosing level. A significantly greater amount of radioactivity above controls was found in spleen DNA for all ETO treatment regimens. In lung DNA increased radioactivity was seen with both 100 ppm x 6 hrs. and 600 ppm x 1 hr. dosing levels. In testes DNA an increase was observed only at 100 ppm x 6 hrs. dose regimen. These findings suggest that previous exposure to ETO might increase the ethylating capability to other ETO exposures even when it occurs 60 days later and that exposure to low levels of ETO for longer time periods may be more deleterious than exposure to high concentrations for short time periods.

GENOTOXICITY OF LEWISITE IN CHINESE HAMSTER OVARY CELLS. R F Jostes, R J Rausch, B M Miller, L B Sasser and J C Bacre. Pacific Northwest Laboratory, Richland, WA and U.S. Army Biomedical Research and Development Laboratory, Ft. Detrick, Frederick, MD.

The cytotoxic, clastogenic and mutagenic effects of the arsenic containing vesicant, Lewisite (L-dichloro(2-chlorovinyl) arsine), have been investigated using Chinese hamster ovary cells. One hour exposures to L were cytotoxic at 0.5µM amounts. The cell survival response yields a D0 of 0.5µM and an extrapolation number of 2.5. The mutagenic response at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus was sporadic and not significantly greater than control values when cells were exposed over a range of 0.125 to 2.0µM. Sister chromatid exchange (SCE) induction, a measure of chromosomal rearrangement, was weakly positive over a range of 0.25 to 1.0µM but the values were not significantly greater than the control response. Chromosomal aberrations were induced at 0.75 and 1.0µM in one experiment and 0.5 and 0.75µM in another experiment. The induced values were significantly greater than the control values. Lewisite appears to be cytotoxic and clastogenic in our investigations but SCE and mutation at the HPRT locus are not significantly greater than control values. Supported by the U.S. Army Medical Research and Development Command Contract BAPP8865 under a Related Services Agreement with the U.S. Department of Energy under Contract DE-AC07-80RLO.


BNOA (CAS NO. 120-23-0) is used as a plant growth hormone analog to promote fruit ripening and prevent premature dropping of fruit from plants. Because residues may remain on food crops, studies were undertaken to examine the genetic toxicity of BNOA. The Chinese Hamster Ovary (CHO) system was used to examine the potential for BNOA to cause chromosome aberrations in vitro. Three concentrations of BNOA (0.5, 0.75 and 1.0 mg/ml) were tested both with and without a rat S-9 metabolic activation system. The incidence of chromatid breaks and fragments was within the normal range. Evaluation of the in vivo clastogenic potential was performed by the peripheral blood microuncleus test, with use of 340 µg/kg, 213 µg/kg, and 106 µg/kg (approximately 80%, 50%, and 25%, respectively, of the LD50) were given to both male and female mice. Peripheral blood polychromatic erythrocytes of the test animals sampled at 30, 48, or 72 hours post-dosing exhibited no dose-related increase in microuncleus. In summary, BNOA was not considered to be clastogenic in either the in vivo mouse microuncleus test or the in vitro CHO system.

GENOTOXICITY OF TETRAETHYLENE GLYCOL (TEG) EVALUATED WITH MULTIPLE IN VITRO AND IN VIVO ASSAYS. R S Slesinski, P J Guzzie, E R Morabit and B Ballantyne. Bushy Run Research Center/Union Carbide Corporation, Export, PA.

Tetraethylene glycol (TEG) [CAS #:12-60-7] was evaluated for genotoxic potential using the Ames test, multiple assessment of gene mutations, sister chromatid exchanges (SCE) and chromosome damage in CHO cells and in vivo tests for production of chromosome damage in mouse peripheral erythrocytes and rat bone marrow cells. In vitro tests were performed with and without addition of an Acoclor 125A-induced, rat-liver S9 activation system. TEG did not produce positive, dose-related increases in mutations in either the Ames test or in the CHO/HPRT forward mutation test. Significant increases in SCEs and chromosome damage were evident in CHO cells both with and without metabolic activation but the low level increases were variable and did not have a dose-related trend. A mouse microuncleus test had only a single statistically significant increase for males given 5 g/kg TEG by i.p. injection. No significant increase of chromosome damage in bone marrow cells was observed within a similar range of doses when administered orally to rats. The lack of concordance in these results suggests the possibility of testing artefacts at high doses which were relatively nocytoxic.

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COMPARATIVE GENOTOXIC AND NONGENOTOXIC EFFECTS OF TWO STRUCTURALLY SIMILAR NITROPHENYLEDIAMINE DYES (HC BLUE 1 AND HC BLUE 2) IN FEMALE MOUSE HEPATOCYTES. SM Driscoll, KM Rudo, FW Kari, S Strom, and R Langenbach. Sponsor: J Bucher. NIEMHS, Res. Tri. Park, NC.

Previously reported chronic evaluations demonstrated that HC Blue 1 produced dose-dependent increases in hepatocellular carcinomas in mice, while HC Blue 2 did not. Comparative metabolism studies also showed that HC Blue 1 yields 3 major metabolites while HC Blue 2 yields a single metabolite. Genotoxic effects were studied to help clarify these differences. Both HC Blue 1 and HC Blue 2 induced significant levels of SCE in female mouse hepatocytes. There was a dose-dependent increase in the number of chromosomal aberrations in the first division cells exposed to HC Blue 1 while no dose-related effect could be demonstrated with HC Blue 2. Cultures examined at harvesting showed those exposed to 100 μg/ml HC Blue 1 had 25-35% fewer cells than the control or those containing the same concentration of HC Blue 2. A significant delay in chromosomal replication time occurred in hepatocytes exposed to HC Blue 1 as compared to controls or cells exposed to HC Blue 2. When studied in the V79 metabolic cooperation system, HC Blue 1 inhibited cell-to-cell communication, while HC Blue 2 did not. These observations indicate that HC Blue 1 is a more potent genotoxic agent than HC Blue 2, and suggest that the carcinogenic activity of HC Blue 1 may involve genotoxic and non-genotoxic mechanisms.

MIREX (MX) INDUCES HEPATIC ORNITHINE DECARBOXYLASE (ODC) ACTIVITY IN RATS. A K Mitra and A P Kulkarni. Florida Toxicology Research Center, College of Public Health, University of South Florida, Tampa, FL.

ODC, the initial and rate-limiting enzyme in the biosynthesis of polyamines, is considered to be a biochemical marker of tumor promotion. The pesticide, MX, was gavaged (at a dose of 180 mg/kg) in female Sprague Dawley rats and the hepatic ODC activity was measured radiometrically at 12, 24, 36, and 48 hrs. post-dosing. It was observed that at 36 hr. posttreatment, an increase of 55-fold in ODC specific activity occurred over controls. In a separate study, MX (120 mg/kg) was gavaged twice at 21 and 4 hrs. before sacrifice and ODC activity was found to be about 70 times the controls. Additional data indicated a 50% increase in serum GPT and no significant change in levels of cytochrome P-450, reduced glutathione, and DNA. Although the exact mechanism of ODC induction due to MX is not clear at the present time it seems reasonable enough to believe that MX is a potent inducer of ODC. Supported by a grant from the Environmental Protection Agency.


The genotoxicity of the spa sanitizer/algicide, lithium hypochlorite (LiOCl), was evaluated. LiOCl was not mutagenic in the Ames Test when tested in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537 or TA1538 with or without metabolic activation by rat liver microsomes (S9). LiOCl did not increase the frequency of mutations at the hypoxanthine-guanine phosphoribosyl transferase (HGPRT) locus of Chinese hamster ovary (CHO) cells with or without S9. LiOCl did not induce DNA damage in the unscheduled DNA synthesis assay using rat primary hepatocytes. Metaphase chromosomes were evaluated in vitro in CHO cells after 12 & 18 hrs exposure without S9 and after 12 and 22 hrs following a 2-hr. exposure to S9. LiOCl induced a statistically significant increase in chromosome aberrations at the high dose only at both harvest times with or without S9. To further assess the effect of LiOCl on chromosome structure, an in vivo cytogenetics study was conducted. There was no increase in chromosome aberrations in bone marrow cells, collected 6, 24 & 48 hrs after a single oral dose of LiOCl to rats (100, 500, 1000 mg/kg in males; 50, 250, 500 mg/kg in females). The weight-of-the-evidence indicates that LiOCl is lacking in genotoxicity.

NO EVIDENCE OF BENZENE INDUCED DNA STRAND BREAKS IN VIVO. E W Lee and C D Garner. Biomedical Science Dept., GM Research Labs, Warren, MI.

The capability of benzene (B), p-benzoquinone (BQ), and 1,2,4-benzenetriol (BT) to induce DNA strand breaks was investigated to determine if B administration results in cellular DNA damage. In the in vitro test, bone marrow (BM) cells obtained from untreated mouse femurs were reacted with BQ or BT for 60 min. DNA strand breaks were measured by labelling the cells and then detecting the DNA with autoradiography. The results showed that BQ and BT induced DNA strand breaks by 100 and 53%, respectively. Catalase completely blocked the effect of BQ, and there was no protection against the BQ effect. Consistent with the protective effects of glutathione and catalase, no induction of DNA SSBs by B was observed in vivo even with a dose (1760 μg/kg) above the LD50 of 990 mg/kg at both 1 and 24 hr post administration. These results suggest that no DNA damage was induced by B in vivo and also that the previously observed inhibitory effect of B on DNA synthesis in vivo was not produced as a result of DNA damage, but through an inhibition of DNA polymerase alpha.
USE OF LIQUID CHROMATOGRAPHY AND P-32 POST-
LABELING TO CHARACTERIZE CARCINOGEN-DNA ADDUCTS.
M Ridley, G Yalamanchili, K Asbury, W Hopkins,
M Dietrich and R Howe. Monsanto Agricultural Co.,
A Unit of Monsanto Co., St. Louis, MO.

An important criterion in determining the muta-
genic and carcinogenic potential of a chemical is its interaction with DNA. The P-32 post-
labeling method for assessing the interaction of nonradio-labeled chemicals with DNA separates normal and modified [P-32-S]3'-5'-nucleoside bisphosphates using thin layer chromatography (TLC). We have investigated the use of high pressure liquid chromatography (HPLC) for the separation of normal and modified P-32 labeled nucleotides. Adducts formed from the reaction of dGMP and DNA with dimethyl and diethyrsulfate were characterized as 5'-monophosphates while bulky adducts formed from benzo[a]pyrene and safrole were analyzed as 3',5'-bisphosphates. Good separations were obtained by conventional C-18 HPLC with a flow through radioactivity
monitor. Using the current system, the sensi-
tivity limits for the detection of N-7 alkyl adducts for methylating and ethylating agents in vitro were 1 adduct in 10^10 nucleotides and 1 adduct in 10^9 normal nucleotides, respective-
ly. Benzo(a)pyrene and safrole adducts were measured in vivo at levels of 1.7 and 2.0-3.3 adducts per 10^9 nucleotides, respectively. These results indicate that the HPLC technique could have application for the assessment of DNA interaction for a variety of chemicals.

ENHANCEMENT OF 2-AMINOFLUORENE Muta-
GENICITY BY 2,4-DIAMINOTOLUENE. Y L Pan,
M J Ryan, and C A Reed. University of Kansas
Medical Center, Kansas City, KS.

2,4-diaminofluorene (2,4-DAT), a high volume inter-
mediate in the production of various dyes, elastomers and polyurethane foams, is moderately carcinogenic to rodents. In addition, 2,4-DAT is activated by cyto-
chrome P-450 to forms mutagenic to S.typhimurium.
As other aromatic amines are activated as mutagens by the peroxidase activity of prostaglandin H synthase (PHS), we attempted to activate 2,4-DAT in Ames' testing using Tween-20 solubilized PHS from ram seminal vesicles. Metabolism initiated with either H_2O_2 or arachidonic acid yields identical results. We find that 2,4-DAT at concentrations up to 100 mM is not mutagenic to S. typhimurium strains TA98, TA97, TA100, or TA102 with the PHS activation system, nor is it toxic to the bacteria. 2,4-DAT, however, does enhance the mutagenicity of 50 uM 2-aminofluorene (2-
AF) to TA98 by up to 230% in the PHS-catalyzed
system. This effect is seen at 2,4-DAT concentra-
tions from 50 uM to 1 mM with half-maximal enhance-
ment observed at about 100 uM. This enhancement appears specific for 2,4-DAT. Isomeric DATs,
toluines, and phenylenediamines are not only devoid of this enhancing ability, but they actually inhibit the mutagenicity of 2-AF. The ability of 2,4-DAT to enhance the genotoxicity of other aromatic amines may be an important activity in regard to the potential health effects of this compound. (Supported by NIH
grant ES-04092.)

ALTERATIONS IN THE METABOLIC CAPABILITIES OF
ADULT RATS EXPOSED NEONATALLY TO ONE OF FOUR
CHEMICAL AGENTS. R C Zanger1,2, D L Springer3,
R A Danovitch2, and D R Buhler1. 1Oregon State
University, Corvallis, OR, and 2Pacific
Northwest Laboratories, Richland, WA.

Adult levels of steroid- and xenobiotic-
metabolizing P-450 enzymes have been shown to be affected by neonatally administered steroids and central-nervous-system
depressants. In order to further investigate long-term effects of neonatal exposure to foreign compounds, one to five day old rats were subcutaneously injected with diethylstilbestrol (DES), pregnenolone-16α-
carbonitrile, dimethylbenzanthracene or phenobarbital, and then examined as adults. In vivo studies of aflatoxin binding to hepatic DNA of male rats showed a 36% decrease in binding in DES animals, indicating altered metabolism of this carcinogen. Unusual, sex-
specific, growth patterns were observed in DES animals. Nine of the 26 male DES animals died between weaning and 23 weeks of age. The results indicate that neonatal exposure to DES may affect viability, growth, and xenobiotic metabolism. Detailed studies of the hepatic microsomal P-450 system are being conducted.

Work supported by Northern College and
University Association for Science (University of Washington) under U.S. Department of Energy
Contract DE-AM06-76-RLO2225.

AGE- AND SEX-RELATED CHANGES IN HEPATIC CO-
SUBSTRATE CONCENTRATION AND SYNTHESIS IN
GERIATRIC FISCHER 344 RATS. T Maziasz, D Mitchell,
C Madhu, B Gemzik, L Sendelbach and C D Kiassen
Univ. of Kansas Medical Center, Kansas City, KS.

The purpose of this study was to examine the effects of aging on co-substrate concentration and synthesis in F-344 rats. The concentration of co-substrates, adenosine
5'-phosphate, 5'-phosphoribosyl-α-d-ribofuranoside (PAPS), UDP-gluconic acid (UDPGA) and glutathione (GSH) were measured in livers of male and female rats at 5, 12, 23 or 26 months (m) of age. Similarly, the activities of PAPS synthetic enzymes, ATP sulfurylase and APS kinase, and the activity of the UDPGA synthetase enzyme, UDP-glucuronide dehydrogenase were also measured, along with the concentration of the precursor of UDPGA, UDP-glucose. Hepatic PAPS concentration was decreased in 26-month males to 55 percent of that in 5-month rats, whereas concentrations in females did not exhibit age-related changes. The activity of ATP sulfurylase was decreased in males and females at 23m to 42 and 64 percent, respectively, of activity in 5-month rats. The activity of APS kinase also exhibited an age-related decrease in males at 23m (45 percent of activity in 5-month rats), but not in females. In contrast, UDPGA and UDP-glucose concentrations, UDP-

glucose dehydrogenase activity and GSH content did not exhibit age-related changes in either sex. These results indicate that significant reductions in PAPS concentration and synthesis occur as a result of aging whereas similar changes do not occur with UDPGA or GSH. Further, these results suggest that a reduced capacity to synthesize PAPS may partially explain the reduced capacity for sulfation observed in senescent organisms. (Supported by USPHS Grants ES-03192 and ES-07079).
It has been shown that the hepatic availability and/or capacity for synthesis of co-substrate is decreased for the sulfation pathway in male and female geriatric F-344 rats; whereas co-substrate availability for glucuronidation and glutathione conjugation appears unchanged. This study examines whether these changes affect the metabolism of AA. Male and female rats at 5, 12, 23 or 26 months (m) of age were given a high dosage of AA (300 mg/kg, iv) and bile was collected for 2 hr to assess drug conjugation capacity. The cumulative biliary excretion of AA-glucuronide (AA-GSH), AA-glucuronide (AA-Glc) and AA-sulfate (AA-SO₄) in 26m males was decreased to 49, 42 and 32%, respectively, compared with 5m rats. In contrast, the biliary excretion of AA-GSH, AA-Glc and AA-SO₄ in 26m females was decreased to 81, 72 and 80% of the respective values in 5m rats. These data demonstrate that age differentially affects the formation of the major conjugated metabolites of AA in males and females. Further, the decreased formation of AA-Glc and AA-GSH suggest that factors other than co-substrate availability govern the decreased capacity of these pathways in aging. However, it is of interest that decreases in excretion of AA-SO₄ that occur with age in males parallel decreases in co-substrate concentration and synthesis capacity; unlike females, in which co-substrate availability and AA-SO₄ excretion are minimally affected. Thus, age-related changes in co-substrate availability are associated with stranding decreases in drug sulfation. (Supported by USPHS Grants ES-03192 and ES-07079).

The use of Valproate (VPA) is associated with a rare but fatal hepatotoxicity. Its metabolite, Δ4-VPA, is also hepatotoxic in rats. In order to understand the potential contribution of Δ4-VPA, this study was designed to quantify and compare the effects of VPA and Δ4-VPA on urinary metabolites of isoleucine and propionic acid. Three groups of Wistar rats received the following treatments: saline (N = 4), VPA (500mg/kg/day ip, 7 days, N = 8), Δ4-VPA (100mg/kg/day ip, 7 days, N = 8). Urine was collected on day 7 for 24hr and assayed for 7 metabolites by GCMS. 2-Methylbutyrylglycine excretion was elevated by VPA (2-fold) and by Δ4-VPA (13-fold). Both VPA and Δ4-VPA did not significantly affect 2-methyl-3-OHbutyrate and 2-methyl-3-oxobutyrate excretion. However Δ4-VPA increased tiglylglycine excretion by 250%. Propionylglycine excretion was enhanced by VPA (127%) and by Δ4-VPA (500%), compared to saline control (22.4 ± 10.2ug/ml creatinine). Propionate (free + alkaline-hydrolysable propionylcarboxylate) excretion associated with VPA and Δ4-VPA was 5.7 ± 4.5 and 12 ± 3.9ug/ml creatinine respectively. Methylmalonate was not increased by VPA but elevated 3-fold by Δ4-VPA. These findings suggest that: (1) both VPA and Δ4-VPA inhibit 2-methylbutyrylCoA dehydrogenase, (2) Δ4-VPA produces effects antigious to those of methylmalonate acidaemia.

Microosomal oxidation of simazine, terbutryn, and atrazine was investigated in rats, mice, goats, sheep, chickens, pigs, and rabbits. All species produced the same products, but rates and percentage of metabolites varied with species. No sex-related differences were noted. In rats, 3-MC induction did not quantitatively or qualitatively alter metabolism. PB induction, however, increased metabolic rates in both rats and mice, but did not alter the ratios of the principal metabolites, as compared to the uninduced product ratios in each species. Similar Km values (27.5 and 27.8 µM) and Vmax values (3.02 and 3.28 nmol atrazine/mmol P-450) were obtained for hepatic microsomes from Sprague-Dawley and Fisher rats. No difference spectrum was observed with atrazine, suggesting that the substrate does not bind tightly to cytochrome P-450. Fenton's reagent (HgNO₃ and FeSO₄) reacted with atrazine to give the diketallylation product plus the dechlorinated (hydroxy) product, indicating that an active oxygen species produced by cytochrome P-450 may catalyze the oxidative degradation of atrazine.

DISPOSITION AND ELIMINATION OF BIOFL-143, AN ANTIVIRAL AGENT. D J Embichon, K M Meekel, P Major and K K Ogilvie. Dept. Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada.

BI013-143, an experimental, purine-based acyclic nucleoside having antiviral activity, was administered iv or ip to young adult male and female Sprague-Dawley rats and to albino New Zealand rabbits to determine the (1) pharmacokinetic disposition, (2) route and rate of excretion and (3) acute toxicity. Analysis of plasma, urine and tissues for drug and/or metabolite residues was carried out by HPLC.

Acute administration elicited no obvious adverse effects in either species. The pharmacokinetic profile retained a monoeponential rate of disappearance from the bloodstream with mean plasma half-lives of 15-23 min for rats and 21-44 min for rabbits treated with dosages ranging from 50 to 250 mg/kg bw. BI013-143 was widely distributed in the extracellular space, with negligible binding to plasma proteins and erythrocytes. Elimination of the parent compound from the body, primarily via the kidney (75-90%), was rapid and degradation products were not detected.
DOSE-DEPENDENT PHARMACOKINETICS OF 5-AMINOSALICYLIC ACID (5-ASA) IN CYMONOLUS MONKEYS K-K Hwang, A Mandagere, D Drees, J Lacz, Marion Laboratories, Inc., Kansas City, Missouri.

5-Aminosalicylic Acid (5-ASA) is the therapeutically active moiety of sulphasalazine (SASP), a drug of choice in the treatment of inflammatory bowel disease. Studies in ulcerative colitis diseased patients suggest that 5-ASA is as effective as SASP. Recent toxicological evaluation of 5-ASA in Cynomolgus Monkeys (CM) revealed that doses higher than 400 mg/kg resulted in significant histological damage to the kidney. A dose-dependent pharmacokinetic study of 5-ASA in CM was conducted to assess the possible role of drug/metabolite bioaccumulation in renal toxicity. We evaluated the absolute bioavailability and the dose proportionality of 5-ASA as measured T1/2, Cmax, AUC and ClO. Ten male CM were used and each received IV or PO doses of 5-ASA ranging from 25-1600 mg/kg. Serial blood samples were taken over a 48 hr period and plasma assayed for 5-ASA and N-Ac-5-ASA concentrations by HPLC methodology. Pharmacokinetic analysis was performed using NONLIN.

Conclusion: 1) the absolute bioavailability of 5-ASA was 48%, 2) doses above 400 mg/kg demonstrated nonlinearity for T1/2, Cmax and AUC; ClO decreased, 3) the potential for dose-dependent kinetics of 5-ASA and changes in renal function in man needs to be considered.

FATE OF HEXACHLOROCYCLOPENTADIENIUM (HEX) IN FISH. A A Podowski, and M A Q Khan. University of Illinois, Chicago, IL.

To develop models for studying rate of volatile and reactive chemicals. Absorption, tissue distribution and elimination of (C-14)-Hex was studied in goldfish and bluegills. The injected radioactivity was highest in liver followed by pancreas, blood and gut with lower levels in other tissues. On Day 8 the highest activity occurred in bile and kidneys indicating excretion routes. Total radioactivity excreted in 3 Days was 19% of the dose with estimated 50 and 90% elimination of 8 and 162 D, resp. A 3-segment linear regression model gave the best fit and a compartment model indicated 2 elimination and 1 reabsorption phases. The 58% of the injected activity recovered from fish (19% unextractable) included 80% organosoluble (hexane 85%, ethyl acetate 8% and chloroform 2 products) and 20% hydrophilic products (several products). Hepatic microsomes metabolized 50% of Hex to organosolubles (17%) and hydrophilic (12%) products. Cystosol plus GSH metabolized 90% of Hex, mostly to water-soluble. The hexane extracts contained 2 products+Hex and water-solubles yielded more ethyl acetate extractables on acid hydrolysis with GSH incubates but equal amounts of hexane- and ethyl acetate-extractables on acid and alkaline hydrolysis with microsomes.


The in-vivo and in-vitro absorption of p-cyanophenol by rat embryos was studied in pregnant rats and in tissue culture media. The rats (GD-11) were dosed by gavage with 100 or 1000 mg/kg of 14C-cyanophenol (ring) and exposed for 1, 6 or 24 hrs. Blood, urine, feces, tissues, and embryos were assayed. The tissue/blood concentration ratios in most cases peaked at 1 or 6 hrs after dosing, while fat peaked at 24 hrs. The ratios were 11, 4.3, 3.3, 2.0 and 0.7 in carcass, fat, kidney, liver and skin, respectively. The embryo/blood ratios increased from 0.01 at 1 hr to 0.15 at 24 hrs. The cyanophenol derived radioactivity in embryos increased with time. Embryo units in culture were exposed to 25 or 50 μg/ml media, with or without hepatocytes for 1, 6, 24, 42 hrs. The uptake of radiocarbon by embryos increased with time. The uptake (0.18 vs 1.4%) and AUC (2.4 vs 7.5%-hrs) at 42 hrs were several-fold lower in the presence of hepatocytes. The embryo exposure in-vitro was 2700 times greater than in-vivo at equivalent effect doses.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

NICOTINE ELIMINATION IN SMOKERS FOLLOWING CIGARETTE SMOKING AND INTRA-VENOUS ADMINISTRATION J D deBethizy, J H Robinson, K T McManus, R A Davis, J H Reynolds, G T Burger, A W Hayes, R.J. Reynolds Tobacco Co., Winston-Salem, NC.

The elimination of nicotine was examined in 8 smokers on three occasions: Once following an intravenous infusion of nicotine (1.9 μg/kg/min for 10 min), and on two other occasions following the smoking of 7 cigarettes that burn tobacco (REFERENCE) and 7 cigarettes that heat tobacco (TEST). Diets were identical for each subject during each pharmacokinetic session. The elimination of nicotine following intravenous infusion was biphasic, but was triphasic when the subjects absorbed nicotine from smoking. The terminal half-life (Mean ± SD) for plasma nicotine following infusion was considerably shorter (117 ± 40 min) than the terminal half-life on each of the two smoking occasions: REFERENCE, 228 ± 77 min; TEST, 192 ± 58 min. The results indicate that following smoking there was a third elimination component for plasma nicotine with a longer half-life than when a lower amount of nicotine was administered intravenously. There was no difference in the rate of elimination of nicotine obtained when smoking either cigarette.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is a potent inducer of hepatic microsomal enzymes. The influence of induction on TCDD pharmacokinetics was studied with TCDD, a radio-labeled TCDD analog. Female C3H/He mice were treated with an inducing dose of TCDD (0.1 μmol/kg) or vehicle, followed by a non-inducing dose (0.1 μmol/kg) of TCDD 3 days later. In naive mice the peak TCDD level was found in the fat (400 pmol/kg). In contrast, in induced mice the highest TCDD level was in the liver (605 pmol/kg). Induced mice attained peak tissue TCDD concentrations earlier than naive mice (3 days vs 4 days after dosing). Whole body excretion was also faster in the induced mice. These results were satisfactorily described by a pharmacological pharmacokinetic model in which induction increased the amount of microsomal TCDD-binding protein and the metabolic rate of free TCDD. In agreement with results from earlier physiological modeling, the primary factor influencing the liver/fat concentration ratio is the affinity and capacity of the microsomal TCDD-binding protein. Risk assessments based on high-dose rodent experiments need to consider these dose dependencies of TCDD distribution.

A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR CHLOROFORM. A R Corley, A L Mendrala, F A Smith, M L Gargas, R H Conolly, M E Andersen, R H Reitz. Dow Chemical Company, Midland, MI; 2AAMRL/TH WPAFB, OH; 3NSIT, Dayton, OH.

A physiologically-based pharmacokinetic model was developed and validated to describe the disposition of chloroform and its metabolites in mice, rats, and humans. Macromolecular binding, which has been associated with chloroform-induced cytotoxicity, was emphasized as a measure of “internal dose.” Metabolic and macromolecular binding constants for rodents were derived from in vivo metabolism studies. Human metabolic constants were estimated from in vitro studies with human tissues. The model successfully described a wide variety of experimental data in several species, including humans, exposed to chloroform by different routes. Recurrent cytotoxicity followed by compensatory cellular regeneration is an important stage by which compounds such as chloroform are believed to influence the process of carcinogenesis in laboratory animals. The PB-PK model for chloroform represents the first stage in the development of a pharmacodynamic cancer model linking mechanistic and kinetic data with cytotoxicity and cellular regeneration.


Physiologically-based pharmacokinetic modeling is dependent on the accurate determination of tissue/blood distribution coefficients (Kp). The present study was undertaken to evaluate the validity of the in vitro estimation of Kps of organophosphate insecticides. Single-pass perfusions of mouse livers in situ with parathion (PS) or methyl parathion (MPS) were performed in order to determine Kp from the following equation t1/2 = 0.693 (VH)(Kp)/Q where t1/2 is the half-life for approach to steady-state of the chemical, VH is the volume of the liver, Q is the rate of perfusate flow, and Kp is the liver/perfusate distribution ratio. Equilibrium dialysis of liver homogenate and perfusate was utilized to estimate Kp in vitro. Kp for MPS was found to be 16.4 ± 7.3 and 7.7 ± 2.3 (MEAN ± SD) from perfused livers and equilibrium dialysis respectively. Estimations of Kp for PS gave 15.6 ± 6.3 and 9.5 ± 2.6 from perfused livers and equilibrium dialysis, respectively. These results suggest that equilibrium dialysis can be utilized to give a reasonably accurate estimation of tissue partitioning of these insecticides. (Supported by NSF/Industrial/Univ. Center for Res. in Hazardous and Toxic Substances, and NIEH Grant ES04335).

A BIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR ORAL DOSING OF ETHYL ACRYLATE. C B Frederick, D W Potter, I M Chang-Mateu, M E Andersen, Rohm and Haas Co., Springs House, PA and AAMRL/TH, Wright-Patterson AFB, OH.

A biologically-based computer model has been developed to describe the metabolic fate of ethyl acrylate (EA) in rats following gavage dosing. The model is based on in vivo rates of ester hydrolysis, protein binding, glutathione conjugation, and blood/tissue partitioning and in vivo rates of glutathione synthesis for 14 tissues. The predictions of the model are consistent with a variety of in vivo metabolic results. For example, the predicted and observed metabolic profile of a high gavage dose of EA is concordant with a toxic response only at the site of dosing that follows severe glutathione depletion. Lower gavage doses are predicted to cause minimal changes in glutathione concentration and no subsequent local toxicity. Rapid systemic detoxification is predicted that is consistent with the lack of toxicity observed in tissues remote from the site of dosing. The model provides a valuable quantitative tool in understanding and predicting the toxic response (or lack thereof) of a tissue based on the rate of EA delivery and the metabolic characteristics of the tissue.
Accurate extrapolation of experimental animal data to man is one of the most difficult areas of toxicology involving both cross-species and cross-dose extrapolation. Given an adequate experimental data base, physiologically pharmacokinetic (PB/PK) modelling can provide a means of predicting the fate of a chemical in man from that known in animals.

Rats and mice were exposed to a range of methylene chloride concentrations from 100-4000 ppm. The rates of elimination of methylene chloride and its metabolites CO and CO2 were measured and in vivo metabolite rate constants K and Vmax calculated for the two known metabolic pathways. These constants were also measured in vitro using liver fractions from rats and mice and found to accurately represent the species differences seen in vivo. Metabolic rates were also measured in vitro with human liver fractions. These in vitro values were used in a PB-PK model to predict the behaviour of methylene chloride in man by scaling from experimental data obtained in the mouse in vivo. Using these techniques it was found that the uptake and metabolism of methylene chloride in man could be predicted for a wide range of exposure concentrations.

Gastrointestinal absorption in PB-PK models is typically described as a one-compartment, first-order process. This approach is adequate for water but not for oily vehicles. In this study a two-compartment description was developed in which the dose moves from the first compartment to the second (first-order rate constant K) and is absorbed from both compartments (first-order constants KAS and KAD, respectively). Blood time course data sets for methylene chloride, chloroform, dichloroethane, and trichloroethylene after oral gavage in water or corn oil were used for model validation.

Optimization of KAS, KAD, and K for each dosing solution allowed accurate simulation of each data set. In general, KAS was 3-4 times higher when water rather than corn oil vehicle was used. KAD and K were similar between the two vehicles. By comparison, a one compartment description resulted in a poor simulation of the oil gavage data. In summary, a simple two-compartment description provides an excellent simulation of absorption from the gastrointestinal tract of a variety of toxicants dosed in water or corn oil. (Supported by Contract No. F33615-85-C-0532).

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Dialyzed tissue homogenates of 14 organs were prepared from male F344/N rats, and Michaelis-Menten rate constants for the carboxyesterase-dependent hydrolysis of [2,3-14C]ethyl acrylate (EA) were determined. After establishing linearity with incubation time and protein concentration, incubations were conducted over a wide range of substrate concentrations to estimate the apparent Km and Vmax for the enzymatic hydrolysis. The extent of hydrolysis was determined by HPLC analysis of [2,3-14C]acrylic acid formation. Although all of the tissues examined hydrolyzed EA, the maximum rate of hydrolysis varied over a 100-fold range with the liver exhibiting the highest rate (28 umol/min/g of tissue). Km values ranged from 2 mM for liver to 20 mM for kidney tissue. The results suggest that the liver, lung, kidney, and fat hydrolyze EA most effectively, and that EA is hydrolyzed very rapidly during systemic absorption and distribution.

Gastrointestinal absorption of xenobiotics in physiologically-based pharmacokinetic models: A two-compartment description. D A Staats and B Conolly. NSI Technology Services Corp., Dayton, OH.

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Trytophan (TRYP) and other cyclic aminoacids undergo a cyclization reaction with formaldehyde (FAL). Such aminoacids would be expected to act as FAL-trapping agents and be useful in methanol (MeOH) poisoning. The effect of FAL-trapping would be decreased formation of the toxic metabolic end product, formic acid (FA). While trying to develop an animal model to test the effect of TRYP and related compounds on FA production following MeOH, we found a toxic interaction between this TRYP and MeOH. In order to block the normally rapid metabolism of FA, male BALB/c mice were given methotrexate (MTX), 1 or 4 mg/kg, ip, one-half to several hours before MeOH. Then, non-anesthetic doses of MeOH (5-3 ml/kg, ip) were administered with or following dl-TRYP HCl, 400 mg/kg, ip, or an equivalent volume of water. Mice given the MeOH and TRYP were much more depressed in activity than were animals given MeOH and water. Blood for plasma FA analysis was collected via retro-orbital sinus puncture under light ether anesthesia two or four hours after MeOH. In all cases, the hematocrit was so greatly increased in animals given the combination of MeOH and TRYP that it was very difficult to collect the blood sample. With the smaller dose of MTX, there were no differences between the FA concentrations (determined by specific enzymatic analysis) in controls and in TRYP-treated mice. Significantly higher concentrations of FA occurred in mice given TRYP after the high dose of MTX (0.96 mM mean ± 0.14 mM SD) than in the mice given the water vehicle (0.44 ± 0.11). n = 6 (pooled samples from two mice per sample) per treatment, p < 0.001.
IN VITRO PRODUCTION OF TRYPTOPHAN DERIVATIVES IN RAT FECES CAPABLE OF BINDING TO THE Ah RECEPTOR. G H Perdew, and C F Babbs (Spon; C G Carlson) Foods and Nutrition and Hillenbrand Biomedical Engineering Center, Purdue University, West Lafayette, IN.

Photooxidized derivatives of tryptophan are capable of binding with high affinity to the Ah receptor. Having observed that feces is a significant source of highly reactive hydroxyl radicals, we designed an assay to determine if radical oxidation of tryptophan by fecal suspensions could produce Ah receptor ligands. Tryptophan (1 mM) was added to 1/1000 fecal suspensions and incubated at 37°C overnight. The suspension was extracted with chloroform to obtain the hydrophobic compounds present for analysis. To test for the presence of Ah receptor ligands, a competition binding assay using [125I]2-iodo-7,8-dibromo-dibenzo-p-dioxin and Hepa 1c1c7 cytosol was developed, capable of detecting 2.5 pg of a competing ligand of similar affinity. Results indicated that fecal suspensions in the presence of oxygen and 1 mM tryptophan were capable of producing greater than 50% inhibition of radioligand binding/10 μg of extracted feces. The data suggest that the colon may be a previously unrecognized endogenous source of tryptophan derivatives capable of binding to the Ah receptor.


Glycol ether solvents produce reproductive, developmental and hematologic toxicities after metabolic activation. To aid in risk assessments, metabolism of ethylene glycol monomethyl ether (EGME), ethylene glycol monomethyl ether (EGEE), and ethylene glycol monobutyl ether (EGBE) was compared in hepatocytes isolated from male F344 rats and a human organ donor. Cell suspensions were incubated with 0.02, 0.2, and 2.0 mM [14C]glycol ether for 4 hr and metabolites determined by reverse phase hplc. The major metabolite of each compound in both species was the respective alkoxyacetic acid (AA). AA increased linearly with dose in rat cells but was saturated in human hepatocytes at 0.02 mM.

Relative rates of AA formation from the glycol ethers were: EGEE > GGEE > EGME. A second major metabolite found in both species was identified as ethylene glycol (EG). Confirming results of in vivo studies (Medinsky et al., 1989).

Relative rates of EG formation were: EGEE > EGGE > EGGE. EG accounted for up to 20% of EGEE dose in rat cells. The fraction converted to EG decreased markedly at higher glycol ether concentrations in both species. These results suggest that the liver metabolizes glycol ethers by two major pathways, AA formation and O-dealkylation, and that EG may contribute to the toxicity of these compounds. (Supported by NIHES ES-55109).

EFFECT OF ETHYLENE GLYCOL (EG) METABOLITES ON ENZYMATIC ACTIVITIES IN TISSUE HOMOGENATES. J P Bercz, J Tauci, S Tauci, L Jones Toxicology and Microbiology Division, HERL USEPA, Cincinnati, OH

Previously we noted unusual depression of serum enzymes in rats subchronically exposed to EG. Suspecting that some metabolites may effectively block the in vitro expression of enzymatic activities we examined the effects of intermediates including glycolic acid, glyoxylic acid (GA), glycolaldehyde, glyoxal (G) and oxalic acid (OA) on aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase (LDH) and creatine kinase activities. These enzymes were studied using calibrated dilutions of tissue homogenates prepared from liver, kidney, heart, brain, skeletal muscle and serum of rats, and quantitated using the Baker Encro (TM) Centrifugal Kinetic Analyzer. The previously known inhibitory effect of OA on LDH has been verified in all homogenates and in serum. Additionally, a uniform inhibition of all examined enzymes by C in all samples was documented, a phenomenon which may be attributable to the extreme reactivity of the bifunctional aldehyde groups of this molecule. In contrast, GA was shown to activate several enzymes. Tissue (isozyme) specificities and relative concentration dependency of activities of these enzyme modulators will be discussed in detail. (Abstract does not necessarily reflect EPA policy.)


The ease with which hydrogen exchanges with highly tritiated solvents or metal hydrides under moderate conditions makes high specific activity tritiated compounds relatively inexpensive to produce, however, the exchange of tritium with environmental hydrogen can be a problem. Fortunately, this is not insurmountable if the extent of the exchange can be measured. [6-3H]AFB1 underwent an almost total tritium exchange with water during penetration through isolated human skin. [8-3H]AFB1 also underwent exchange but to a much lesser extent. For AFB1 the process was not enzymatic and the site of exchange appeared to be within the epidermis. The mechanism which mediated this extensive exchange was not determined.

However, the tritium in [6-3H]AFB1 was found to be very susceptible to chemical conditions which favored carbonation formation at the α-carbon of the cyclopetentene ring. The relative effectiveness of the various solvents in mediating the loss of the tritium label was 1 N NaOH >> methanol > 1.0 N HCl > water. This work serves as a warning that [6-3H]AFB1 can easily undergo significant changes in specific activity in biological tissues and under relatively mild experimental conditions. It is possible that conditions within the skin favor carbonation formation.
959 METABOLISM OF 7-ETHOXYCOUMARIN (7-EC) BY ADULT F-344 RAT SKIN. S Thohan, J Barr and L G Sipes. Department of Pharmacology & Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ.

Literature on the biotransformation of xenobiotics in adult rat skin in vitro is limited. The resilient structure of this organ, coupled with the extremely low levels of drug metabolizing enzymes have severely impeded biotransformation studies. The purpose of this work was to develop a sensitive technique for the assessment of in vitro cytochrome P-450 [P-450 mediated metabolism in adult rat skin. The O-deethylation of 7-EC yields 7-hydroxy coumarin (7-HC), a fluorescent metabolite, thus providing a highly sensitive measure of P-450 activity. Subcellular fractions of F-344 (180-220g) rat skins were prepared by minor modifications of existing procedures. P-450 content could not be determined in any subcellular fraction by conventional means. NADPH cytochrome e reductase was found to be localized in skin microsomal fraction (9 nmol/min/mg protein). Production of 7-HC by skin microsomes was found to proceed in a time and by protein dependent manner. Optimal reaction conditions allowed for linear metabolism over a 90 min incubation (17 pmol/min/mg protein). The administration of Aroclor 1254 (500 mg/kg, ip) produced a 4 fold increase in hepatic microsomal 7-EC metabolism and resulted in a 6 fold increase in the 7-HC production by skin microsomes. Further elaboration of this technique may provide consideralbe insight into P-450 mediated metabolism in skin and may be useful for biotransformation studies with human skin. (Supported by NIH No. NO1-ES-55112.

960 THE EFFECT OF NEAR-ULTRAVIOLET LIGHT ON THE METABOLISM OF BENZO(A)PYRENE BY MOUSE SKIN MICROSOMES. W B Peirano and D Warshawsky. Department of Environmental Health, Cincinnati, OH 45267 and Environmental Criteria and Assessment Office, U.S. Environmental Protection Agency, Cincinnati, OH 45268.

Near ultraviolet (NUV) light has been reported to alter the carcinogenic response of benzo(a)pyrene (BaP) in dermally exposed mice. Our data indicate that in the C3H/HeJ mouse, 3-methylcholanthrene (3-MC) induced skin microsomes have -2% BaP metabolizing capability relative to induced liver microsomes. Concurrent 365 nm light irradiation of 3-MC induced skin microsomes and BaP at 37°C greatly enhanced the total conversion of BaP to its products, -3-fold for C3H/HeJ and -8-fold for DBA/J mouse microsomes, compared to induced mouse skin microsomes and BaP alone. HPLC analyses of organic extracts indicated a more than additive enhancement of the formation of most of the individual products, due to the combined interaction of 365 nm light with BaP and skin microsomes. Similar interactions were observed using benz(a)anthracene in this system. These data show that NUV light alters the metabolic profile of polycyclic aromatic hydrocarbons produced by mouse skin microsomes.

961 THE METABOLISM AND CLEARANCE OF NIFEDIPINE AND 4-4H-NIFEDIPINE IN THE ISOLATED PERFUSED RAT LIVER. D L Kaplan, N Harper, and J L Born. The University of New Mexico College of Pharmacy, Albuquerque, NM. Sponsor: G B Corcoran.

Nifedipine is a dihydropyridine calcium antagonist utilized in the treatment of hypertension and in the treatment of angina pectoris. Rapid first pass metabolism of nifedipine produces a ring oxidized pyridine metabolite, which is pharmacologically inactive. Nifedipine is metabolized by cytochrome P450 via two one electron oxidations. The metabolism and clearance of nifedipine and 4-4H-nifedipine were studied using isolated perfused rat liver preparations. Livers were perfused with nifedipine or 4-4H-nifedipine at a concentration of 100 μM. Concentrations of nifedipine and its ring oxidized metabolite were determined by HPLC. Deuteration at the 4 position slowed both nifedipine clearance and the appearance of the ring oxidized pyridine metabolite. The clearances of nifedipine and 4-4H-nifedipine were 0.24 ± 0.03 ml/min and 0.12 ± 0.02 ml/min, respectively. This represents a kinetic isotope effect of 2, which is statistically significant at p < 0.05. The magnitude of the kinetic isotope effect in this preparation indicates that the half-life of nifedipine in rats may be increased by deuteration substitution.

962 N-ACETYLYATION IN HUMAN AND RABBIT LIVER SLICES. L Gunawardhana, J Barr, A J Weir, K Brandel and L G Sipes. Department of Pharmacology and Toxicology, University of Arizona, Tucson, AZ.

N-acetylation is the major route of biotransformation for arylamine drugs. A genetic polymorphism in the acetylation of drugs is known to exist in both humans and rabbits. An attempt has been made to develop the New Zealand White rabbit as an animal model to predict human metabolism of arylamine drugs using an in vitro tissue culture system. Precision-cut human and rabbit liver slices were incubated in dynamic organ culture in the presence of 100 μM para-aminobenzoic acid (PABA) or sulfamethazine (SMZ). % Acetylation was determined by monitoring the disappearance of parent from the culture media using a colorimetric procedure. Initial studies indicated that % Acetylation for PABA at 2 hours to be 27% and 48%, and for SMZ at 4 hours to be 14% and 48% for human and rabbit liver slices, respectively. HPLC analysis of culture media from both human and rabbit liver slice incubations, over time, revealed a decrease in the concentration of parent associated with an increase in the concentration of N-acyetyl conjugate. Media cultured in the presence of rabbit liver slices also showed an additional peak which was not found in the human samples. Treatment with glucuronidase and sulfatase had no influence on this peak. These results show that both human and rabbit liver slices in dynamic organ culture system are capable of N-acetylation of arylamine compounds. Furthermore, this system appears useful for species comparison of hepatic N-acetylation of arylamine drugs. (Supported by NIH NO1-ES-55112).
SULFOXIDASE ACTIVITY OF HEMOGLOBIN. B H Magee and M A Marletta. E C Jordan Co., Wakefield, MA. Sponsor: Thomas F Schrager

While hemoglobin's (Hb) primary role is to carry out reversible oxygen transport, it can participate in xenobiotic metabolism. Human Hb is shown to act as a monooxygenase, leading to the sulfoxidation of chlorpromazine (CPZ) and other phenothiazines when reconstituted with the methemoglobin reducing system of the human erythrocyte:NADH/cytochrome b5 reductase/cytochrome b5. The in vitro reaction rate for CPZ was 6-13 mmoles/mole Hb/min. The in vitro rates of Hb-mediated sulfoxidation of several phenothiazines were only slightly lower than rates with cytochrome P-450 (rat liver microsomes) by a factor of 5-20. Thiouridazine, a molecule with two sulfides, displayed different regiospecificities of sulfoxidation with the two hemoproteins, suggesting an experimental approach for investigating Hb's role in vivo. Hb mutants were studied because their redox properties suggest that they might have elevated monooxygenase activity. Hb A, succinyl Hb, which has a high autooxidation rate, is 2.8 times more reactive as a CPZ sulfoxidase than Hb A. Sickle Hb is 3.5 times more reactive than Hb A. Results from studies of other Hb M mutants suggest that both Hb chains act as catalysts.

METABOLISM AND CYTOTOXICITY OF 2-METHYL-NAPHTHALEN (2MN) IN MURINE ISOLATED HEPATOCYTES (IH) AND DISSECTED BRONCHOCLAR AIRWAYS (BA). A Pang, C Suverkropp, D Morin, A Buckhalt and C Plopper. Veterinary Anatomy and Pharmacology and Toxicology, UC Davis, Davis, CA.

2MN causes an organ selective lesion of murine nonciliated bronchiolar epithelial (Clara) cells. IH and BA have been used to model sensitive and nonsensitive cell populations to examine the relationship between metabolism and cytotoxicity in an in vitro system with potential applicability in human tissue. Based on light and electron microscopy, the control dissected tracheobronchial airway epithelium remained intact for at least 4 hrs postdissection. A concentration-dependent Clara cell injury was evident in explant tissue incubated with 2MN at levels as low as 0.1 mM for 4 hrs. Preincubation with piperonyl butoxide (0.5 mM, 15 min) did not inhibit 2MN cytotoxicity. Significant loss of cell viability in IH was observed at 1 but not 0.1 or 0.5 mM 2MN for 4 hrs. 2MN resulted in concentration dependent depletion of hepatocell GSH; GSSG levels were unaltered. Cobalt-protoporphyrin (Co-proto) pretreatment (125 µmol/kg, s.c., 48-hr prior to hepatocyte isolation) failed to protect the cells from 2MN cytotoxicity. Moreover, 2MN depletion of GSH levels was unaltered in hepatocytes from Co-proto pretreated mice. The 2 models described provide a tool for examining factors critical to toxicity in target and non-target cell populations. Supported by NIEHS 04311.


Previously we described a method for isolation and maintenance of the mouse lung in vivo. Physical (wet/dry weight, pulmonary arterial pressure), biochemical (cytochrome P450, GST etc.) and morphological (light and electron microscopic) tests revealed that the lung is viable for up to 5 hrs. These studies have examined the metabolism and cytotoxicity of naphthalene (NA) in a system where possible contributions of hepatic metabolism are eliminated. LM examination of lungs perfused for 1 hr with NA followed by 4 hrs with medium alone showed time and concentration dependent loss of Clara cells from the bronchiole epithelium. Clara cells comprise 70% of the cells in controls; this percentage decreased to 50 and 30% with infusion of 1 and 10µ mole, respectively. Reactive NA metabolites were bound covalently both to lung tissue and to BSA in the perfuse. Binding was concentration dependent (varying from 60 to 1050 molecules/bound/mg lung protein at 1-10 moles of NA). Binding to BSA in the perfusion medium was substantially lower (7 pmol/mg protein at 10 mole NA). NA infusion decreased pulmonary GSH levels in a concentration-dependent manner. We conclude that NA toxicity can be mediated entirely by processes resident in the lung. Supported by NIEHS 04311.

PERFUSED ORGAN TECHNIQUES FOR DETERMINING RECYCLED FRACTION OF DRUGS WHICH UNDERGO REVERSIBLE METABOLISM. M P Carver, C T Gombar, E Burak and B R Smith. Drug Metabolism Department, Smith Kline and French Laboratories, King of Prussia, PA.

The role of reversible metabolism and the effect of drug recycling on the determination of classical pharmacokinetic parameters have recently been questioned. SK&F 86468 is a substituted benzoazepine which undergoes both N-demethylation and N-oxidation. The latter process results in the formation of an N-oxide (SK&F 102102) which may subsequently be reduced to the parent compound. In this study, areas under the perfusate concentration-time curves were determined for both parent and metabolite in isolated perfused (portal perfusion) livers. The recycled fraction of drug was calculated according to the simple interconversion model and the associated clearance equations described by Ebright and Jusko [J. Pharmacokin. Biopharm. 14: 557-593, 1986]. In livers perfused under normoxic conditions, the recycled fraction was relatively low (5.4%). However, when similar experiments were conducted under partial hypoxia, a condition which favors redox interconversion, the recycled fraction increased to 11.0%. This increase was consistent with results from other studies involving anaerobic incubation of SK&F 102102 with rat liver microsomes in which a maximum of 30% reversion to parent (SK&F 86468) occurred. These findings suggest that isolated perfused organs may be useful for examining reversible metabolic processes in vitro.
ASSESSMENT OF CUTANEOUS IRRITATION OF FORMALDEHYDE, SALICYLIC ACID AND SODIUM LAURYL SULFATE USING TRANSDERMAL LASER DOPPLER VELOCIMETRY. LE BLALOCK AND NA MONTEIRO-RIVIERE. COLLEGE OF VET. MEDICINE AND TOXICOLOGY PROGRAM, NORTH CAROLINA STATE UNIVERSITY, RALEIGH, N C.

Primary skin irritation resulting from topical application of chemicals is characterized by erythema and edema. Transdermal laser Doppler velocimetry (LDV) was used to measure cutaneous blood flow, velocity and volume changes at 4 and 8 hrs after topical application of 15, 25 or 35% formaldehyde (F) in distilled water; 15, 20 or 25% salicylic acid (SA) in dimethyl sulfoxide (DMSO); or 25, 30 or 35% sodium lauryl sulfate (SLS) in DMSO on the ventral abdomen of pigs. Erythema was noted after all treatments except for vehicle controls, with SLS showing the most severe response. A significant treatment effect occurred for LDV-measured flow and velocity. (F) significantly decreased flow and (SLS) and (SA) primarily altered velocity compared to control and vehicle. These results suggest that LDV might be a useful noninvasive technique for assessing the vasculal response to primary skin irritation. (Supported by the Johns Hopkins Center for Alternatives to Animal Testing.)

CLOSED-PATCH REPEATED INSULT DERMAL SENSITIZATION STUDY IN GUINEA PIGS WITH GLYOXAL. R E Duell ette, C S Auletta, and R A Davis. Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ; Bio/dynamics Inc., East Millstone, NJ; American Cyanamid Company, Wayne, NJ.

This study was designed to define the dose response of glyoxal for induction of dermal sensitization in guinea pigs. Animals received 6-hour dermal induction exposures to glyoxal at concentrations of 1.25, 5 and 20%, 3 times weekly for 3 weeks. Two weeks later, animals received 6-hour dermal challenges with 5 different concentrations of glyoxal (0.01, 0.03, 0.1, 0.3 and 1.0%). A second challenge was conducted 1 week later at 0.3, 1.0 and 3.0%. Essentially no response was seen with challenges of 0.01, 0.05 or 0.1% at any induction dose. Although some responses were seen with the 0.3% challenges, there was no clear dose response for induction. With 1.0 and 3.0% challenges, responses were increased up to 5.0% induction, but increased no further at 20%. Responses were greater at 3.0% than at 1.0% for all induction concentrations. Thus, glyoxal exhibited a potential to induce dermal sensitization in guinea pigs at concentrations of 1.25, 5 and 20%, and to elicit responses to challenge concentrations of 0.3% and higher.

EVALUATION OF POTENTIAL OF DIOCTARYL DIMONIUM CHLORIDE IN GUINEA PIGS. C E Dick and H I Maibach. S C Johnson & Son, Inc., Racine, WI and University of California, San Francisco, CA

While it is important to test new compounds in guinea pig assays for potential sensitization, it is also known that guinea pig allergic contact dermatitis prediction assays have several sources of variation that may produce misleading results. The quaternary compound, dioctaryl dimonium chloride (DODC) initially tested positive on a guinea pig maximization test; the purpose of this study was to validate this result by comparison to other guinea pig tests. The maximization test was repeated plus the Buckner method plus the open epicutaneous method. Each test incorporated multiple controls-irritation control, sham control, vehicle control, untreated control; multiple induction doses; double cross-challenges with benzalkonium and DODC; both sexes. These tests which accounted for irritancy, for "excited skin", for non-specific effects, for solubility, and for sex all showed no sensitization. On the basis of these data, DODC was incorporated into several commercial skin products, which have been sold in large numbers with only minor consumer complaints. It is thus established that a single guinea pig test, especially when performed with adequate controls, cannot give reliable results; questionable tests should always be checked and protocols should always be performed with full attention to solubility, irritation levels, cross-reactions, etc.


A new fabric softener active, ditalow imidazoline (DI), was extensively evaluated for contact sensitization potential. Assessment involved analytical characterization, predictive animal and human testing, product use tests, diagnostic patch tests, evaluation of consumer exposure, and diagnostic follow-up of consumer comments.

Guinea pig skin sensitization tests (Buehler patch method) indicated that DI possessed weak sensitization potential. With appropriate precautions, DI was evaluated in human repeat insult patch tests (HRPT). Over a two-year period, DI has been tested in HRPTs in over 4500 subjects in the U.S. and Europe, with only one case of confirmed sensitization. This sensitized individual completed product use tests without adverse reaction. To evaluate the potential of a DI-containing product to induce sensitization through typical use, a diagnostic patch test was conducted among persons routinely using the product for 6 months. No evidence of sensitization was observed in the 454 subjects tested. Diagnostic patch tests among 250 subjects in Europe were also negative. A comparison of estimated consumer exposure with DI concentration in HRPTs established adequate margins of safety.

Following market introduction consumer comments were carefully monitored. Individuals alleging product-related skin reactions suggestive of contact dermatitis or urticaria were encouraged to participate in diagnostic testing. To date over 50 individuals have been evaluated via open application or the diagnostic patch test. All such diagnostic testing has been negative. The results of this program provide support for the long-term dermal safety of ditalow imidazoline in a fabric softener.
The local irritation potential of hypertonic saline/Dextran 70® (HSD) and its constituents, 7.5% hypertonic saline (HS) and 6% Dextran 70® (D70), was evaluated after intravenous (IV), paravenous (PV), intra-arterial (IA), intramuscular (IM), and subcutaneous (SQ) injections in male New Zealand White rabbits. Ringer's lactate (RL) was evaluated as a control. Observations were made 4, 24, 48, and 72 hours after injections. IV of HS or D70 produced an increased bruising versus HSD or RL. All four regimens produced a transient bleb formation and HSD, HS, and D70 produced isolated bruising after PV. All four regimens produced hematoma and bruise formation after IA. No effects were observed after IM and only transient bleb formation after SQ. Findings from gross and microscopic evaluation of injection sites were consistent with blood leakage associated with needle insertion. These results suggest that hypertonic saline/Dextran 70® is nonirritating following parenteral injection and support its use as a field-expedient resuscitation fluid.

Subcutaneous Tissue Reaction to Silicone in the Rat. C. L. Smith, J. Wosu and G. J. Davis.
Research Laboratories, Ortho Pharmaceutical Corporation, Raritan, N. J.

Studies were performed in Charles River CD rats to determine the fate of silicone fluid after subcutaneous injections. Rats received 0.0, 0.1, or 0.2 ml of silicone solution subdivided into multiple microinjections of 0.01 ml each, within an area 1/2 inch in diameter on the back. The injection sites were excised and examined by light microscopy at intervals from 1 day to 18 months. Necropsy examination up to 4 weeks after a single injection revealed raised subcutaneous clear foci (determined to be silicone fluid) at the injection site. Microscopically, the silicone droplets appeared as clear irregular spaces or vacuoles in the cutaneous or subcutaneous tissue. The vacuoles were bordered by intact connective tissue soon after injection and at later periods (1, 3, and 12 months) were encapsulated by a thin layer of collagen. The droplets produced minimal compression of adjacent tissue, no necrosis and mild to moderate acute inflammation up to 4 months post injection. Cellular infiltrates were present in a few animals 12 months post injection. After 3 and 12 months, the vacuoles were observed primarily in the panniculus adiposus, striated muscle fibers and dermis and rarely in the adipose tissue beneath the striated muscle. Vacuoles were absent from some treated animals, perhaps due to migration of silicone.


The Food and Drug Administration has recently held public meetings to discuss issues pertaining to OTC sunscreen products. Among the more controversial topics discussed in these forums is the "risk versus benefit" of sunscreen products with sun protection factors (SPF) greater than 15. At question is whether the increased sun protection offered by sunscreen products with SPF > 15 outweighs their allegedly greater potential to cause dermal toxicity. Although an earlier clinical irritation study demonstrated that there was no positive correlation between SPF and cumulative dermal irritation, concern about the phototoxicity of these high SPF sunscreens remains unresolved.

The purpose of this research was to investigate the phototoxicity of sunscreens as a function of their SPF. In this study human subjects were exposed to ten different commercial sunscreen products with and without concomitant exposure to UVA/UVB radiation.

It is critical that valid and predictive models be employed to determine the phototoxic potential of consumer products designed for topical exposure and outdoor use. For assessment of PA, a mouse ear swelling (MES) model was used. PA is assayed in cyclophosphamide treated mice by applying the test material to the shaved backs for induction on days 0, 1 and 2 and to the ears on day 7 for challenge. The test sites are irradiated with UVA/UVB. A response is determined by measuring ear thickness before and after challenge. With this model, we have examined various parameters which influence PA and the histological characteristics of a phototoxic reaction. We have detected the phototoxic potential, or lack thereof, in 10 human photogenizers and 4 negative controls.

A rapid test for the test material is applied to both ears. At 60 minutes post application, the right ear is irradiated with UVA/UVB radiation using a solar simulator. Using this model, we have examined factors which influence PA and the histological characteristics of a phototoxic reaction. We have detected the phototoxic potential, or lack thereof, of 8 known human phototoxins and 4 negative controls.

A major advantage of these MES models is that they are quantifiable and more objective than models based on subjective evaluation of skin changes. We feel that these MES models, with further validation, offer potential as methods for predictive PA and PT testing.


Evidence suggests that assays using cultured mammalian cells can provide a measure of ocular irritation potential for certain materials. Thus a simple cell culture dose/response cytotoxicity assay was developed as an in vitro, non-animal screen for evaluating ocular irritation potential of surfactants and surfactant-based formulations. Cells of the 3T3 mouse embryo fibroblast cell line are incubated for 1 hr with serial dilutions of test material, and 24 hrs later surviving cells directly enumerated in 96 well microtitre dishes using an automated colony counter adapted to an inverted microscope. Cytotoxicity (LC50) is determined using computer-driven linear regression and graphical statistical analysis programs. For 10 mild or non-irritating materials, the mean LC50 was 566 µg/ml (range: 48 - 2000) while for 31 moderate to severe irritants, the mean LC50 was 70 µg/ml (range: 4 - 252). While there is some overlap in these ranges, these results indicate good agreement between relative cytotoxicity (LC50) and relative in vivo ocular irritation, suggesting that a simple cytotoxicity assay could be developed for screening of surfactants and surfactant-based products for potential ocular irritation.

977 EYE IRRITATION PREDICTED WITH TWO IN VITRO ASSAYS - CYTOTOXICITY AND PROTEIN DENATURATION. H E Kennah II, J D Dorko, S Hignet and C E Barrera. PPG Industries Inc., Environmental Sciences Center, Pittsburgh, PA

Eye irritation results from at least two different mechanisms described as chemical and physical. In vitro cytotoxicity assays can predict the eye irritancy potential for chemicals acting via physical mechanisms. The objective of this study was to develop a protein denaturation assay as a measure of chemical mechanisms and use it in concert with cytotoxicity to predict in vivo eye irritation. Denaturation of Ribonuclease A, Type III was followed by fluorescent changes. Urea (6.4 M) was added to ribonuclease (0.01%, pH 7.0 in 0.03 M MOPS buffer), so that the native denatured equilibrium constant was approximately 0.25. Alcohols (hexanol, 2-ethyl-1-hexanol, isopropanol, and isobutanol) were then added to shift this equilibrium. Linear relationships (r > 0.90) were established between the volume of alcohol added (0-1000 µl) and the fluorescence increase to the denatured state. Regression equations were used to calculate the volume of alcohol required to reach 50% denaturation (V50). The V50 values exhibited a linear correlation (r = 0.74) with in vivo eye irritation quantitated in rabbits as corneal swelling. Agreement with in vivo eye irritation was attained by correlating V50 with previously published in vitro cytotoxicity results. This novel system shows promise as an in vitro alternative to the Draize eye test.

978 COMPARISON OF TEST METHODS FOR EVALUATING EYE IRRITATION POTENTIAL. M C Capdeville, D Bagley, B M Kong, S J DeSelva, Colgate-Palmolive Co., Piscataway, NJ

The need for alternatives to the Draize Eye Irritation Test is important for the reduction of animals used in safety testing. This study is a comparison of 3 in vivo and 2 alternative tests; Standard Draize, two low volume modifications of the Draize, the Choroidallantoic Membrane Assay (CAMZ), and the Choroidallantoic Membrane Vascular Assay (CAMVA). The results from these tests were used to determine regulatory labeling needs (FHSA & EEC) for 4 commercially available light duty liquid detergents. In the low volume in vivo tests, volume (0.01ml) and application site (corneal surface versus conjunctival) were found to affect irritation. It was found that the materials were eye irritants with the Draize Standard Test (FHSA & EEC) but with the lower volume methods several could be considered non-irritants. With the CAMZ and the CAMVA assays all products were irritants. Since the alternative tests (CAMZ & CAMVA) were calibrated against the Draize Standard, the findings are consistent with the standard. It is important to recognize that there are differences in outcome from the standard and the modified Draize tests. The significance of this report is that labeling for eye irritation potential is influenced by the method of assay and the regulatory prerequisites applied to the results.
DEVELOPING AN ALTERNATIVE TO THE DRAIZE SKIN TEST: COMPARISON OF HUMAN SKIN CELL RESPONSES TO IRITANTS IN VITRO. G S Lamont, D M Bagley, B M Kong, S J De Salva, Colgate-Palmolive Co., Piscataway, NJ.

Arachidonic acid metabolites have been implicated in skin irritation. This observation is the basis for the development of an in vitro skin cell culture assay using 3H-arachidonate labeled cells to assess skin irritation potential (Kam et al., Proc. Soc Exp. Biol. Med. 184:477). To optimize assay conditions, different skin cell types and culture conditions were evaluated for response to a variety of test materials. The response (release of radioactivity) was independent of cell type (fibroblasts vs keratinocytes), cell line (primary vs immortal), cell density and passage number, and dose-dependent in all cases. The assay was reproducible based on intra- and inter-laboratory studies. Rank order of in vitro responses correlated well with in vivo data with a single false positive. These results indicate the potential of this assay as an alternative method to in vivo skin irritation testing.


Studies were initiated in albino (Crl:CD) rats and New Zealand white rabbits to determine the exposure route specificity for the ocular injury produced by 2-phenyl-APB-144. This aromatic diamine produces ocular lesions consisting of diffuse, bilateral disorganization and degeneration of the retina with atrophy and loss of the rod and cone cell processes and nucleus. 2-Phenyl-APB-144 was administered to test animals either by gavage, occlusion on shaved skin, instillation onto conjunctival sacs or by inhalation. In albino rats, ocular lesions were produced after a single dermal application (77 mg/kg), oral administration (58 mg/kg) or inhalation (89 mg/m^3); approximate lethal doses (ALD) for these exposure routes were >7000 mg/kg, 570 mg/kg and 980 mg/m^3, respectively. Similar ocular injury occurred in albino rabbits after oral administration at 1600 mg/kg (oral ALD >1600 mg/kg); no lesions were found in rabbits after conjunctival application of 0.01 g/eye or dermal administration of 7000 mg/kg (dermal ALD >7000 mg/kg). Blood samples taken from selected rats showed that significantly increased MetHb concentrations were found only in rats exposed to lethal levels of 2-phenyl-APB-144; MetHb values were not predictive of ocular injury. Overall, rats were more sensitive to the acute toxicity and ocular injury produced by 2-phenyl-APB-144 than rabbits.

EVALUATION OF THE IN VITRO EYTEX® SYSTEM AS AN ALTERNATIVE OR ADJUNCT TO IN VIVO OCULAR IRRITANCY TESTS. H J Powers, K C Norbury, G J Davis. Research Laboratories, Ortho Pharmaceutical Corp., Raritan, N.J.

The objective of this work was to conduct an evaluation of the EYTEX® System as a potential alternative to the Draize ocular test and secondarily to conduct a validation of this system. Developed by National Testing Corporation, the EYTEX System is a chemical in vitro method designed to predict ocular irritancy potential of a diverse group of chemicals and yields results that correlate to those in vivo tests. The method incorporates protein denaturation, measured as a change in optical density, as a primary component of chemical induced ocular damage. The evaluation of seven test articles (acetic acid, acetonitrile, benzonilum chloride, ethanol, M-butyl alcohol, propylene glycol and trichloracetic acid) yielded ocular irritancy classifications comparable to those in published literature that ranged from non-irritating to severely irritating. In addition, gel and cream formulations were evaluated to determine the efficiency of the EYTEX System in predicting the impact of minor topical formulation excipient changes on their ocular irritancy potential. Results of our evaluation indicate a correlation between EYTEX results and in vivo data for a variety of test articles.
Localized eye area sensitivity syndrome (LEASS) refers to a collection of subjective and objective symptoms which occur when susceptible individuals apply specific topical products to the periciliar area. Patients experiencing this syndrome complain of symptoms ranging from itchy, burning, stinging and watery eyes to fuming and foreign body sensations. Ophthalmologic and dermatologic examinations reveal erythematous skin reactions and varying degrees of bulbar conjunctival chemosis and injection.

A strategy was developed to investigate LEASS. Individual chemicals found in experimental products shown to produce LEASS were evaluated in three tests: an animal model for non-immunologic contact urticaria (NICU), human periciliar application and cutaneous scratch test.

Eight chemicals gave positive responses in the animal model. Application of these chemicals to the human periciliar area correlated with results from the animal model. Cutaneous scratch test results were negative for the eight chemicals.

**USE OF ANIMAL EYE TEST DATA AND HUMAN MARKETING EXPERIENCE FOR DETERMINING THE OCULAR IRRITATION POTENTIAL OF SHAMPOOS.** GS Allgood. The Procter & Gamble Co., Cincinnati, OH.

The decision criteria for determining the eye irritation potential of shampoos are reviewed. Results of rabbit low volume eye irritation testing and human marketing experience are used to determine the ocular safety of shampoos. These data are reviewed for four marketed shampoos and show that the shampoos have a low eye irritation potential. Shampoos tested in the rabbit low volume test that have a maximum average score of less than 40.0 (tested undiluted without rinsing) and that clear within two weeks have been shown to have a low level of human eye irritation potential. The criteria for a low level of human eye irritation potential include 1) no permanent injuries from accidental exposure and 2) accidental exposures clear in a short time (median time to clear < 4 day, 95% within 1 wk.). This review further supports the use of the rabbit low volume test in determining human eye irritation potential of new shampoo formulations and 2) the use of consumer accidental exposure information in product safety assessments.

**SPONTANEOUS CORNEAL OPACITIES IN MICE.** D. Abrutyn, L. Hagerman, G. E. Korte and A. N. Johnson, Research Laboratories, Ortho Pharmaceutical Corporation, Raritan, N.J.

Pretest ophthalmoscopic examination of 5 week CF-1 mice revealed approximately 43% with corneal opacities. Since this spontaneous lesion can confound the safety assessment of test substance induced lesions, we evaluated the progression of this lesion in selected mice. Forty mice (6 with no corneal opacities, 22 with unilateral opacities, and 12 with bilateral opacities) were maintained for 15 months. No opacities developed in mice free of this change at study initiation. Sixty percent of mice with the opacities (unilateral or bilateral) remained unchanged and the severity in the remainder increased only slightly. Electron microscopy of selected eyes demonstrated granules at the corneal endothelial border and in the adjacent stroma. Similar spontaneous findings have been reported in rats. Careful assessment of the eyes of mice prior to the initiation of a toxicity study is essential for the recognition of the change and, ideally, the selection of lesions-free mice. The substance has the potential of affecting the eyes, this could confound the assessment of the test substance. We recommend not using animals with this lesion. Potential mechanisms of this lesion will be presented.
METHANOL INDUCED OCULAR TOXICITY IN THE RAT. J I Eads, Medical College of Wisconsin, Milwaukee, WI.

Methanol poisoning in primates is characterized by metabolic acidosis and ocular toxicity. These symptoms occur coincident with the accumulation of formate in body fluids and tissues. Rats do not accumulate formate following methanol and do not display signs of methanol toxicity. However, the anesthetic gas N₂O₂, renders the rat sensitive to the metabolic aspects of methanol toxicity. The objective of this study is to establish a rodent model in which to study the pathogenesis of methanol-induced ocular toxicity. The development of formic acidemia and visual dysfunction was studied in N₂O₂-treated rats following the administration of methanol. Rats were exposed to N₂O₂/O₂ (50/50) for 4 hr prior to the administration of methanol (4 g/kg) and for 4 hr per day thereafter. Accumulation of blood formate (peak values 5.2 ± 0.5 mM) was observed in these animals. Alterations in the P20-N30 component of the flash-evoked cortical potential (FEP) following methanol were used as an index of visual dysfunction. FEPs were recorded prior to methanol administration and at predetermined time intervals thereafter for 84 hr. The amplitude of the FEP decreased (27 ± 11% of control value) coincident with the accumulation of blood formate indicative of a temporal relationship between methanol-induced metabolic and visual disturbances. This study is the first step in the investigation of the ocular toxicity of formate in a rodent model which may prove valuable in elucidating the mechanisms involved in methanol-induced ocular toxicity. (Supported by Fight for Sight GA 88-048).

COMPARATIVE TOXICITY OF 2-ETHYLBENZENES (2EH) IN THE FISCHER 344 RAT BY DIFFERENT ROUTES OF ADMINISTRATION. E V Weaver, M W Gill, E R Fowler, C M Trupm. Bushy Run Research Center, Export, PA.

The potential for toxicity from 2EH following percutaneous and oral (gavage and drinking water) administration was determined in Fischer 344 rats. Rats received 9 daily doses of undiluted 2EH at 0, 0.5, and 1.0 ml/kg percutaneously or undiluted 2EH at 0, 0.1, 0.3, 1.0, and 1.5 ml/kg by gavage over an 11 day period or 2EH in drinking water at 0, 308, and 636 ppm for 9 days. Treatment-related effects of percutaneous exposure included lymphopenia and decreased spleen weight at the high dose, and minor local skin irritation at both doses. Dose-dependent effects of treatment with undiluted 2EH by gavage included decreased food consumption and body weights (0.3 ml/kg/day and above), decreased leukocytes, lymphocytes, and spleen weights (1.0 ml and 1.5 ml/kg/day), and increased liver and stomach weights (1.0 and 1.5 ml/kg/day). Histological findings included decreased extramedullary hematopoeisis, thymic atrophy, and evidence of local forestomach irritation at doses of 0.3 ml/kg/day and above. There were no treatment-related effects from 2EH when administered in the drinking water. Under the conditions of this study, 2EH was more toxic following gavage administration than percutaneous application and not toxic when administered via drinking water. Sponsored by the Chemical Manufacturers Association.

SUBCHRONIC INHALATION TOXICITY OF ETHYLBENZENE IN MICE, RATS, AND RABBITS. S T Cropp, E A Clarke, I W Daly, R R Miller, J B Terrill, R E Ouellette; 1WESTON, West Chester, PA, 2SOMA, Washington, DC, 3Bio/dynamics, E Millstone, NJ, 4USW, Midland, MI, 5Hastings, Washington DC, 6Hoechst-Roussel, Somerville NJ.

The subchronic inhalation toxicity of ethylbenzene (EB) was evaluated in mice, rats, and rabbits (five/sex/group) exposed to EB vapors 6 hr/d, 5 g/week for 26 weeks (20 exposures). Rats and mice received 0, 95, 362, or 782 ppm EB; rabbits received 0, 382, 782, or 1,610 ppm. No changes occurred in mortality, clinical chemistries, urinalyses, or gross/microscopic (including ophthalmologic) lesions. In rats, sporadic lacrimation and salivation were seen at 382 and 782 ppm EB and small but significantly increased leukocyte counts at 782 ppm. Males at this level also showed marginal elevations in platelet counts. At 382 and 782 ppm EB, rats of both sexes exhibited increased liver weights without histopathology and no effects at 99 ppm EB. In mice, females showed increased liver weights at 382 and 782 ppm EB while males had increased relative liver/brain weight ratios only at 782 ppm. Mice exhibited no liver histopathology or any other EB-related effects. Female rabbits showed a slight decrease (not significant) in body weight gain only at 1,610 ppm EB. Male body weights trended downward at 1,610 ppm EB after one week (not evident at sacrifice). These data indicate a subchronic inhalation NOAEL of 382 ppm for rats and mice, and 782 ppm for rabbits.


Acetone (AC) is a widely used industrial solvent and chemical intermediate that has been detected in the groundwater near chemical wastewaters. Male and female F344 rats and female B6C3F1 mice (10/sex/group) were administered AC in their drinking water (0, 0.25, 0.50, 1.00, or 2.00 mg/kg/day) while male B6C3F1 mice received 0, 0.125, 0.25, 0.50, 1.00, or 2.00 mg/kg/day of AC by the same route for 13 weeks. No mortality was noted in either study and reduced body weight gains were noted only in the 5.0% male and female rats. Hematological effects were restricted to rats. Changes in RBC parameters (+RBC, +MCV, +MCH, +Reticulocytes) were noted among males exposed to 2.0 and 5.0% AC. Platelet counts were depressed among males and females exposed to 5.0% AC. Centrilobular hepatic cell degeneration was restricted to 2/10 female mice in the 0.0% AC group and was consistent with elevated liver weight and liver/ body weight ratios among 5.0% females. Minimal to mild pigmentation of the spleen (hemosiderosis) and an increased incidence and severity of nephropathy were associated with exposures to 2.0 and 5.0% AC among male rats. There were no lesions related to AC exposures among male mice and female rats.
Carbon disulfide (CS₂) is widely used as an industrial solvent primarily in the production of viscose rayon fibers. To evaluate exposures of workers to CS₂ and possible health hazards of this exposure, a total of 18 air samples of CS₂ were obtained in workers’ breathing-zones over a 14-month period. Air samples ranged from 13 ppm to 166 ppm (x=69); ceiling values ranged from 43 ppm to 500 ppm (x=97); and peak values ranged from 110 ppm to 701 ppm (x=191). Eleven samples were above the current Federal Standard of 20 ppm. Ten samples were above the maximum peak exposure limit of 100 ppm which should never be exceeded. Two samples exceeded the IDLH level of 500 ppm. All samples exceeded the ceiling limit of 30 ppm. Urine specimens from 47 workers exposed to CS₂ were examined using the iodine-azide test. Twelve workers had E (exposure coefficient) values < 5 indicating excessive exposure. All workers had values in the range of 5-6.5 indicating some exposure. All remaining values were normal. Based upon environmental and biological iodine-azide data, it was concluded that a potential health hazard existed to all cutters due to excessive exposure to CS₂. Engineering controls, improved ventilation, and use of respirators, were recommended.

Three glycol ethers, 2-methoxymethanol (2-ME), 2-ethoxyethanol (2-EE), and 2-butoxyethanol (2-Be), are candidates for chronic NTP studies. 14-day toxicity studies were designed to establish target tissues, no-effect levels, sex/species sensitivity, and slopes of dose-response curves. F344 rats and B6C3F1 mice (5/sex/group) received nominal doses in drinking water of either 2-ME (0-1500 mg/kg), 2-EE (0-2500 mg/kg), or 2-Be (0-650 mg/kg). Only 1 mouse died post-exposure to 900 mg/kg 2-EE. Higher doses of 2-ME and all doses of 2-EE reduced body weights in rats. Decreased water intake and dehydration occurred in female mice at higher 2-ME and 2-EE doses and in female rats exposed to higher levels of all glycol ethers. Testes weights were reduced in mice exposed to 2400 mg/kg 2-ME or to 2500 mg/kg 2-EE; and reduced thymus weights occurred in male mice exposed to ≥1000 mg/kg 2-ME or ≥2400 mg/kg 2-EE, and in female mice at 1200 mg/kg 2-ME. Doses of 2-ME (≥2200 mg/kg), 2-EE (≥2600 mg/kg), and 2-Be (≥2400 mg/kg, males) reduced thymus weights in rats. Reduced testes weights occurred in rats post-exposure to ≥2400 mg/kg 2-ME or ≥1500 mg/kg 2-EE. In this study, the testes and thymus were most sensitive to glycol ether toxicity; relative toxicities were 2-ME > 2-EE > 2-Be.
MONOCHLORACETATE (MCA) INCREASES TOXICITY OF CARBON TETRACHLORIDE (CCL₄) AND HEXACHLORO-
BUTADIENE (HCBD). H E Davis and W O Barrie,
West Virginia U. Health Science Ctr, Morgantown
WV

Contaminated drinking water often contains more
than one chemical; the present studies consider
interactions between co-contaminants. Sprague-
Dawley rats were used. MCA was administered by
gavage (94 mg/kg for females and 188 mg/kg for
males) 1 hour before ip injection of either HCBD
or CCL₄ (365 mg/kg); in males a low dose of
HCBD (30 mg/kg) increased plasma creatinine
(PGc) equally in MCA (.337 ± .035 vs .235 ±
(BUN) were not significant. 200 mg/kg of HCBD
increased BUN significantly more in the MCA
group (104 ± 20 mg/dl vs 22 ± 2) than the NaCl
group (68 ± 16 vs 22 ± 1). In males, BUN and PGc
were increased similarly in both groups of fe-
males after the low dose of HCBD; however after
the high dose of HCBD, both were significantly
greater in the MCA group (BUN 205 ± 24 vs 64 ±
13 in NaCl+HCBD). In females, CCL₄ increased
plasma glutamate-pyruvate transaminase (GPT)
in MCA group (590 ± 201 SPU/ml vs 12 ± 2)
compared to NaCl (189 ± 52 vs 10 ± 2). The
increases of GPT were not significant for male
rats at this low dose of CCL₄. BUN and PGc
were not affected by CCL₄. MCA increased
susceptibility to both hepato- and nephrotoxi-
city, likely by impairing defenses.
(Supported by AF Contract F49620-86-C-0096)

INFLUENCE OF PRETREATMENTS WITH KETONIC SOLVENTS
ON THE METHENOLOBINEMIA (mBH) INDUCED BY N,
N-DIMETHYLANILINE (DMA). X Krishnan, J Broderi,
F du Souich and C L Pina. Dép. de pharmacologie
et Dép. de médecine du travail et d’hygiène du
milieu, Université de Montréal, Québec, Canada.

DNA, a tertiary amine, produces mBH after being
metabolically transformed. Groups of male
Sprague-Dawley rats (m=6) were pretreated (p.o.
with 7.5 mmol/kg of acetone, methyl isobutyl
ketone (MIBK), methyl n-butyl ketone (MnBK) or
2-octanone (OCT), given either as a single dose
or as three consecutive doses. This was follow-
ed, 18 hr later, by the i.p. administration of
0.8 or 2.4 mmol/kg of DMA. Pretreatments with
MIBK, MnBK and OCT enhanced significantly the
mBH produced by DMA. The latter was related
positively (peak mBH concentration, duration of
response) or negatively (time to reach peak mBH)
to the carbon chain length of the ketones. The
3-day pretreatments exerted a greater influence
than the 18-hr pretreatments for all ketones.
In other experiments (3-day pretreatments p.s.
followed by DMA, 8 mmol/kg, i.p.), mBH was
related to the pretreatments as follows: OCT
(7.5 mmol/kg) > phenobarbital (50 mg/kg) > 3-me-
thyl cholanthrene (25 mg/kg) > pregnenolone-16α-
carbonitrile (20 mg/kg) > corn oil = saline. These
results indicate that various ketones can
increase the toxicity of DMA and suggest that
they might act like other microsomal enzyme
inducers. (Supported by NSERC)

INHIBITION OF MITOCHONDRIAL ATP SYNTHESIS BY
DIBASIC ESTERS IN VITRO. M S Bogdanoff, and T
Londergan. E I du Pont de Nemours & Co. Inc.
Haskell Laboratory for Toxicology and Industrial
Medicine, Newark, DE.

Dibasic esters (DBEs) are a solvent mixture of
dimethyl-succinate (DMS), -glutarate (DGC), and
-glutarate (DGC) in the infant and coating
industry. Inhalation studies have shown these
compounds to be mildly toxic to the nasal
olfactory mucosa and rapidly hydrolyzed by
olfactory mucosal esterases. Structural
similarity of the acid metabolites to Krebs
related intermediates prompted the current
investigation into the effects of DBEs on
mitochondrial ATP synthesis. Hepatic
mitochondria were used as a model for nasal
tissue. DBEs were found to inhibit
mitochondrial ATP synthesis 11% to 27% at 100
µM. The order of potency was DMA > DGC > DMS
and paralleled the Vₐ₅₀/Kₑₐ values for the
hydrolysis of the DBEs by nasal monoester
esters. Pretreatment of rats with 100 mg/kg
bis-nitrophenyl phosphate for 3 days decreased the
rate of hydrolysis of the DBEs approximately 50%
and protected the mitochondria from DBE-induced
inhibition of ATP synthesis. The results of
these experiments support the hypothesis that
DBE-induced cytotoxicity of results from
esterase-mediated hydrolysis to acid metabolites
and interference with intermediary metabolism.
Studies with nasal mucosal mitochondria need to
be performed to verify this mechanism in the
target tissue.

SUBCHRONIC TOXICITY STUDY OF 2,3-DICHLOROPROP-
ANOL IN RATS. C DeRosa, H Choudhury, S
Irene, A Bathija, B Sonawane and R

Lack of adequate toxicity data prompted U.S.
EPA to evaluate dichloropropanol for its
potential toxicity as a part of the RCRA Land
Disposal Program. White albino rats (30/dose/sex) were gavaged daily with 0, 10,
35 and 100 mg/kg/day 2,3-dichloropropanol in
denitized water for 30 to 90 days. Ten
rats/sex/group were sacrificed after 30 days
dosing for interim evaluations and the remaining
rats were dosed for 13 weeks. At 100
mg/kg/day, rats were progressively hypoactive,
and emaciated with compounds-related deaths
attributable to myocardial degeneration; body
weights and food consumption were severely
depressed and pathological lesions were noted
in kidneys and liver of both male and female
rats. Myocardial degeneration, as well as
other toxic effects were minimal at 35
mg/kg/day. In this study, the toxic potential
of dichloropropanol appears similar to that of
other halogenated alliphatic alkanes and
alcohols. The 10 mg/kg/day level was a
noted-observed-adverse-effect-level for 2,3-
dichloropropanol applying a factor of 1000 (10/10x10x10) results in a
dosage reference of 1.2 mg/kg/day.
This study was undertaken to investigate the effect of simultaneous exposure to toluene (TOL) and a mixture of isomers (o:m:p = 15:60:25) of xylene (XYL) on some aspects of their respective metabolic disposition. Three groups of rats (n=8) were exposed during 5 hr to TOL (150 ppm), XYL (150 ppm), or a mixture of TOL and XYL (150 ppm each), respectively. Urinary excretion of hippuric acid (HA) and isomers of methylhippuric acid (MHA) over a 24-hr period was measured by HPLC, while concentration of solvents in blood (B-TOL, B-XYL) was determined by head-space-GC at 5, 30 and 60 min following exposure. Compared to single exposure, simultaneous exposure to TOL and XYL decreased the excretion of HA (0.4 x) and MIA (0.6 x) and increased B-TOL (2.3 x) and B-XYL (6.0 x). Elimination of solvents by exhaled air in an exposure room was also measured. Simultaneous exposure resulted in a higher rate of pulmonary elimination of TOL (1.7 x) and XYL (6.2 x). These results strongly suggest mutual metabolic interactions (inhibition) between TOL and XYL that should be taken into account when interpreting biological monitoring data for exposure.

Supported by IRSST, RS-86-46.

**The Metabolism of Benzoene and Trans, trans-Muconaldehyde to Urinary Trans, trans-Muconic Acid in DBA and CD-1 Mice.** W M Manara, V B Hylavarapu, J Joselevitz, T A Kirby, D D Goldstein, and C Witz. Joint Graduate Program in Toxicology, Rutgers University/UMDNJ-Robert Wood Johnson Medical School, Piscataway, N.J.

The present studies were undertaken to examine whether the differences in sensitivity towards benzene (Bz) observed in DBA and CD-1 mice are related to differences in metabolism to ring-opened products, trans, trans-muconic acid (MA), a known ring-opened metabolite of Bz excrated in the urine, was chosen as the endpoint. For the present studies, a 90-100% efficient extraction procedure of MA from urine was developed and MA was quantitated using HPLC. DBA and CD-1 mice treated i.p. with 100 mg/kg Bz excreted 1.4 ± 0.6 and 1.9 ± 0.6% of the administered dose, respectively, as urinary MA. With decreasing benzene dose, the % dose excreted as MA increased correspondingly, up to 6.9 ± 0.7 and 9.5 ± 1.0% in DBA and CD-1 mice, respectively, treated with 1.0 mg/kg Bz. Mice administered trans, trans-muconaldehyde, a ring-opened hemotoxic metabolite of benzene and possible precursor of MA, excreted 20-40% of the dose (0.5, 1.0 and 2.0 mg/kg) as MA. The latter was excreted as such as 55-90% of administered doses ranging from 0.03 to 5.6 mg/kg. Supported by NIH grant ES02558.

**Toluene Decreases Rat Synaptosomal Phosphatidylyethanolamine (PE) But Stimulates PE Synthesis.** C Lebel and R Schatz, Toxicology Program, Northeastern University, Boston, MA.

Toluene is an organic solvent with classical CNS depressant properties that likely result from an interaction with neuronal membranes. The purpose of this study was to determine whether specific solvent--membrane phospholipid (PL) interactions occur. Toluene (lg/kg, i.p.) significantly decreased synaptosomal PE (2%), while phosphatidylcholine (PC), serine (PS), inositol (PI) and sphingomyelin (SM) were unaltered. Phospholipid methylation (PLM), a membrane associated event that uses PE as substrate, was also decreased (+35%) in synaptosomes from toluene treated rats. The observed decrease in PLM after toluene exposure was reversed by the addition of exogenous PE. To determine whether decreases in PE result from inhibition of PE synthesis, synaptosomes were incubated in [H]-ethanolamine ("H-ETH). Toluene did not alter "H-ETH incorporation into either phosphoethanolamine or CDP-ethanolamine, but stimulated formation of "H-ETH labeled PE (27%). The stimulation of PE synthesis in the face of decreased steady state PE levels may reflect a compensatory mechanism, but does not explain the mechanism for decreased synaptosomal PE in toluene treated rats. Further studies are underway to investigate the effects of toluene on base-exchange enzymes and those enzymes involved in the degradation of PE. Research was supported by a grant from GE Plastics Div. and BRSG S07RR03830-08.
THE EFFECTS OF XYLENE ISOMERS ON GLUTATHIONE METABOLISM. T AuCoin and R Schatz, Toxicology Program, Northeastern Univ., Boston, MA.

Xylene's ability to decrease MFO activity has been linked to solvent induced microsomal membrane alterations. The formation of aldehydes from these solvents has been suggested as the causative agent in these membrane and MFO alterations. Since glutathione (GSH) has a role in membrane homeostasis and serves as a donor in xenobiotic conjugation reactions, we investigated the effects of these compounds on GSH metabolism. o-Xylene (1g/kg, i.p.) depleted rat hepatic GSH levels by 25 and 50% at 3 and 12h, respectively. Cysteine (Cys), a precursor of GSH, was also decreased (32%, 1hb). The rate of GSH synthesis was investigated using $^7$ -l-methionine. The specific activity of $^7$-S-GSH was reduced after o-xylene administration with the maximal reduction (40%) at 12h, while that of Cys was increased. The increase in the specific activity of the Cys pool paired with the decrease in the specific activity of the GSH pool suggest that xylene administration inhibits GSH synthesis. Interestingly, p-xylene (1g/kg, i.p., 3h) similarly decreases $^7$-S-GSH specific activity in liver, but induces a substantial increase in $^7$ -S-GSH specific activity in lung. We are presently investigating the mechanism whereby the xylene isomers produce this organ selective alteration in GSH metabolism. Supported by NIH-NCI #CA47671.

ORGANIC SOLVENT TOXICITY IN RAT PULMONARY MICROSONES: ROLE OF METABOLIC ALDEHYDE FORMATION IN VIVO. G Furman, J Stickney and R Schatz, Toxicology Program, Northeastern Univ., Boston, MA.

Toluene and p-xylene are metabolized to aldehyde intermediates which have been proposed to be responsible for the pulmonary toxicity of these organic solvents. The metabolism of p-nitrotoluene (pNT), a structural congener of toluene and p-xylene, does not, however, involve aldehyde formation. If aldehyde formation is requisite for solvent toxicity, then pNT should be less toxic than either toluene or p-xylene. Therefore, we tested the effects of pNT, toluene and p-xylene on MFO components of lung microsomes. p-Xylene decreased aryl hydrocarbon hydroxylase (AHH) activity 43% and reduced cytochrome P450 to non-detectable levels after 1h; these parameters decreased by 41% and 63%, respectively after 24h. Administration of 250 or 500mg/kg p-tolualdehyde directly resulted in a 32% and 44% reduction in AHH activity, respectively. Toluene (1g/kg) inhibited AHH 33% at 1h and 38% 4h after administration. P450 content was decreased 44% and 49%, respectively, at these time points. pNT administered at 200 or 400mg/kg or 1g/kg (approx. LD$_{50}$) did not alter AHH activity or P450 content. 1g/kg pNT decreased AHH 43% only after 4h but did not affect P450 content. Taken together, these data support the hypothesis that pulmonary toxicity of organic solvents may require metabolic activation of the parent compound to reactive aldehyde intermediate. Supported by NIH-NCI #CA47671.

THE VALUE OF AN INTERNATIONAL TOXICOLOGY DATA BASE IN CHALLENGING REGULATORY GUIDELINES FOR NEW MEDICINES C E Lumley & S R Walker. Centre for Medicines Research, Woodmansterne Road, Carshalton UK. Sponsor Dr S Gargoll.

An adequate data base is necessary to support any challenge of the scientific rationale behind regulatory guidelines for animal toxicity tests of new medicines. Pharmaceutical companies in Europe have therefore provided information for a unique toxicology data base. A comparison of data from short- (1., or 6 months) and long-term (12 months or longer) tests for 88 studies does not support the need for animal toxicity studies of longer than 6 months' duration, excluding those to identify carcinogens - in line with the EEC requirements. This resulted in the Canadian authorities decreasing the recommended duration of chronic toxicity tests from 18 to 12 months, and caused the Japanese to revise the duration requirements in their new draft guidelines. This data base now contains information from 270 case studies provided by 23 international companies. Short- and long-term tests for 146 studies have been compared and the results reinforce the previous conclusion. Currently, the need for conducting repeat-dose toxicity tests longer than 3 months in more than one species is being evaluated. This data base is a valuable resource for participating pharmaceutical companies.
MORPHOLOGICAL CHANGES INDUCED IN RATS BY CHRONIC ADMINISTRATION OF THE ANTI-
DEPRESSANT DRUG FEZOLAMINE. RJ Fabian, BA Mayes, TA Barbolet and MA Egy. Sterling-Winthrop Research Institute, Rensselaer, NY.

Fezolamine, a non-tricyclic antidepressant, was administered orally to rats for six months at daily dosages of 0, 15, 60 or 180 mg base/kg. No effects were seen at 15 mg base/kg. Salivation and increased liver and kidney weight were observed at 60 mg base/kg. At 180 mg base/kg postmedication salivation, depressed food intake and growth, occasional tremors or convulsions, reduced RBC, Hgb, and hematocrit, increased ALT, AST, alkaline phosphatase, phosphorous, urea nitrogen, creatinine, and bilirubin, increased organ/body weight ratios for liver, kidneys, heart and adrenals, and mortality were observed. Histomorphological findings included degeneration/regeneration in the diaphragm and vastus lateralis, alveolar histiocytosis, pyelonephritis and centrilobular hypertrophy of hepatocytes. Ultrastructurally lamellar inclusion bodies were seen in the cells of several tissues. These findings suggested generalized phospholipidosis similar to that seen with other cationic amphiphilic drugs such as imipramine, chlorpromazine, and iprindol. The rat is particularly sensitive to these effects as similar findings were not seen in cynomolgus monkeys medicated daily with this drug for one year.


Trimetrexate (CI-898) is a non-classical anti-
folate that is being developed as an anticancer drug and as a treatment for Pneumocystis carinii pneumonia. To assess the toxicity of CI-898, rats were administered single (x1) or multiple (daily x5) doses by either the oral or IV route. In the oral studies, toxicity was delayed and consisted of a generalized debilitation, weight loss, gastrointestinal lesions, bone marrow suppression, lymphoid depletion, and death. These effects were most severe in animals administered CI-898 at 65 and 85 mg/kg in the oral x5 study (65% mortality). Single oral doses up to 375 mg/kg caused moderate changes and low mortality (1/20 @ 375 mg/kg). In contrast, single IV doses at 60 mg/kg resulted in immediate deaths (20%) due to CNS depression. Doses below 60 mg/kg in both the x1 and x5 IV studies were essentially asymptomatic. Toxicity was minimal and limited to decreased WBC counts, lymphoid depletion, and testicular degeneration. Except for the testicular changes, most of the effects were reversible within four weeks after dosing in all of the oral and IV studies. The results show that the dose-limiting toxicity of CI-898 in rats is dependent on its route of administration.


Single- and multiple-dose toxicologic studies were conducted with BM-25801 to assess the acute and subacute toxicity of this antiemetic agent. The minimal lethal intravenous single dose of BM-25801 was greater than 40 mg/kg in mice, rats, and dogs. In the rabbit, the minimal lethal intravenous dose was approximately 15 mg/kg by bolus injection, but greater than 30 mg/kg by slow (20-
inute) intravenous infusion. Daily doses of 5, 15 and 30 mg/kg were administered intravenously during one- and 3-month subacute, toxicologic studies in rats and dogs. Acute drug-related deaths occurred in 3/30 and 4/20 high dose rats during the one- and 3-month studies, respectively. Emetis occurred in dogs in both studies. Body tremors that occurred in dogs given BM-25801 at 30 mg/kg/day have been reported at 1/100 of this dose in metoclopramide-treated dogs. Light microscopic examinations did not reveal any significant organ toxicity in rats and dogs. BM-25801 did not affect fertility (Segment I), was not a teratogen (Segment II), nor was it a mutagen.

CARDIOVASCULAR TOXICITY IN DOGS GIVEN INOTROPIC AGENTS BY CONTINUOUS INTRAVENOUS INFUSION. G E Sandusky, J R Means, and G C Todd. Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN.

A comparative study of the toxicity of isoproterenol hydrochloride (IP), l-norepinephrine bitartrate (NE), dopamine hydrochloride (DP), and dobutamine hydrochloride (DB) was conducted in beagle dogs (2/sex/dose/group). The doses were continuously infused for 96 hr and were 0.025, 1.25, and 2.5 µg/kg/min IP, 2.5 and 5 µg/kg/min NE, and 25, 50, and 100 µg/kg/min DP and DB. Three of four dogs that received 50 µg/kg/min NE and one of four given 100 µg/kg/min DP died. Pronounced tachycardia was observed at all doses of IP. DB produced a moderate tachycardia at 25 µg/kg/min which was not increased at higher doses. Significant bradycardia occurred when NE was infused at 2.5 µg/ 
kg/min and DP caused moderate bradycardia at 50 µg/kg/min. The higher doses of the 4 inotropes produced small, focal pale areas in the myocardium which histologically were identified as focal myocardial necrosis. These agents also produced coronary vascular injury that consisted of segmental medial hemorrhage and necrosis mainly in the muscular branches of the coronary arteries. An additional arterial lesion consisted of perivascular cellular infiltrate and fibrosis. The cardiovascular lesions produced by DB were less severe compared with those of the other 3 agents.

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Aqueous solutions of CGP 31608, a penem antibiotic, were administered sc at daily doses of 30, 100 or 300 mg/kg (dogs: 6-8/sex/group) or iv at daily doses of 30, 60 or 120-90 mg/kg (dogs: 6-8/sex/group) for 13 or 26 weeks. The high-dose level was decreased from 120 to 90 mg/kg in the dogs during week 4 due to severe, overt signs of toxicity. Bilaterally pale or enlarged kidneys, and minimal to marked toxic nephrosis with secondary changes of tubular dilatation and basophilic, interstitial fibrosis, mononuclear cell foci or interstitial nephritis were evident following 13 weeks (300 mg/kg:rats; ≥ 60 mg/kg:dogs) and 26 weeks (≥ 100 mg/kg:rats; ≥ 30 mg/kg:dogs). Also observed were: polyuria, proteinuria, glycosuria and/or the presence of urinary casts. The renal lesions were largely irreversible in the high-dose recovery rats, while in the high-dose dogs, only occasional focal areas of tubular epithelial hyperplasia were present at the end of the recovery period. Local changes at the injection site in the rats were consistent with a sensitization and chronic irritative response. Based on the proposed maximum clinical dose of 4 gm/day in man, a "no-effect" level approximating or higher than the projected human dose was not demonstrated in either species.

CGS 10787B, a non-steroidal anti-inflammatory agent, was administered by gavage to CD rats at doses of 20, 80, 300, or 3000 mg/kg orally for at least 52 or 104 weeks. The 80 mg/kg dose was reduced to 60 mg/kg during week 45 due to high mortality. After 52 weeks, the 100 mg/kg group was terminated and selected rats from the control and 100 mg/kg groups were placed on recovery for four weeks. All other groups continued on treatment until 104 weeks. Of compound-related oncogenicity was noted. Doses of 20 and 40 mg/kg were well-tolerated with only minimal effects; however, doses of 80-160 and 100 mg/kg were ulcerogenic and resulted in increased mortalities that were due to gastrointestinal intolerance. The GI lesions were characterized by ulcerations, adhesions, inflammation, and peritonitis. The ulcerogenic effects were accompanied by a variety of secondary clinical changes. Although the majority of effects in the 100 mg/kg group were reversible during the recovery period, intestinal ulcers were still evident in several animals of this group following recovery. However, there was no apparent increase in the incidence or severity of the GI lesions or ulcers in the 80 mg/kg group during weeks 52 through 104. GI ulceration and its sequelae are common side effects of long-term treatment with relatively high doses of non-steroidal anti-inflammatory agents.

Comparative Subchronic (91-Day) Peroral Toxicity of NE-11740 in Rat and Dog.
D B Mitchell and D J Dobrozsi. The Procter & Gamble Company, Cincinnati, OH.

NE-11740, a di-tert butylphenol anti-inflammatory drug, was administered by gavage for 91 days to Sprague-Dawley rats and beagle dogs to determine target organ toxicity and establish the NOEL. Dose levels were 0, 5, 15, or 50 mg/kg. Absolute and relative liver and kidney weights increased in rats at doses ≥ 15 mg/kg. In dogs, only the liver/body weight ratio increased. Clinical chemistry changes were generally restricted to 50 mg/kg groups. There were slight decreases in RBC, HGB, and WBC in rats but no hematological changes in dogs. Serum total protein, globulins, and GGT were increased in rats. In dogs ALT and ALKP were both serum proteins were decreased. In rats, centrilobular hepatocellular swelling was observed microscopically in 4/15 males and 13/15 females at 50 mg/kg dose levels. In dogs at 50 mg/kg/day there was an increased incidence/severity of brown pigment accumulation in hepatocytes and bile canaliculi. There were no renal or gastric lesions observed in either species. The NOEL was defined as 5 mg/kg in both species.


CGS 18320B, an aromatase inhibitor, was administered orally by gavage as a suspension in aqueous 3% cornstarch to three groups of beagle dogs (3-6/sex/group) at daily doses of 0.1, 0.5 or 5 mg/kg for at least 13 weeks. Doses ≤ 0.5 mg/kg were well-tolerated, although compound-related effects occurred at all doses. The changes were most pronounced at the high-dose level, and included: mortality in one high-dose dog; gingivitis at all doses; slight reductions in mean body weight parameters at 5 mg/kg; slight, nonspecific clinical laboratory changes at ≥ 0.5 mg/kg; electrocardiographic evidence of arrhythmia, decreases in heart rate and increases in QT interval at 5 mg/kg; and organ weight alterations and microscopic evidence of cystic ovaries, uterine and mammary gland atrophy, atrophy of the seminiferous epithelium with Leydig cell hypertrophy/hyperplasia, and inflammatory changes of the ginviva generally at all doses. The majority of these findings were considered to be secondary to the pharmacologic activity of the compound and were largely reversible in the high-dose recovery animals. Based on the functional cardiac changes observed in the dog at the 5 mg/kg level (250x the proposed maximum human dose of 0.02 mg/kg/day), the heart was considered to be a potential target organ at high doses.
SUBCHRONIC TOXICITY AND PHARMACOKINETICS OF SPIRONOLACTONE IN RATS AND MICE. K M Mackenzie1, B Boynton1, R Hall1, W Field1, S Gad2, and C Cook2, 1) Hazleton Laboratories America, Inc., Madison, WI; 2) G.D. Searle & Co., Skokie, IL.

Spiironolactone was administered in the diet for 13 weeks at doses of 0, 30, 150, and 300 mg/kg for Crl:CD®(SD)BR rats and 0, 50, 100, 150, and 300 mg/kg for Crl:CD®:1(ICR)BR mice. There were 20 animals/sex/group in the main study. Another set of animals (10/sex/group) was designated for pharmacokinetic evaluation, and blood samples were collected during Weeks 2, 6, 9, and 12 (rats) or 6 and 13 (mice). Survival was 90% to 100% for all groups. Alopecia was slightly greater in treated male rats; there were no treatment-related in-life observations for females or for male mice. Based on statistically lower body weights, the no-observable-effect levels for antemortem data in males and females, respectively, are 30 and 300 mg/kg in rats, and 100 and 150 mg/kg in mice. The lowest-effect levels for pathology variables are 30 and 50 mg/kg for rats and mice, respectively. Spiironolactone was absorbed at all doses, and proportionally more drug was absorbed at higher doses. There was a sex difference in the metabolic profile in rats (males were more active) but not in mice. Although steady state was achieved by Week 2 for rats, the pattern of metabolism changed for mice during the course of the study.

TOXICITY OF A FLUORINATED-BIPHENYL HMG COA REDUCTASE INHIBITOR IN BEAGLE DOGS. H L Allen, R J Gerson, J S MacDonald, and D L Bokelman. Merck Sharp and Dohme Research Labs, West Point, PA.

L-645,164, a potent inhibitor of HMG CoA reductase is a structurally unique synthetic fluorinated biphenvyl which was administered to beagle dogs at doses of 2, 10, or 50mg/kg/day for 14 weeks to evaluate its toxic potential. Unlike previously tested semisynthetic or fermentation-product inhibitors (i.e. lovastatin and simvastatin), administration of L-645,164 produced a significant spectrum of toxicity. Subcapsular lens opacities were produced in 75% of the dogs receiving 50mg/kg/day within 8 weeks. One dog receiving this dose experienced increases in serum ALT activity to 10X baseline. Light and electron microscopy of a wedge biopsy obtained within two days of this elevation failed to reveal any significant changes and the elevation resolved despite continued drug administration. Myelolynotic lesions of the optic tract, nerve and several tracts in the pons were observed in several dogs receiving 50mg/kg/day. Optic tract changes were generally mild, consisting of small to medium vacuoles without apparent myelin loss. Lesions in the pons ranged from barely visible to prominent vacuolation. No corneal degeneration clinical signs were observed. Plasma drug levels were >5µg/ml at least one order of magnitude greater than those attained after administration of other previously tested inhibitors. These findings suggest that HMG CoA reductase inhibitors producing high plasma levels are associated with a significant degree of systemic toxicity.

TOXICITY OF AZIDOTYLMIDINE (AZT) TO CATS. W N Haschek, C de Vera, R Wetgel, R Fienmehl, G Scherba, P Solter, M H Tongkina, and W A F Tompkins. Department of Veterinary Pathobiology, Univ. of Illinois, Urbana, IL.

Feline leukemia virus (FeLV) infection of cats is a model for the acquired immunodeficiency syndrome (AIDS). AZT toxicity was evaluated in SPF cats infected with Rickard strain of FeLV. Several were viremic and all antibody positive for FeLV antigens with subclinical infections. Thirteen, 6 to 10 mth old cats were divided into 5 groups and given AZT orally at 0, 7.5, 15, 30 or 60 mg/kg daily, in 3 divided doses for 33 days. Cats were clinically evaluated daily. Hematology and clinical chemistry were performed weekly. Cats were necropsied at the end of the study. All cats remained alert and clinically healthy. AZT caused a progressive anemia, dependent upon dose and time. Anemia was induced by day 6 with 60 mg/kg and by day 22 with 7.5 mg/kg AZT. Progressive absolute neutropenia was present in some cats at > 30 mg/kg. Clinical chemistries were normal. Lesions consisted of hypererythrocytosis of the bone marrow at > 30 mg/kg and splenic extramedullary hematopoiesis at > 15 mg/kg AZT. Thus oral AZT is toxic to cats at > 7.5 mg/kg. It induces a dose-related neutropenia and macrocytic normochromic anemia, suggestive of an abnormality of maturation of the erythroid series.
ACUTE AND SUBACUTE TOXICOLOGIC EVALUATIONS OF LIPOSOMAL FORMULATIONS OF METAPROTERENOL SULFATE (MPS)
P. P Hinman, N Drocik. Sponsor: R Hill
Liposome Technology Inc., Menlo Park, CA, Cooper Labs, Mountain View, CA

Liposomal formulations of MPS have prolonged bronchodilator activity and reduced cardiovascular side effects. The safety of liposomes comprised primarily of lecithin and cholesteryl, both with and without MPS, was assessed after inhalation by mice and monkeys. Mice received 4 hr, nose only exposures to 2 different liposome formulations or saline at maximal attainable liquid aerosol concentrations (2 mg/l, MMAD approx. 1µm). Animals were sacrificed at 0 and 24 hr, and 14 days after exposure. No differences in body weight, wet lung weight, gross necropsy observations, or pulmonary histology were seen between test and control animals. Monkeys were exposed by oronasal mask daily for 28 days to liquid aerosols (MMAD<4µm) of liposomal-MPS (L-MPS), and control solutions. L-MPS doses of 1x, 2.5x, and 7x daily human dose were attained. Complete clinical, biochemical, hematologic, and gross and histologic pathology observations, including oil-red-O lung staining, revealed no differences between treatment and control animals. Thus acute exposure to maximal attainable concentrations of liposome aerosols had no adverse effects on mice, and inhalation of up to 7x daily human dose of L-MPS for 28 days had no adverse effects on monkeys.

PROTECTION AGAINST TETRAPLATIN TOXICITY IN THE FISCHER 344 RAT. P F Carfagna, B S Chaney, D J Holbrook and J Chang. University of North Carolina, Biochemistry Department, Chapel Hill, NC
Tetrachloro(d,1-trans),1,2-diaminocyclohexane platinum(IV) (tetraplatin) (TP) is an anticancer drug recently approved as a phase I/II clinical trial. The limiting factors in TP use are nephrotoxicity, myelosuppression and gastrointestinal toxicity. The nephrotoxicity and gastrointestinal toxicity of TP (16.5 mg/kg iv) in male Fischer 344 rats was effectively reduced by treatment with either diathyldihiolocarbonat (DDT) (750 mg/kg ip) 0.5 hr after TP treatment or by S-2-(3-aminopropylamino)ethylphosphorothioic acid (WR-2721) (200 mg/kg ip) 0.5 hr before TP. Rats receiving TP alone had blood urea nitrogen (BUN) and creatinine of 40.9 mg/dl and 1.09 mg/dl, respectively. When rescued by DDT, the BUN and creatinine were 16.3 mg/dl and 0.42 mg/dl, respectively. WR-2721 protection resulted in BUN of 19.4 mg/dl and creatinine of 0.55 mg/dl. Gastrointestinal toxicity was evident in 95% of the rats treated with TP while it was not evident in any of the DDT- or WR-2721-protected rats. Only DDT was moderately effective in increasing platelet count. Additionally, mortality was decreased from 33% in rats treated with TP alone to 0% in both the DDT- and WR-2721-protected rats. The results suggest that DDT and WR-2721 may allow for increased dosages of TP by ameliorating the toxic side effects of the drug. (Supported by ACS Grant CH-393)

ACUTE AND TWO-WEEK ORAL TOXICITY EVALUATIONS OF A LIPOXYGENASE INHIBITOR IN COMBINATION WITH THEOPHYLLINE IN MICE AND RATS. R K Majors, B Burton, M B Friedman, L W Krasula, P K Coakley, and D R Patterson. Drug Safety Evaluation Division, Abbott Laboratories, Abbott Park, IL.

Clinical trials of ABBOTT-61589, a 5-lipoxygenase inhibitor, may include administration to patients who also are treated with theophylline. Thus, an acute oral toxicity study was conducted in male mice to test for possible adverse interactions between these drugs. Pretreatment of mice with 80 mg/kg of theophylline slightly increased the acute toxicity of ABBOTT-61589. The LD50 value for ABBOTT-61589 in non-pretreated mice was 4.23 g/kg, whereas the LD50 in theophylline-pretreated mice was 2.53 g/kg. Therefore, a two-week oral toxicity study was conducted to test for possible adverse effects of ABBOTT-61589 and theophylline administered concurrently to rats for two weeks. Groups of rats (10/sex/group) received ABBOTT-61589 at doses of 25, 50, and 100 mg/kg/day. These dosages are about 5 to 20 times the clinical dosage. The rats were treated concurrently with theophylline at a dosage of 65 mg/kg/day or about 5 times the clinical dosage. In this study, concurrent administration of ABBOTT-61589 and theophylline produced no adverse effects that were not observed with the individual drugs.

SALMONELLA MUTAGENICITY ASSAY OF BLEACH-DEACTIVATED DOXORUBICIN. TM Sullivan, TE Lawlor. Adria Labs, Columbus OH and Microbiological Associates, Inc., Rockville MD
A 1985 WHO report recommended a potassium permanganate/sulfuric acid deactivation procedure for doxorubicin (dox) wastes. Bleach deactivation was said to not fully eliminate mutagenicity, although no data were given. Since the bleach procedure is widely used and more practical for clinical situations, its efficacy was assessed by standard Ames mutagenicity procedures. The deactivation protocol was 0.25 mL of bleach per mL of dox solution at 2 mg/mL. Mutagenicity was determined of the non-deactivated dox and the deactivated solution after 5 minutes and 2 hours of reaction. Excess bleach was neutralized with metabolite and the pH adjusted to avoid bleach-induced cytotoxicity. The solutions were nitrogen-purged of dissolved chlorine gas. Test strains TA97, TA98, TA100, TA102, and TA1538 were selected for maximum sensitivity. To avoid significant alterations in the test system, 200 µL was the maximum plating volume. A positive response was defined as a doubling (or, for TA1538 only, a tripling) of the negative control mean revertant rate. Non-deactivated dox was positive in strains TA97, TA98, TA100, and TA102. Bleach-deactivated dox after 5 minutes or 2 hours of reaction produced no increase in mutagenicity in any test strain at dox-equivalent concentrations of up to 320 µg/plate.

In a sensitive and well-controlled Ames test, bleach-deactivated doxorubicin residues retained no detectable mutagenic activity.
INTRADERMAL AND VASCULAR IRRITATION ASSAYS OF DOXORUBICIN PRESERVATIVE-FREE SOLUTIONS: PRECLINICAL DEMONSTRATION OF SAFETY. R. Landes, L. Wong, T. Sullivan, Adria Labs, Columbus, OH.

A preservative-free, pH 3 doxorubicin solution (ADRIAMYCIN PFST, dox PFS) was recently introduced in clinical practice. Preclinical studies were conducted to compare the irritation potential of dox PFS to that of the established lyophilized product (dox lyo) reconstituted with saline for injection without pH adjustment (pH 4.5-6). ICR mice were given 0.2 mL of either solution by intradermal injection (id), to simulate an inadvertent extravasation, and lesion development was monitored. In a second series of experiments, NZ rabbits were given 2 mL of either solution via a marginal ear vein once a day for five days, to study vascular irritation. Edema and erythema in the injection area were scored at 24 hours after each dose.

All mice given dox id developed dermal lesions. Mean maximum lesion sizes were:
- Males: 211 dox PFS 291 mm²; dox lyo 227 mm²
- Females: 223 dox PFS 232 mm²; dox lyo 241 mm²

Group means of time to maximum lesion size ranged from 6-12 days and time to complete healing was 37-42 days. Similarly, all rabbits receiving dox iv had positive irritation scores, with group means of 2.7 (dox PFS) and 3.0 (dox lyo) on a grading scale of zero to 8 (Draize criteria). In both animal models, the response to pH 3 dox PFS was comparable to that of the lyophilized product, suggesting that the ready-to-use formulation presents no added irritation or necrotizing potential.


Cyclamates (Cycl) are consumed worldwide but regarded as a health hazard in U.S.A. In genetically prone hamsters (Nature 223, 407, 1969), Cycl caused cardiopathies. We have shown toxic dose-response signs and EKG changes in syrian hamster strains (Pharmacologist 13,241,1971). This report confirms these effects on heart and kidney lesions from twice daily oral doses of Cycl but not from equimolar Ca salts (chloride, acetate, lactate) and to a lesser extent from NaCyc but not acetate. Kidney tubular degeneration was seen with CaCyc (1-2 gm/kg) and NaCyc (1 gm/kg). Focal myocardial degeneration, mineral deposition, and myocarditis were seen with CaCyc at these doses. No heart lesions were noted with the Ca salts or due to NaCyc or Na acetate at these high doses. Grossly, the hearts showed so-called ischemic light surface plaques and the kidneys were swollen, pale, granular, and congested indicating pathological lesions. These data suggest that CaCyc is more toxic than NaCyc and the effects appear to be caused by indirect actions of these agents on inducing diarrhea, fluid loss, and hypokalemia rather than a specific cardio-nephrotoxicity of Cyclamate overdose.

DIET AND STRESS INFLUENCES ON THE PROLIFERATIVE EFFECT OF SODIUM SACCHARIN ON THE RAT URINARY BLADDER. E M Garland, T Sakata, M C Jackson, T Masut, M Cano, and S M Cohen. Univ. Nebraska Medical Center, Omaha, NE.

Proliferation of rat bladder epithelium was assessed by labeling index (LI), histology, and scanning electron microscopy (SEM) following feeding of large doses of sodium saccharin (NaS). In Experiment (Exp) 1, male F344 rats, 5 wk old, were placed on a diet of 0, 5, or 7.5% NaS mixed in Prolab 3200 (Pro), NIH-07 (NIH), or AIN-76 (AIN) diet for 4 or 10 wk. In Exp 2, 5 wk old F344 rats or 4 wk old Sprague-Dawley (SD) rats were fed 0, 5, or 7.5% NaS in Pro or Purina 5002 (Pur) diet for 10 wk. In Exp 1, at both the 4 and 10 wk intervals, NaS had a greater effect on the urothelium when administered in Pro compared to NIH, and there was little or no response with AIN. Eight of 10 rats fed 7.5% NaS in Pro for 4 or 10 wk had bladders with simple or nodular hyperplasia, and 8/9 bladders were clearly abnormal by SEM. At 10 wk, the average LI for all control animals was 0.05. For rats fed 7.5% NaS diets, LI was 0.42 for Pro, 0.14 for NIH, and 0.04 for AIN. In Exp 2, the response to NaS was considerably greater in F344 rats than in SD rats fed the same diet, and for both strains, the response to NaS was greater in Pro than NIH. In conclusion, the proliferative effect of NaS on male rat urinary bladder was variable and dependent on rat strain as well as type of diet. Supported by ILSI-NF.

LACK OF ABSORPTION OF OLEstra (OL) FOLLOWING CHRONIC FEEDING TO RATS AND MONKEYS. B R Detrull, F March, J J Hollenbach, and R M Kaffenberger. Proctor & Gamble, Cincinnati, OH. Sponsor: G P Danner.

OL consists of a mixture of hexa-, hepta-, and octaesters of sucrose with edible-grade fatty acids. It has physical and taste properties similar to those of triglycerides, but is not hydrolyzed or absorbed. The lack of absorption is supported by studies using thoracic duct cannulation, radiolabel disposition, and fat-balanced techniques. Further evidence for the lack of absorption was obtained by analyzing livers from rats and monkeys fed OL chronically. The methodology is based on the fact that the liver is the primary site for the localization of OL when it is introduced systemically by intravenous injection. Using thin-layer chromatography (detection limit [DL], 10-30 μg/g), no OL was detected in the livers of 14/14 monkeys fed OL as 8% of the diet for 29 months. Using high-pressure liquid chromatography (DL, 1-3 μg/g), no OL was detected in the livers of 10/10, 9/10, and 10/10 rats fed OL as 1, 5, or 9% of the diet, respectively, for 18 months; and 19/20 rats fed OL as 3% of the diet for 24 months. The levels of OL detected were near the DL, and correspond to 0.001X of the daily dose. The extremely low levels and isolated nature of the findings suggest possible contamination at necropsy. The results support the non-absorbability of OL.
Olestra consists of a mixture of hexa-, hepta-, and octaesters of sucrose with edible-grade fatty acids. It has physical and taste properties similar to those of triglycerides, but is not hydrolyzed by pancreatic or intestinal bacterial lipases, and is not absorbed. Olestra was fed at levels up to 9% of the diet for two years to male and female Fischer 344 rats in two separate studies. Daily observations, food consumption and body weights, organ weights, serum chemistry, hematology, urinalysis, and histopathological evaluations revealed no evidence of any adverse effects. The only histopathological observation related to the administration of olestra was an earlier appearance of spontaneous basophilic foci of hepatocellular alteration in females, with no difference between groups at the end of the study. There was no increase in hepatic nodules or tumors. A panel of expert pathologists concluded that these foci were qualitatively the same as those which occurred in the untreated groups and which occur spontaneously in aging rats, and that they did not represent a neoplastic response. Isolated instances of other statistically signficant differences were noted; however, none of these were considered to be related to the administration of olestra.

**THE EFFECT OF COOKING METHODS ON THE MUTAGENICITY OF FOOD AND ON URINARY MUTAGENICITY OF HUMANS FOLLOWING CONSUMPTION.**

D J Doolittle, C A Rahn, E A Reed and C K Lee.
R.J. Reynolds Tobacco Co., Winston-Salem, NC.

Certain foods have been shown to contain protein pyrolys products, which are mutagenic in vitro and carcinogenic in animals. The formation of mutagenic pyrolys products during the cooking process has been reported to be temperature-dependent, with higher cooking temperatures resulting in greater mutagenicity. The objective of this study was to prepare food by different methods and to quantitatively compare the mutagenicity of individual foods, the mutagenicity of meals containing those foods and the mutagenic exposure of human volunteers following consumption of the meals. A boiled diet, a baked diet and a fried diet were prepared. The mutagenicity of meats, but not carbohydrates or vegetables, was markedly affected by the method of cooking. The mutagenicity of human urine following consumption of the meals was related to the mutagenicity of the meals themselves. The present results demonstrate that the mutagenicity of our meals may be markedly altered by the method used to prepare them and that the mutagenicity of our diet is reflected in the mutagenicity of our body fluids.

**AN EVALUATION OF FOOD FLAVORING INGREDIENTS IN A GENETIC TOXICITY SCREENING BATTERY.**


Sixty-three widely used food flavoring ingredients were screened for genetic activity in the Salmonella mutagenesis (SAL) assay. Many of these ingredients were also tested in the rat hepatocyte unscheduled DNA synthesis (UDS) assay and the mouse lymphoma L5178Y TTK/6- cell mutagenesis (MLY) assay. All assas except UDS were performed with and without an Arctor 1254-induced rat liver s9 activation system. All 63 ingredients were inactive in Salmonella strains TA1535, TA1537, TA1538, TA98 and TA100. Of the 48 ingredients tested in the UDS assay, only p-cresol methyl ether yielded a positive response; this material was not tested in the MLY assay. Pipersonal gave discordant UDS responses in replicate assays and was inactive in the MLY assay. Seventeen of 33 flavorings tested in the MLY assay gave positive responses. Anethole, benzaldehyde, dihydrocoumarin, ethyl vanillin, vanilloyl acetate, heptanoic acid, hexanoic acid, isobutyric acid, linalool, g-nonalactone, prune juice, sage oil and vanitrope were active in the MLY assay only with S9 activation. Licorice extract, valerian extract, orange oil, and veratraldehyde were MLY positive with or without S9. Additional studies are needed to clarify the basis for the positive MLY responses.

**ACUTE, SUBCHRONIC, CHRONIC AND FERTILITY ASSESSMENT STUDIES OF TURMERIC.**

OLEORESIN IN F344 RATS AND B6C3F1 MICE.

J French*, R Fleischman, MJ Esber, AB Russfield, and LE Sendelbach.

*NIEHS/NTF, Research Triangle Park, NC and EGG & Mason Research Institute, Worcester, MA.

Turmeric, oleoresin is used extensively as a food coloring agent and has significant antiinflammatory properties. Because of its wide spread use and human exposure turmeric, oleoresin was administered to both sexes of F344 rats and B6C3F1 mice to assess potential toxicity. Single oral doses (0.62 to 10 g/kg, by gavage) produced minimal hepatic and renal changes in both species. Treatment for 13 weeks in the feed (0.1 - 5%) had no effect on mortality, clinical chemistry parameters or microscopic findings. Fertility assessment at doses up to 5% demonstrated no adverse effect on sperm morphology, vaginal cytology, reproductive function, or gross and microscopic morphology of reproductive organs. Administration for 15 months in feed (0.2 - 5%) produced gastrointestinal tract pigmentation and cecal ulcers and inflammation. Further studies are underway to determine carcinogenicity following 24 months of treatment.
EVALUATION OF THE PROTEIN QUALITY OF MEAL FROM DETOXIFIED CANAVALLA MARITIMA SEEDS. H. D. Graham, C. McGowan, and L. Matthews. Chemistry Department, Univ. of Mayaguez, Mayaguez, PR and Food Science and Human Nutrition Department, Univ. of Florida, Gainesville, FL.

Canavalla maritima (Beach bean), a potential food source, has a protein content of >26%. Like other legumes, it contains a number of growth-inhibiting factors (GIFs). Its potential as a protein source was investigated using rat protein efficiency ratio (PER) assays. The meal from dry C. maritima seeds was either untreated (Exp I), soaked in 2M sodium bicarbonate and cooked for 1 hour (Exp II), or dehulled, soaked, and cooked (Exp III). For each PER assay, 2 groups of male weanling rats (10/group) were maintained for 28 days on either a 10% casein diet (control) or one in which 10% bean meal protein (test) replaced the casein. In Exp III, the test diet was supplemented with 0.3% methionine (MET) at the end of the 28 day period and all rats were fed 2 additional weeks. The PER values were as follows: Exp I = -1.7 ± 0.4; Exp II = -0.17 ± 0.6; Exp III = 1.35 ± 0.3. The data showed a response to MET (Exp III) and suggested an influence of GIFs in the seed coat (e.g. polyphenols) on growth. Thus, the supplementation of dehulled, soaked, and cooked seeds with SAA may dramatically improve the protein quality of C. maritima.

NATURAL OCCURRENCE OF FUMONISINS IN CORN ASSOCIATED WITH EQUINE LEUKOENCEPHALOMALACIA (ELEM). K. A. Voss, C. W. Bacon and J. K. Porter. Russell Research Center, ARS/USDA, Athens, GA and *Northern Regional Research Center, ARS/USDA, Peoria, IL.

Corn contaminated with Fusarium moniliforme (FM) causes the fatal brain syndrome, ELEM, in horses, and produces toxic hepatitis and hepatocarcinoma when fed to rats. We have shown that aqueous extracts of culture material prepared from a South African isolate of FM (MRC 826) cause liver toxicity in rats, and Gelderblom et al. (Appl. Environ. Micro. 54:1806, 1988) recently purified a water-soluble metabolite from MRC 826, Fumonisin B1, which caused hepatitis and induced γ-glutamyl transpeptidase positive foci in rats. We prepared methanol-water extracts of MRC 826 culture material and of corn screenings naturally contaminated with FM. The corn screenings caused field cases of ELEM and were hepatotoxic to rats. Fumonisins B1 and B2 were isolated from MRC 826 and the identity confirmed by TLC, NMR and MS. Fumonisins were also detected in extracts of the corn screenings by TLC. Examination of crude extracts of both MRC 826 and corn screenings by liquid secondary ion MS revealed the presence of protonated molecules for fumonisins B1 and B2 at m/z 722 and 706, respectively.


Flavonoids are a common constituent of diets and are found in fruits and vegetables. Total dietary intake is approximately 10 daily. Until recently, the majority of these compounds have been thought to be beneficial due to their antioxidant properties. However, there is recent evidence that they are toxic to cells and that the toxicity is due to toxic oxygen species (TOS) production. We have found a number of dietary flavonoids are toxic to isolated intestinal cells and that this toxicity correlates with their ability to produce TOS on autoxidation. Additionally, using both spin trapping and oxygen consumption techniques, we have found that the production of TOS is increased in the presence of iron and is, in some cases, increased by SOD. These reactions occur at physiologic pH. Finally, we have EPR and O2 consumption evidence that some of these compounds redox cycle to produce TOS, a reaction also increased by iron.

Supported by a grant from the National Foundation for Ileitis and Colitis.


Ingestion of Fusarium moniliforme (FM) contaminated grain has been associated with human esophageal cancer and equine leukoencephalomalacia (ELEM). Renal lesions, toxic hepatitis and hepatocellular carcinomas have been found in rats fed FM contaminated diets. This study is part of an ongoing effort to identify heretofore unknown hepatotoxins produced by FM and assess their human health significance. Aqueous and chloroform/methanol (1:1) extracts of hepatotoxic FM strain MRC 826 corn cultures and/or the extracted culture material (20%) were mixed with commercial diet and fed to five male rats for four weeks. Decreased weight gain, increased serum transaminase and alkaline phosphatase activities and microscopic hepatic and renal lesions typical of FM toxicity were present only in those groups fed aqueous extract or the chloroform/methanol extracted culture material. Recently, Gelderblom et. al. (Appl. Environ. Micro. 54:1806, 1988) isolated a novel, water soluble hepatotoxic, fumonisin B1, produced by MRC 826 in culture. Efforts to verify the presence of fumonisin B1 in the aqueous extract and further evaluate its toxic effects are in progress.

Male Sprague Dawley rats were fed (4 wks) diets supplemented (16%, w/w) with corn cultures of FM (NRC 826) or ELEM associated corn screenings (CS-1 & CS-2) naturally contaminated with FM. Animals on the FM (NRC 826) diets had lower body weights (30%, P<0.01) along with increased brain weight/body weight (BW/BW) ratios (40%, P<0.01). As measured by high performance liquid chromatography with electrochemical detection, brain serotonin (5HT) and 5-hydroxyindoleacetic acid (5HIAA) in the rats on the FM diets were increased (11%, P<0.02 & 60%, P<0.01, respectively) as were their 5HIAA/5HT ratios (45%, P<0.01). The rats on the CS-1 diet had lower BW (17%, P<0.01) and higher BW/BW (14%, P<0.01) ratios. In addition, the CS-1 rats had increased brain 5HIAA levels (42%, P<0.02) and 5HIAA/5HT ratios (24%, P<0.07). There were no differences in the rats on the CS-2 diet and their controls. These preliminary results suggest a possible FM (ELEM associated) induced dysfunction in neurotransmitter metabolism in rats. Monitoring these compounds (in rats) may be a useful bioassay in the detection and subsequent identification of the ELEM toxins produced by FM.

TOXICITY OF VENOMS FROM OPISTHOGLYPHE SNAKES: AN OVERVIEW. R. A Young. Health and Safety Research Division, Oak Ridge National Laboratory, Oak Ridge, TN.

Opisthoglyphous (rear-fanged) snakes have been classified as mildly venomous and, with exception of Dipsopholidus typus, Rhadophis tigrinus, Rhadophis subminatus, and Thelotornis kirtlandii, are not considered dangerously poisonous to humans. Recently, additional species including Thamnophis elegans, Heterodon nasicus and Coelophanes sp. have been implicated in serious envenomations of humans. Previous work characterized the venoms of R. typus, T. kirtlandii, and Rhadophis sp. and revealed toxic components with coagulopathic activity. Whole venoms of H. platirhinos and S. blandings (100 µg/ml) each produced an irreversible neuromuscular blockade of the frog sacral nerve-gastrocnemius muscle. The venom of R. typus produced a dose-dependent excitatory effect on the isolated guinea pig ileum that was blocked by prior addition of atropine (10^-6 M). A higher interest profile for the venoms of opisthoglyphous snakes may be justified by their implication in serious human envenomations and as a source for biologically active components potentially useful as pharamacological tools. (Supported in part by a Sigma XI Grant-in-Aid of Research).


Rhodamine 123 (Rh 123) and two analogs of carboxyfluorescein diacetate (CFDA) were used in an in vitro toxicity assay system to indicate mitochondrial and cell membrane damage in response to heavy metals and detergents. Madin Darby canine kidney (MDCK) cells and human epidermal keratinocytes (HEK) grown on collagen coated microporous membranes exhibit a differentiated in viva-like ultrastructure characterized by cuboidal morphology, basal nucleus, desmosomes, tight junctions (MDCK), and stratification (HEK). Cell monolayers were stained with either Rh 123 or one of the CFDA derivatives. There is a dose response efflux of the fluorescent probes when exposed to 0-100 µM mercucir or cadmium chloride or to dilutions of 1-10% sodium dodecyl sulfate (SDS) or Tween 20. Dye efflux was quantified in the Fluoroscan II spectrofluorometer excitation 455 nm, emission 538 nm. We found that with heavy metals, mitochondrial toxicity preceded disruption of cell membrane integrity. For 10% SDS, assay sensitivity extended to dilutions of 1 in 10,000. Rh 123 and CFDA are sensitive to quantifiable probes of cell membrane and organellar toxicity in response to heavy metals and detergents.


When normal human keratinocytes (Clonetics, Inc.) are exposed to a variety of irritant compounds, we find that their metabolic rates alter in characteristic ways that correlate with the severity of the irritants as determined by other assays such as the in vivo Draize test. To measure these rates we have constructed a device based on a flow chamber and a light-addressable potentiometric sensor (Science 240:1182,1988). Signals are obtained from about 1,000 cells. When flow is temporarily interrupted, the rate of acidification of the medium by the cells is a measure of their overall metabolic activity. This process is detected by the sensor, which forms one surface of the chamber. Resumption of flow introduces fresh medium and resets the pH for subsequent measurements. Exposure times to irritants and metabolic measurement times as short as tens of seconds currently can be achieved. We find that 300sec exposure to a variety of irritants depresses metabolism in a dose-dependent manner. An increase in metabolic rate is often observed for irritant concentrations slightly below those which cause metabolic depression. Several log units of concentration separate lethal doses of severe and mild irritants. The kinetics of recovery from exposure to some irritants have been measured. This device may be the basis of a rapid, flexible, and automated system for general in vitro toxicity assays. Partial support by ARO contract DAAL03-86-C-0009.

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An in-vitro technique has been developed for assessing the skin corrosive potential of chemicals. In an initial validation (44 corrosives, 24 non-corrosives) the proportion of corrosives detected (sensitivity) was 92% whilst non-corrosives excluded (specificity) was 74% (Oliver et al., 1988). This initial validation was extended to 162 chemicals (44 corrosives, 61 irritants and 57 non-irritants) with little loss in sensitivity (80%) or specificity (72%) (Pemberton et al., 1986). The test has been used blind as a prescreen in a sequential approach to skin hazard testing and shown that for 88 substances (51 solids, 39 liquids), 9 corrosives, 63 irritants and 16 non-irritants sensitivity was 89% whilst specificity was 89% for irritants and 100% for non-irritants. This data shows that the in-vitro technique used as part of a sequential approach to testing may provide objective measurements and contribute to a reduction in the use of animals in toxicity testing. Oliver G J A, Pemberton M A and Rhodes C, (1988) Toxic In-vitro 2 No. 1 pp 7-17. Oliver G J A, Pemberton M A and Rhodes C. (1986) Fd. Chem. Tox 24 6/7 pp 513-516.


Skin irritation testing is an essential part of the assessment of the potential human health hazard of chemicals and is currently done in vivo in rabbits. An in-vitro technique has been developed as a means of both introducing objective measurement and reducing the number of animals used (Pemberton and Oliver, 1986). Epidermal (keratome) slices of young rat skin and full thickness young rabbit skin have been maintained in a viable state at an air/liquid nutrient medium interface for up to 72 hours. Chemicals or mixtures of a wide range of physico-chemical type were applied to the epidermal surface and tissue toxicity measured by the inhibition or leakage of tissue enzymes (AST, MDH and GLDH) and lactate. A preliminary intralaboratory validation with 59 substances (ranging from non-irritant to severe irritant in-vivo) showed that all severe and a proportion of moderate irritant chemicals were distinguished and these correspond closely to those substances which are regarded as irritant and require labelling.


An in-vitro technique has been developed for the assessment of chemical corrosion of skin (Oliver et al., 1988). Studies with this technique using skin from cadavers and young rats indicate that human skin is less susceptible to the corrosive action of chemicals in-vitro (Oliver and Pemberton, 1986). Three substances, a proprietary surfactant, an industrial solvent and an Agrochemical formulation, which have been shown to be corrosive to animal skin in-vivo and in-vitro were tested under double blind conditions in a standard four-hour occlusive patch test in six human volunteers. In the clinic the responses observed were limited to transient slight inflammatory changes and desquamation. This study demonstrates that current standardised animal models may overestimate the dermal hazard to man of some substances and that the in-vitro technique can be used to identify these instances.


IN VITRO CELL LINE SENSITIVITY MAY NOT CORRELATE WITH IN VIVO TARGET ORGAN TOXICITY OF SELECT XENOBIOTICS. D A Linseman and T J Racznik, Drug Safety Research, The Upjohn Company, Kalamazoo, MI.

The potential of cell lines to predict the in vivo target organ toxicity of a heterogeneous subset of xenobiotics was examined. Gentamicin, an aminoglycoside nephrotoxin, Galactosamine, a hepatotoxic aminosugar, and Chloroquine, an antimalarial ocular toxin were used as models. Porcine proximal kidney (LLC-PK1), human liver (Chang), and rabbit corneal (SIRC) cells were exposed for 48 hrs to Gentamicin Sulfate (5, 10, 15, 20, or 25 mg/ml), Galactosamine chloride (1, 2, 4, 6, or 8 mg/ml), or Chloroquine Diphosphate (0.05, 0.10, 0.15, 0.30, or 0.60 mg/ml). Cytotoxicity was assessed by morphology, cytoplasmic LDH leakage, and cell proliferative capacity. The rank order of cell line sensitivity for each of the compounds tested was as follows: Gentamicin; Chang > SIRC > LLC-PK1, Galactosamine; LLC-PK1 > SIRC > Chang, and Chloroquine; LLC-PK1 > SIRC > Chang. No apparent correlation exists between the sensitivity of the continuous cell lines and the in vivo target organ for each xenobiotic. The results suggest that in vitro systems chosen solely on the basis of in vivo target organ toxicity of a compound, may not always provide the most sensitive model. The implications of the finding may be important in both mechanistic studies as well as in vitro screening systems when continuous cell lines are used.

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Hemosiderin (aggregated ferritin), derived from hemoglobin, was deposited in the red pulp of the spleen during a 13 week feeding study in Sprague-Dawley rats administered the herbicide, chlorotoluron. This insoluble form of storage iron was detected initially by standard H & E sections; subsequently, an attempt was made to quantify the hemosiderin with an image analyzer after staining with Prussian Blue. Sections, 4–5 microns, were examined with a Quantimet 900 (9000), composed of a Zeetepan microscope, Newcomv Camera tube, scanner and a DEC LSI 11/23 computer which digitalized the analog video signals. Average values of five fields per animal (10/sex/group) were obtained and group means compared with in-life hematologic parameters. Regression analysis showed a linear relationship (p<0.001) between dose and relative stained area. Both males and females were affected; however, decreased hemoglobin, RBC count and increased reticulocyte count were noted in males only. Congruing hemolysis in males was effectively countered by responsive bone marrow. This technique provides a rapid means of quantifying in microslides changes which may be differentially stained. Hematological changes which may not be detected by usual means have been unequivocally demonstrated.

DEVELOPMENT OF A VIBRATION SENSITIVITY BASELINE FOR OCCUPATIONAL HEALTH. P.J. Maurissen and G.J. Charzan. Mammalian and Environmental Toxicology Research Laboratory, Health and Environmental Sciences, The Dow Chemical Company, Midland, MI.

Vibration sensitivity reflects the functional status of the peripheral nervous system. Numerous chemical and physical agents, as well as diseases that affect peripheral nerves, and vibration sensitivity has been used to follow the course of peripheral nerve dysfunctions. The reliability, sensitivity and specificity of the computerized Dow vibration sensitivity system have been documented. The purpose of the present study was to establish normative values for a sample of 415 Michigan Division Dow workers selected for the absence of neurological dysfunctions, of conditions potentially associated with neurological disorders and of potential exposure to neurotoxins. Data analysis showed that the tested subjects constituted a representative sample of the Dow worker population from the points of view of gender and job distribution. Vibration sensitivity thresholds followed a Gaussian curve and varied with age in a linear fashion. The data provided an age-corrected baseline (derived from a relatively large sample) that can be used as a reference for future comparison. This study shows the potential for the use of vibration sensitivity testing in an occupational setting.


Experiments were conducted to determine if extending the induction phase of the modified Buheher test for contact sensitization would lower the elicitation concentration for DNBC. Groups of 10 animals were exposed via occluded patch (1 patch/week) to DNBC for 3, 7, 11, 15 and 19 weeks. Two induction doses were used; 43 nmol, a marginal effect level when used at both induction and challenge in the standard (3 week induction) Buheher test, and 4.3 nmol, a no effect level. Two weeks after completing induction, animals were challenged with 1.2, 12 and 120 nmol of DNBC. There were no positive responses at 3 weeks. For animals induced for 7 or 11 weeks at 43 nmol, 3 of 10 showed positive responses at the 120 nmol challenge dose (a level 3-fold higher than the induction dose), and 1 of 10 was positive after 7 induction weeks at the 12 nmol challenge dose. For animals induced at 4.3 nmol, 1 of 10 showed positive responses at 120 nmol after 11 or 15 weeks of induction. No other positive responses were observed. Results indicate that 7 to 11 weeks of induction were required to elicit positive responses. A longer induction time did not progressively decrease the effect level for elicitation.


There is considerable evidence that exposure to xenobiotic agents may significantly affect temperature regulation in rodents. We have shown that acute administration of compounds such as nickel, cadmium, lead, and some pesticides causes a reduction in body temperature of mice when tested at normal room temperatures. When provided with the option of selecting their preferred ambient temperature, the toxicated animals generally select cool temperatures which augment the hyperthermic effect of the toxic compounds. It would appear that many of the xenobiotic compounds have central as well as peripheral effects on the control of body temperature. That is, the hyperthermic animals select cooler temperatures, a condition indicative of a centrally mediated decrease in the set-point. This decrease in set-point, or regulated hyperthermia, may be beneficial to survival since the lethality of most xenobiotic compounds increases with rising body temperature. Our observation that acute doses of various compounds leads to behaviorally and autonomically mediated changes in body temperature may have significant implications for the measurement of other biological effects of these chemical agents.
A method for the extraction, separation, and isolation of perfluorodecanoic (PFDA) and perfluorooctanoic (PFOA) acids from biological samples along with tissue lipids was devised. A two-stage lipid extraction procedure, utilizing sulfuric acid to decrease the pH of the solvent systems was employed. Extraction from liver of free fatty acids, including the perfluorinated fatty acids (PFA), was complete using this method. Silica gel chromatography was used to separate neutral lipids from complex lipids. Neutral lipids were eluted first, using diethyl ether-trifluoroacetic acid (100:1, v/v), while complex lipids remained bound to the column. The neutral lipid fraction containing the PFA was then separated by thin-layer chromatography. A unidimensional, two-solvent system utilizing glacial acetic acid was used to move the PFA from the origin, whereas their non-perfluorinated analogs migrated with palmitic acid. Replacement of acetic acid with trifluoroacetic acid caused PFDA and PFOA to behave similarly to decanoic and octanoic acid. Final recovery from liver samples was 96%, 74%, and 94% for PFDA, PFOA, and palmitic acid, respectively. (AFOSR 85-0207)

We have developed a thermal extraction GC/MS method which permits direct monitoring of dieldrin and aldrin in soils and tissues. Dried humic soils and avian tissues (25 mg) were contaminated with aldrin and dieldrin over a 500-fold (ppb to ppb) concentration range. Samples were heated from 30 to 340°C (10.3 min) in a fused quartz cell under a helium carrier gas stream. Volatilized material, cryoconcentrated on a head of a DB-5 capillary column (0.25), was chromatographed over 30 minutes from 50°C to 300°C; eluting GC fractions were identified and quantitated by on-line ion trap MS. Total analysis time is 57.5 minutes. No thermal degradation of aldrin or dieldrin was observed; each compound was dose-dependently separated and identified over the concentration range. These results support thermal extraction coupled with on-line GC/MS as a rapid method for direct analysis of polychlorinated and polycyclic hydrocarbons in soils and tissues requiring no sample preparation.

A comparison of physiological and biochemical parameters in two species of minipig,

Five Normel-Hamford and five Yucatan minipigs of comparable size and age were used in the study. The pigs were fitted with a catheter in the vena cava, then placed in a Proneplato sling. The mean arterial blood pressure (MABP) and heart rate (HR) were monitored by a Dinamap Research Monitor using a neotrol cuff attached to the tail. Blood specimens were obtained as measurements of cardiovascular parameters were made, and plasma levels of (total) catecholamines and cortisol were determined at each sampling time. Following three control sessions to obtain baseline values, each pig was administered angiostat (A) (to raise blood pressure). After a rest period of several weeks, each pig was administered kynurenin (V) (to lower blood pressure). With A treatment, catecholamine levels increased, then decreased; cortisol levels were increased in the Yucatan minipig. V generally increased catecholamine and cortisol levels over controls. The Normel-Hamford strain of minipig showed a statistically significant higher control plasma catecholamine level. Because of these differences, the Yucatan strain may be preferable to the Normel-Hamford if plasma catecholamine levels are to be determined in an experiment.


The toxic effects of hypervitaminosis A syndrome at effective doses presents a severely limiting factor in the clinical usefulness of the retinoids in the treatment of skin diseases and possible treatment and prophylaxis of neoplasia. The design of retinoid acid analogs with high activity together with low toxicity is a major goal. An in vivo retinoid screening system in the mouse comparing the ED50 value in the chemically induced skin papilloma assay (the dose which, when given i.p. once weekly for 2 weeks, causes a 50% regression of papillomas) with the lowest dose which, when given i.p. daily for 14 days, causes a defined degree of hypervitaminosis A, provides a model for defining the therapeutic ratio of a retinoid and detecting a dissociation of efficacy from toxicity.

The CASE program is a sophisticated quantitative structure activity relationship method that identifies molecular fragments that are either activating (biophores) or inactivating (bioinhibitors) with respect to a specific biological activity. CASE generated related fragments that were activating for antipapilloma activity and inactivating for hypervitaminosis A activity. The results suggest possibilities for the design of novel retinoids with improved therapeutic indices.
A COMPUTER APPLICATION FOR IMPLEMENTING CURRENT STATISTICAL ANALYSIS TECHNIQUES FOR CHRONIC STUDIES INVOLVING TUMOR PREVALENCE DATA.
G S Bieler, Research Triangle Institute, RTP, NC
Sponsor: Douglas E. Rickert.

Standard statistical methods for analysis of tumor prevalence data arising from long-term animal experiments involve the arbitrary formation of time intervals and the use of non-parametric techniques to adjust for survival differences among the dose groups. Recently, Dinse and Lagakos (1983) introduced an alternative technique based on logistic regression procedures. This technique offers many advantages over earlier methods, in that it eliminates the need for arbitrary interval selection, provides a framework for testing assumptions underlying both methods, allows for the direct inclusion of covariates such as sex, and estimates the strength of dose-response relationships. This paper illustrates a statistical computer program for implementing these regression techniques, accompanied by auxiliary analysis of estimated survival data, which constitutes the first step in evaluating any carcinogenicity study. To accomplish these goals, the user can request any or all of the following: a summary table of death times and tumor prevalence, a determination of the effect of exposure to the test compound on mortality and/or of estimated survival curves, tests of the proportional odds and other regression assumptions, and the sex-specific and/or sex-adjusted regression analyses of tumor prevalence data. An example is included.

STATISTICAL DESIGN CONSIDERATIONS FOR STAGEWISE ADAPTIVE DOSE ALLOCATION STUDIES. P I Feder, D W Hobson, C T Olson, and R L Joiner. Battelle Columbus Division, Columbus, OH.

A principal objective of many dose-response studies is to estimate extreme percentiles of a dose-response distribution for a drug therapy as precisely as feasible using the smallest number of experimental animals possible. This design requirement necessitates dosing in a stagewise fashion to maximize the information obtained from each subsequent experimental observation in light of what has previously been determined.

This presentation describes specialized algorithms and associated computer programs to evaluate, on a stagewise basis, the anticipated relative sensitivities of alternative experimental plans in the case of dichotomous responses. Following each stage, the dose-response distribution parameters, as well as the uncertainties, are estimated and used to assign subjects to dose levels for the next stage of testing. Competing dose allocations are compared with respect to anticipated improvement in estimation precision; that allocation which results in the smallest average variability of estimation, weighted over all plausible combinations of parameter values, is selected for the next stage. The entire process is iterated. [Supported by USAMRDC DAMD17-83-C-3129.]

EMPIRICAL DEFINITION OF BIOLOGICAL MEANINGFUL DIFFERENCES IN TOXICITY STUDIES AND INTEGRATION INTO STATISTICAL TESTS. C Y Meng, A J Roth, J T Stevens and C B Breckenridge. CIBA-GEIGY Corporation, Summit, NJ, Greensboro, NC.

A statistical definition of the historical normal range for parameters evaluated during routine toxicity testing in laboratory animals was integrated into standard tests of the null hypothesis. A variance components model for historical data was used to estimate between experiment variability, between animal variability, and residual variability. The normal range was related to these variance components and represented by the interval:

\[ \pm c \sqrt{\text{Total variance}} \]

The empirically derived constant c, was used to define the magnitude of a minimum difference that was considered biologically significant. Computer simulations were performed to limit the sensitivity of the statistical tests such that only "biologically meaningful" differences between treated groups and the concurrent control group would be flagged statistically. The utility of this method for defining biologically meaningful differences in toxicity studies was compared to other more arbitrary approaches used by regulations in establishing no observable effect levels.

ANALYSIS OF FREE AND BOUND MALONALDEHYDE IN LIVER FROM CCl4 TREATED RATS. T Ichinose, K Dennis, M Miller, and T Shibamoto. Department of Environmental Toxicology, University of California, Davis. Sponsor: B Wilson.

Direct analysis of malonaldehyde (MA), which is known as a product of lipid peroxidation and prostaglandin biosynthesis, is extremely difficult. The 2-thiobarbituric acid (TBA) method is commonly used to determine MA for the investigation of biological damages caused by lipid peroxidation. However, because it is not specific for MA, the TBA method results in overestimation of MA. In the present study, free MA formed in rat liver was derivatized into N-methylpyrazole and was subsequently analyzed by a gas chromatograph equipped with a nitrogen-phosphorus specific detector. Free MA determined in normal rat liver was 0.428 \( \mu \text{g} \)/g liver. Bound MA, which was freed by acid hydrolysis, was 0.934 \( \mu \text{g} \)/g liver. Total MA, which is a sum of free and bound MA, was 1.464 \( \mu \text{g} \)/g liver. The level of bound MA increased approximately two fold with CCl4 treatment. On the other hand, the values obtained by TBA method were not changed by CCl4 treatment.
In our search for in vivo biomarkers for toxicity, we monitored the volatile carbonyl compounds produced during CCl₄-induced lipid peroxidation. Sprague-Dawley rats dosed with CCl₄ (3 ml/kg) were placed into a glass chamber through which air was passed continuously at 120 ml/min. Two different techniques were used to trap the volatile aldehydes, ketones, and related carbonyl compounds produced. In the first method, aldehydes and ketones were derivatized to thiaaldehydes by passing the effluent gas stream through an aqueous cysteamine solution. The derivatives were then extracted and analyzed by gas chromatography and GC/MS. The second method used methyl hydrazine in place of the cysteamine. Methyl hydrazine reacts with carbonyl compounds, including malonaldehyde and acrolein, to form specific hydrazones, pyrazole, and pyrazoline derivatives that can be measured by gas chromatography. Increased levels of several cysteamine and methyl hydrazine derivatives of carbonyl compounds were found in CCl₄-treated rats relative to control rats. Results of the two methods suggest acetone to be the major volatile carbonyl compound produced by CCl₄-induced peroxidation.

Evaluation of large scale non-isotopic immunoassay drug testing was performed on pre- and post-race specimens taken from equine athletes. The technologies employed to accomplish this task were Particle Concentration Fluorescence Immunoassay (PCFIA) and Enzyme Linked Immunosorbent Assay (ELISA). On introduction into post-race testing, these rapid and inexpensive immunoassays exposed acepromazine, buprenorphine, oxyphorone, minidole, sufentanil and cocaine as undetected agents of drug abuse.

PCFIA and ELISA assays have been introduced into pre- and post-race testing programs in Illinois. Evaluation of frozen samples unmasked seventy ELISA "positives", twenty-five of which were further confirmed by mass spectrometry. PCFIA has been modified into human drug tests in order to enable racing authorities to test jockeys and other race track personnel. Therefore, we propose that state programs incorporate these flexible, broad spectrum immunoassay techniques to detect such high potency drugs of abuse.

Supported by grants from the KY State Racing and KY Harness Racing Commissions, the KY Equine Drug Council, the American Horse Shows Assoc., I.D.S. Corp., the Oklahoma State Racing Commission and the U.K. Graduate Center for Toxicology.

Detomidine is a potent non-narcotic alpha-2 adrenoceptor agonist which is pharmacodynamically similar to xylazine. Detomidine is currently in the process of being approved for veterinary clinical use in the United States. A radioimmunoassay for detomidine in equine blood and urine was developed since no effective screening method currently exists. Cross-reactivity studies were conducted with acepromazine, epinephrine, haloperidol, promazine, metdetomidine, xylazine and the hydroxyl and carboxyl metabolites of detomidine. Pharmacokinetic data indicates first-order kinetics. The diuretic effect of detomidine was taken into account in analyzing urine data. Detomidine administrations from 3 to .5 mg/horse are readily detectable with this method.

Supported by grants from the Kentucky State Racing Commission, Kentucky Harness Racing Commission, the Kentucky Equine Drug Council, the American Horse Shows Association, I.D.S. Corp., the Oklahoma State Racing Commission and the U.K. Graduate Center for Toxicology.

A one step enzyme-linked immunosorbent assay (ELISA) and particle concentration fluorescent immunoassay (PCFIA) test for furosemide was evaluated for use in racing horses. The ELISA test is sensitive to furosemide with an I-50 of about 20 ng/ml. The test is rapid and can be read either by eye or by an inexpensive spectrophotometer. Both the PCFIA and ELISA tests readily detect the presence of furosemide in equine blood for at least five hours after administration of the recommended therapeutic dose. The principal utility of these tests lies in the ability for rapid screening to determine whether or not a horse has been administered furosemide or whether or not permitted plasma concentrations have been exceeded. Pilot trials with these systems in Kentucky and Illinois suggest that these tests are economical and effective and can substitute for the currently used detention barn system of monitoring furosemide administration.

Supported by grants from the Kentucky State Racing and Kyle Harness Racing Commissions, the Kentucky Equine Drug Council, the American Horse Shows Association, I.D.S. Corp., the Oklahoma State Racing Commission and the U.K. Graduate Center for Toxicology.


Antibodies raised to fentanyl and sufentanil were incorporated and evaluated in immunoassays. The tests were designed to detect and monitor sufentanil administration in both equine and human subjects. The development of these immunoassays will allow for the detection of illegally administered sufentanil in either equine or human athletes, as well as an aid to clinicians with respect to the use of sufentanil as an analgesic in pre- and post-operative care. During the evaluation of RIA (Radioimmunoassay) and ELISA (Enzyme Linked Immunosorbent Assay), both tests were found to have an I-50 for sufentanil of about 1 ng/ml. These tests readily detect the presence of sufentanil and its metabolites in blood and urine after administration of sub-therapeutic doses. The antibodies evaluated also cross-react with fentanyl and its analogs, when introduced into routine screening, these immunoassay tests are capable of improving the quality and efficiency in both equine and human drug screening.

Supported by grants from the Kentucky State Racing and Kyle Harness Racing Commissions, the Kentucky Equine Drug Council, the American Horse Shows Association, I.D.S. Corp., the Oklahoma State Racing Commission and the U.K. Graduate Center for Toxicology.

1061  DETECTION AND QUANTITATION OF PHENYL BUTAZONE IN EQUINE BLOOD AND URINE BY PARTICLE CONCENTRATION FLUORESCENCE IMMUNOASSAY AND ELISA. T Tobin, S Krzatkowski, J McDonald, C A Frange, H H Tai, and D Watt. Sponsor: L Robertson.

We have developed Particle Concentration Fluorescence Immunoassay (PCFIA) and Enzyme Linked Immunosorbent Assay (ELISA) tests for phenylbutazone (PB) as part of a panel of immunoassay tests for drugs in racing horses. Since PB is legal in most American racing jurisdictions, the utility of this test is likely to be quantitation of plasma levels of PB. Our PCFIA test has an I-50 for PB of about 1 ug/ml, close to the widely used 5 ug/ml plasma limit for PB. Our ELISA test is essentially more sensitive to PB, with an I-50 for PB of about 10 ng/ml. Both tests are based on our anti-phenylbutazone antibody, which shows limited cross-reactivity with oxyphenbutazone and does not cross-react significantly with other acidic drugs used in horses. Quantitation of PB in equine blood and urine by these assays correlates well with GC/MS quantitation of PB. Both assays are sufficiently sensitive to detect the presence of PB or its metabolites in equine urine for up to 48 hours, and in equine plasma for up to 24 hours after therapeutic doses of PB.

Supported by grants from the Kentucky State Racing and Kyle Harness Racing Commissions, the Kentucky Equine Drug Council, the American Horse Shows Association, I.D.S. Corp., the Oklahoma State Racing Commission and the U.K. Graduate Center for Toxicology.

1063  COMPONENTS OF VARIATION IN RAT PLASMA AND ERYTHROCYTE CHOLINESTERASE RESULTS. M C Carakostas, M L Draton, L A Knight, M A Lardis. E L du Pont de Nemours & Co. Inc. Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE. Sponsor: D B Warrell

Physiologic and analytical factors may affect the interpretation of cholinesterase (CHE) results in safety assessment studies. Variability in rat plasma CHE results was primarily due to intra- and inter-animal factors. The analytical component was small indicating that repeat measurements on the same plasma sample are unnecessary. Increasing the number of rats sampled and the number of times each rat is analyzed will reduce the effects of high intra- and inter-animal variability. Analytical sources were responsible for most of the variability in erythrocyte CHE results. Intra-animal variation was also moderately large, but the inter-animal variation was small. The large intra/inter animal variance ratio suggests that inherent within animal variability could obscure significant treatment related effects. The large amount of analytical variation increases the difficulty of distinguishing true differences in erythrocyte CHE activity between groups of animals without a larger number of measurements per group. Increasing the number of times each rat is sampled and performing replicate analyses on each sample will help reduce unwanted variability in erythrocyte CHE results. Sampling a large number of animals from each treatment group appears necessary. This is a somewhat different situation from that encountered with plasma CHE results indicating the same experimental design is probably not appropriate for both these tests.
RECENT DEVELOPMENTS IN REDUCING AND REPLACING ANIMAL USE IN TOXICOLOGIC RESEARCH. S. C. Gad, G. D. Searle & Co., Skokie, IL.

Significant progress has been made in toxicology/safety assessment in both replacing animals with in vitro systems and in reducing the number of animals used. Review of annual reports of the numbers of animals used in testing in the U.S., U.K. and Japan shows a continuing reduction in the numbers for all species. Multiple in vitro systems have been developed for screening/testing for eye and skin irritation, skin sensitization, teratology and other endpoints, and a scientific consensus has been formed on requirements and process for validation.

Innovative designs have been developed for in vivo tests which reduce both the numbers and the pain and distress of animals used in testing. Progress and dialogue continue in modification of both U.S. and international requirements and guidelines for testing, and for defining an "approach" process for alternatives and innovations.

In summary, significant progress has been made across the entire spectrum of applying the "3 Rs" to animal use in toxicology. Careful consideration of the actual objectives behind such testing remains essential to future progress.

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The potential reproductive toxicity of BOA was evaluated using the continuous breeding protocol. BOA was given to male (M) and female (F) Swiss (CD-1) mice at concentrations of 0, 1000, 4500, or 9000 ppm in the feed. During 18 weeks of cohabitation and continuous access to BOA-diet, fertility was completely or partially impaired at 4500 or 4450 ppm, respectively. Among the litters at 4500 ppm, litter size and body weight (BW) were significantly reduced compared to controls. Males at 4500 ppm also showed decreased sperm motility and concentration and decreased testis weights. Reproductive parameters were unaffected at 1000 ppm BOA. A crossover mating trial of control and 4500 ppm groups confirmed the male as the affected sex: fertility values were: 0 ppm M x 0 ppm F, 74%; 4500 ppm M x 0 ppm F, 54%; 0 ppm M x 4500 ppm F, 65%. In a mating trial of control and 1000 ppm F1 offspring at 10 weeks of age, fertility was unaffected, but the mean BW of F2 pups was depressed in the BOA group. This study confirms that BOA is a reproductive toxicant in mice, primarily through an effect in the male. Supported by NIEHS/NTP Contract No. N01-ES-65141.

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Reproductive Assessment by Continuous Breeding (RACB) is a NTP protocol for reproduction toxicity testing, originally used in CD-1 mice. In order to extend the RACB design to CD rats, two variations of the RACB protocol were tested using ethylene glycol dimethyl ether as a positive chemical. Protocol I followed the standard mouse RACB design, with a continuous cohabitation allowing delivery of up to five litters; the last litter was weaned and used for F1 evaluation. In Protocol II, rats were separated at delivery of their second litter (L2) and this litter used for F1 evaluation. After weaning L2, the F0 rats were re-cohabited to allow delivery of up to three more litters. In both protocols, after all litters were delivered the F0 rats were used in a cross-over mating trial to determine sex affected by chemical treatment. Fertility and litter size of F0 control pairs declined over time using Protocol II, compared to Protocol I. Results of the F1 evaluations did not differ between the two protocols. Protocol I was chosen as the design for future rat studies.

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THE SENSITIVITY OF FERTILITY TO OTHER REPRODUCTIVE ENDPOINTS. L. E. Gray, J. S. Ostby, J. P. Perrell, R. Linder, R. Cooper, J. Goldman, G. Rhembein and R. Signorn, RBG, ERG, USEPA, RTP, NC.

The assessment of reproductive risk typically relies upon fertility studies using rodents. However, fertility is difficult to disrupt and more sensitive reproductive endpoints need to be identified. The present study compares fertility to other measures following oral administration of four compounds. Rats were dosed from weaning, through breeding and lactation with methoxychlor (M) (25, 50, 100, and 200 mg/kg/d), carbendazim (C) (50, 100, 200, and 400), dibutyl phthalate (DBP) (250, 500, and 1000), and Lindane (L) (5, 10, 20 and 30). For M, vaginal opening (25) and cyclicity (50) were altered at lower doses than those that blocked implantation. F1 females were less fecund at 50. For C, testicular (50) and teratologic (100) measures were also altered below doses that caused infertility. For DBP, fertility and testicular endpoints were affected at the same dose (500), but the fertile males, at 500, had smaller testes and reduced sperm counts. L-treated females were fertile but estrous cyclicity and neonatal viability were altered. These results provide a data base that can be used to identify sensitive reproductive endpoints and demonstrate that the utility of certain measures varies from compound to compound.
INTERSPECIES COMPARISON OF A/D RATIOS: Fugu nov. 1, CR Ritchie1, GP Daston2, JM Rogers2, and TD Sabourin2.
1Procter & Gamble, Cincinnati, OH, U.S. EPA, Research Triangle Park, NC, and Battelle-Columbus, OH.

The A/D ratio (Adult Toxicity / Developmental Stage Toxicity) has been suggested as constant across a wide phylogenetic range. This hypothesis is attractive as it would allow information generated rapidly on submammalian organisms to be used in decision making. As part of a larger study, we determined the A/D ratio for 14 chemicals with the fathead minnow, *Pimephales promelas*. Test compounds selected covered diverse chemical properties and toxic modes of action to develop a representative data base. Fathead minnow embryos and adults were exposed to test material dissolved in water for seven days. Embryo exposures were initiated immediately after fertilization. The A/D ratio was based on no observed effect levels, survival and terata for the embryo; and survival for the adult. A/D ratios ranged from 0.1 for 2-methoxyethanol to >1,000 for retinoic acid. Little agreement with A/D ratios for other species, including mice, were noted. Information obtained for the fathead minnow were not useful for extrapolation to other organisms. For a more complete discussion of A/D ratios and the data for this and other species, see the abstract by Daston et al.

INTERSPECIES COMPARISON OF A/D RATIOS: Drosophila: D Baines1, GP Daston2, JM Rogers2, TD Sabourin2 and DJ Versteeg1. 1Procter & Gamble, Cincinnati, OH, U.S. EPA, Research Triangle Park, NC, and Battelle-Columbus, OH.

The hypothesis has been stated that the ratio of a chemical's adult toxicity (A) to its developmental toxicity (D) is constant across animal species. The purpose of this study was to determine A/D ratios for a group of 14 chemicals in the fruit fly Drosophila melanogaster, and compare them to A/Ds for the same chemicals in mice, frogs and fish. Drosophila was chosen as a test species because it is in use in toxicology, and its development is well characterized. Chemicals were selected to represent a range of potencies and mechanisms of developmental toxicity. Larvae and adult flies were exposed to test agents via the medium which serves as food and substrate. Larvae were exposed from before hatching through pupation. These are the most relevant stages for comparison since the imaginal disks which form the adult are developing during this time. Developmental toxicity was assessed as larval/pupal death, developmental delay, or abnormalities. Adults were exposed for the same period of time as larvae, with death as the endpoint of toxicity. Of 14 agents tested, only 3 had A/D ratios within the same order of magnitude in all four species. Comparisons between Drosophila and mice only indicate that A/Ds were within an order of magnitude for B.
The ratio of adult developmental no observed adverse effect levels (NOAEL), "A/D", has been recommended as an index of developmental hazard. We have conducted a computer simulation study of statistical properties of A/D's determined in Segment II protocols, with a control and four doses at log2-spaced intervals. There were 500 replicate determinations of maternal and developmental NOAEL for all combinations of 7 maternal weight gain, 7 fetal weight, and 9 embryo/fetal death dose-responses, and 3 choices for the high dose for each combination. The responses, their variances, and the litter effect were selected to mimic those of CD-1 mice. The average A/D from any set of conditions agrees with what we would expect from the underlying dose-responses. However, the ratios of the A/D values that bracket at least 95% of the sampled A/D's from a set of conditions range from 2 to 32, with values of 4 and 8 common. Furthermore, even for sets of conditions with average A/D less than two, the percent change from control values at the NOAEL divided by a safety factor of 100 was often an order of magnitude greater for a developmental response than for the maternal response. These observations suggest that A/D be used with extreme caution for developmental hazard assessment.

Maternal and developmental toxicity of 14 compounds (aminopterin, bromodeoxyuridine, cadmium, caffeine, congo red, dinocap, dinoseb, 2-methoxyethanol [Nagno, et al., Toxicology 20:335-343, 1981], ethylenethiourea, epinephrine, mirex, phenytoin, all-trans retinoic acid and trypan blue) were assessed in CD-1 mice using a Segment II protocol. Pregnant mice were dosed by gavage or s.c. injection on gestation days 7-16. At least 3 dose levels and a control were used to establish maternal and developmental NOAELS and LOAELS for each compound. Dams were killed on day 18, fetuses were removed and weighed, and half were preserved for visceral examination and half for skeletal examination. Maternal endpoints were death, net weight gain during dosing and clinical signs; developmental endpoints were mortality, malformations and fetal weight. About half of the compounds had A/D ratios near 1, and the others ranged from 6 to 16. Fetal weight was the most sensitive endpoint of developmental toxicity for most of the compounds, while malformation without concomitant fetal weight effects was the most sensitive endpoint for only three compounds. For comparison to other species, see abstract by Daston, et al.

2-METHOXYPYRINOL (2-ME) TERATOGENICITY FOLLOWING BOLUS AND CONSTANT-RATE ADMINISTRATIONS IN MICE. D O Clarke and F Welsch. CIIT, Research Triangle Park, NC.

2-ME, an ethylene glycol ether used extensively in industry, is teratogenic in several animal species. CD-1 mouse embryos are maximally susceptible to 2-ME on gestation day (gd) 11 and display highly specific digit malformations. Oxidation of 2-ME to 2-methoxyacetic acid (2-NAA) is a prerequisite for induction of these anomalies and occurs rapidly in the pregnant dam following gavage with a teratogenic dose of 2-ME (250 mg/kg; 99% conversion by hr). Present studies using this regimen measured peak 2-NAA concentrations in the embryo of about 5.5 µmole/g after 1-2 hr. High levels persisted at 6 hr (24.0 µmole/g) and dropped to 1.0 µmole/g and about 0.5 µmole/g at 12, 24 and 24 hr post-administration. Since 2-NAA is cleared from the dam and embryo slowly we are investigating the effects of low doses of 2-ME administered over extended time periods. While a sc 250 mg/kg bolus dose of 2-ME produced abnormal digits in 92% of fetuses, studies using sc implanted osmotic minipumps show that 2-ME doses of 25 or 50 mg/kg/h delivered over 12 hr on gd 11 produce digit malformations in 25% or 75% of fetuses, respectively. These preliminary data suggest that extended exposure to 2-ME in the workplace may pose a potential teratogenic hazard.

2-Methoxyethanol (2-ME) is an ethylene glycol ether that is teratogenic in mice. The developmental toxicity of 2-ME is commonly attributed to its oxidative product, 2-methoxyacetic acid (2-NAA). Amino acids such as serine and glycine which provide precursors for purine and pyrimidine bases of DNA and RNA attenuate the paw dysmorphogenesis caused by 2-ME and 2-NAA. In vitro studies have shown that 2-NAA inhibits the conversion of sarcosine (S) to glycine. We found that a dose of 16.5 mmol S/kg given concomitantly with a teratogenic dose of 2-ME (3.3 mmol/kg) or after a 6 hr delay significantly decreased the incidence of digit malformations. Following administration of 2-ME, slight but nonsignificant increases in endogenous S concentrations were observed in embryos, yolk sacs and maternal serum (HPLC method). Although whole embryo S levels were not increased, regional and target tissue differences may still exist. S levels were also measured after dosing with 16.5 mmol S/kg and remained greatly elevated for at least 6 hr. The marked rise (100-fold after 6 hr) of S in embryos could increase glycine pools and enhance conversions for new DNA/RNA synthesis. This might explain the replacement of 2-ME damaged cells and the resultant attenuation of digit malformations by exogenous sarcosine.

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The purpose of the present study was to compare the teratogenicity of EGME in the rat following dermal application both as a pure compound and in aqueous solution. Two teratology studies were conducted simultaneously. In the first study, timed mated Fisher 344 rats were dermally exposed to neat EGME on days 7 through 16 of pregnancy at doses of 0, 150, 300, and 600 mg/kg/day applied dermally in five divided doses over an 8-hour period. In the second study, doses of 0, 37, 75, 150 and 300 mg/kg/day EGME in solution with water were administered over equivalent dosing intervals. C-sections were performed after 20 days of gestation. Maternal body weight, fetal body weight and viable fetuses per dam were significantly reduced, moreover, the post implantation losses per dam were found to be significantly increased. No abnormalities were observed in those dams receiving EGME in water versus dams receiving the neat compound. Examination of the fetal tissues revealed similar but significantly increased numbers of both visceral and skeletal abnormalities in fetuses from dams receiving EGME in water. It appears that water enhances the ability of EGME to penetrate the skin.

A retrospective comparison of results suggested that EGME, a commonly used test vehicle, might not be an appropriate agent to use in studies of the increased incidence of fetal malformations as compared with DW (Kimmel et al., Toxicologist 5, 185, 1985). DW or CO was given to timed pregnant CD-1 mice (10 ml/kg/day DW; 3 or 10 ml/kg/dy CO: -5/group) and CD rats (10 ml/kg/day DW or CO: -90/group) on gestational days (gd) 6-15. Animals were killed on gd 17 (mice) or gd 20 (rats). Live fetuses were examined for malformations. After treatment, maternal food consumption was depressed at 10 ml/kg CO vs. DW in mice, but no differences in other maternal or developmental parameters were observed. In rats, maternal food and water consumption were slightly reduced in the CO group vs. the DW group during the treatment period. No abnormalities of other maternal or developmental parameters were observed. Thus, in contrast to the retrospective study of pooled controls, the current study did not show a difference between CO and DW on fetal development when administered during organogenesis in amounts consistent with their use as vehicles in developmental toxicity studies. Supported by NTP/NIHES Contract No. N01-ES-55080.


ST-281, an antiarrhythmic agent, has been shown to induce a ventricular septal defect (VSD) in the fetus when administered orally to maternal rats during the organogenesis period of gestation. In the present study, factors affecting ST-281-induced VSD were examined. Experiment 1: To determine the critical period of VSD induction by ST-281, the agent at doses of 900 or 1800 mg/kg/day was administered orally to rats as single dose (9, 10, or 11 of gestation) or double (6-9, 10-11, or 11-12) or triple (6-9, 10-11, or 11-12) doses. The fetuses were examined by microscopic methods as GD 20. As a result, the most critical period was around GD 9 with the incidence of 16-40%. Most cases of VSD were isolated types located in the membranous septum. Experiment 2: To assess strain differences in susceptibility to induction of VSD, ST-281 was administered to rats of three strains: Jcl:Wistar, Jcl:SD and Crl:PHF 34 using the double dose regimen. Among the strains, SD and 239) was less sensitive than Wistar (18-39%) for induction of VSD, while F344 was more susceptible to the reproductive toxicity of ST-281. These data indicate that the effects of ST-281 are dependent on the gestational day of administration and on the strain of rats used.

DEVELOPMENTAL TOXICITY OF 2-ETHYLMETHANOL ACID (2-EGA) BY GAVAGE IN FISCHER 344 RATS AND NEW ZEALAND WHITE RABBITS. L M Fisher, R W Tyl, L J Fosnight, M F Kubena and M A Vrbanic, Bushey Run Research Center, Export, PA.

2-EGA (CAS No. 149-57-5) in certified corn oil was administered by gavage at 0, 100, 250 or 500 mg/kg/day [rats, 25/group, gestational day (gd) 6 through 15] or 0, 5, 25 or 125 mg/kg/day [rabbits, 15/group, gd 6 through 18]. Sacrifice was on gd 21 for rats and gd 29 for rabbits. In rats, maternal clinical signs were observed, liver weights were increased and fetal weights/litter were decreased at 500 mg/kg/day. There were no treatment-related fetal malformations. Fetotoxic variations, indicative of toxicity, were observed at 250 and 500 mg/kg/day. In rabbits, maternal toxicity was noted at 250 mg/kg/day: one doe died at 250 and 250 mg/kg/day, one abort at 125 mg/kg/day, and mean weight gain and food consumption were reduced at 250 mg/kg/day. There were no treatment-related fetal malformations or variations. The NOEL for maternal toxicity was 250 mg/kg/day in rats and 25 mg/kg/day in rabbits. The NOEL for developmental toxicity was 100 mg/kg/day in rats and at least 250 mg/kg/day in rabbits. Sponsored by the American Pharmaceutical Association, Ethylhexanoic Acid Program Panel.
DEVELOPMENTAL TOXICITY OF ETHYLHE GYCOL (EG) AEROSOL BY WHOLE-BODY EXPOSURE IN CD RATS AND CD-1 MICE. E W Ten, B Ballantine*, L C Fisher, D L. Halt, T A Savine, D L. Kitchens, I M Pritts and D E Dodd, Bushy Run Research Center, Export, PA and Union Carbide Corp.*, Danbury, CT.

EG (CAS No. 107-21-1) is teratogenic to rats and mice at 750-5000 mg/kg/day, po. To evaluate exposure to EG aerosol, a major mode of human exposure, pregnant rats and mice (25% species/group) were exposed to a respirable EG aerosol on gestational days (gd) 6-15, 6 hr/day, at target exposure concentrations of 0 (air), 150, 1000, or 2500 mg/m3 and sacrificed on gd 21 (rats) or gd 18 (mice). Rats had increased liver weight at 2500 mg/m3 Food and water consumption, body weight, weight gain, and the incidence of individual, pooled or total fetal malformations were unaffected by exposure. Reduced ossification in various fetal skeletal districts was seen at 1000 and 2500 mg/m3. In mice, maternal toxicity (reduced body weight and weight gain) and developmental toxicity (decreased viable implants and fetal weight/litter) were seen at 1000 and 2500 mg/m3. Malformations of the head, face and skeleton (like those seen after po dosing) were seen at 1000 and 2500 mg/m3. The maternal toxicity NOEL was 1000 mg/m3 for rats and 150 mg/m3 for mice; the developmental toxicity NOEL was 150 mg/m3 for both species. Analysis for EG on the fur of “satellite” animals indicated 65-95% (at 90-10% pulmonary retention of the inhaled aerosol) of the calculated total EG dose could have been from ingestion after grooming and or percutaneous absorption in both species. Subsequent work has shown that, in mice, nose-only exposure to the aerosol (2500 mg/m3) or cutaneous (0.1 ml/mouse) of EG produces essentially no teratogenicity. Exposure to large doses of EG via the GI tract appears to be the only route which results in teratogenicity in rodents.

MATERNAL AND FETAL TOXICITY OF NITROGEN ANALOGS. B M Francis, University of Illinois, Urbana, IL.

Seven analogs of nitrofen [CL] were compared for effects on liver to body weight ratios [L:BW] in adults; maternal and prenatal morbidity; perinatal mortality; and occurrence of malformations in surviving pups. L:BW increased after administration of 2,3,4,5-tetrachlorophenyl (tric1) 4'-nitrophenyl ether (4'N) [234-TCN], 2,3,5-tric1-4'N [235-TCN], 2,3-tric1-4'N [236-TCN], 2,4,5-tric1-4'N [245-TCN], 2-4,6-tric1-4'N [246-TCN], nitrofen [CL], 2,4-dibromophenyl-4'N [BR], and 2,4-difluorophenyl-4'N [FL] in the order: 245-TCN > BR > 235-TCN & 246-TCN > 236-TCN & 234-TCN > FL & CL, at 500 mg/kg/d. Litters/female were in the order: 236-TCN & BR < 246-TCN & 245-TCN < 235-TCN < FL < 234-TCN & 235-TCN, at 250 mg/kg/d; survival among liveborn pups was: 245-TCN < 236-TCN, CL & BR < 235-TCN < 234-TCN < 246-TCN, 345-TCN and FL at 100 mg/kg/d. All compounds decreased Harderian gland size; absent glands were seen at 10 mg/kg/d 245-TCN, 50 mg/kg/d CL & BR, and 100 mg/kg/d 234-TCN and 246-TCN.

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EVALUATION OF ESPERAMICIN (BMY-28175) FOR DEVELOPMENTAL TOXICITY IN RATS. D T Liao*, B A Burker*, D E Rodwell, Bristol-Myers Company, Syracuse, NY.

Esperamicin is a cytotoxic antineoplastic agent. This drug was administered intravenously at dose levels of 0.01, 0.05, 0.1, 0.2, and 0.20 micrograms/kg/day during the major period of organogenesis (gestation days 6-15) of rats. The vehicle control and each treated group consisted of 30 mated female rats. Dams were observed for clinical signs, body weight and food consumption and a Cesarean section was performed on gestation day 20. Fetuses were weighed, sexed, and examined for morphological alterations in development. Maternal survival was 100% in all groups. Uterine stasis, reduced food consumption and body weight gains were dose-related in all treated groups. Intrauterine survival was significantly reduced at the highest dose. Reduced fetal weight occurred in a dose-related pattern. Malformations of the primitive gut, urogenital system and the skeleton were observed in all esperamicin-treated groups. The incidence of fetal findings in the low dose group was lower than the other two dose groups.

PERINATAL AND POSTNATAL STUDY OF CEFEPIME (BMY-28142) IN RATS. M D Merseia, T J. Davidson*, J C Siglin1 and D E Rodwell, Bristol-Myers Life Sciences, Inc., Spencerville, OH and Bristol-Myers Co., Syracuse, NY.

Cefepime (BMY-28142) is a new semisynthetic cephalosporin with excellent broad spectrum antimicrobial activity. In the present study, the drug was formulated as a dihydrochloride salt blended with L-arginine at the approximate ratio of 1:0.72 by weight. Cefepime was administered twice daily by subcutaneous injection to 3 groups of 25 mated females rats from gestation day 16 through lactation day 20 at dose levels of 250, 500 and 1000 mg/kg/day. A concurrent control group received saline on a comparable regimen. Clinical signs, body weights and food consumption were measured during gestation and lactation. All females were allowed to deliver. F1 pups were weighed, observed and examined for developmental, functional and behavioral parameters. Selected F1 offspring were mated and allowed to deliver. Cefepime produced dose-dependent dermal irritation at the site of injection and a decrease in F0 body weight gain and food consumption from gestation days 16-20 at all levels tested. F1 pup body weights during lactation were slightly decreased at 1000 mg/kg/day and marginally decreased at 500 mg/kg/day. No treatment-related effects were observed in F0 parturition and lactation data, or in F1 litter size, viability, developmental and behavioral indices and reproductive capabilities.

Cefepime (BMY-28142) is a new semisynthetic cephalosporin with excellent broad spectrum antimicrobial activity. In the present study, the drug was formulated as a dihydrochloride salt blended with L-erythine at the approximate ratio of 1:0.72 by weight. The teratogenic potential of cefepime was evaluated in rats. Cefepime was administered twice daily by subcutaneous injection to 3 groups of 25 mated females from gestation days 6 through 17 at levels of 250, 500, and 1000 mg/kg/day. A control group received saline under similar experimental conditions. Clinical signs, body weights and food consumption were evaluated during gestation. Females were sacrificed on day 20 for necropsy and cesarean section. Uterine implantation sites were classified and viable fetuses were weighed and examined externally. One-half of the females were examined vaginally (Wilson's technique) and the remaining fetuses were examined skeletal ly. Cefepime produced dose-dependent dernal irritation at the site of injection at all levels tested. Maternal body weight gain and food intake were significantly decreased at 1000 mg/kg/day. Other than dernal irritation, no definitive maternal effects were observed at 250 and 500 mg/kg/day. No teratogenic or fetotoxic effects were produced at any level.

ORAL GENERAL REPRODUCTIVE STUDY OF CITRAL IN FEMALE RATS. A M Haberman, M S Christian, M B Bennett,[*] and T A Vollath.[*] Argus Research Laboratories, Inc., Horsham, PA and *Lorillard Inc., Greensboro, NC.

Citral, a fragrance and flavor additive, has been reported to affect reproductive performance in rats after in utero or maternal exposure. This study assessed the effects of oral administration of citral on reproduction, including ovarian function, estrous cycles, mating and fetal and pup development. Citral was given daily to rats (30/week) at dosages of 0 (corn oil), 50, 160 and 5000mg/kg/day for two weeks prior to mating and through day 20 of gestation. Approximately 1/2 of the rats were Caesarean-sectioned on day 20G while dosage of the remaining rats continued through parturition and a 21-day lactation period. Dosage-dependent incidences of mortality, adverse clinical signs and reductions in body weight gain and feed consumption occurred at the two highest dosages. The 50mg/kg/day dosage was an apparent maternal NOEL. No effects on estrous cycling, mating, fertility or gestation time were produced by any dosage of citral. The only reproducively-developmental effect was significantly (P<0.05) decreased pup body weight at birth in the 500 mg/kg/day dosage group. A slight but not significant decrease in fetal body weight also occurred. Based on these results, the NOEL in the offspring was greater than 50 mg/kg/day. In conclusion, citral did not affect reproductive performance or development of the offspring in the rat in the absence of significant maternal toxicity.

PLACENTAL PASSAGE AND MATERNAL-FETAL DISTRIBUTION OF PHENAZOPYRIDINE (PAP) IN THE RAT. A S Butler, B H Thomas and J H Moffatt. Bureau of Drug Research and Health Protection Branch, Ottawa, Canada.

PAP is a nonanalgesic drug used in the management of discomfort of genitourinary tract infections. Single doses of an aqueous solution of 100mg/kg (PAP) (100 mg/kg) were given orally to 20 pregnant rats. Dams were kept in individual metabolism cages where the blood levels of 14C and its excretion into urine and feces were followed up to 24 h. Radioactivity was detectable in tail blood at 5 min, peaked at 9 h and declined slowly after 12 h. Urine was the main channel of 14C excretion, and only 5% of the administered dose was recovered in feces during 24 h. At 24 h, 14C levels were highest in the maternal kidney, liver, adrenal gland and cecum, but lowest in the brain. The 14C-PAP derived radioactivity concentrations in amniotic fluid were 4.4 times greater than maternal plasma (6.3 mg/l), whereas the amounts of 14C in eviscerated fetus and placenta were similar to that of the maternal plasma. In comparison with the maternal tissues, the amounts of 14C were markedly higher in fetal fat and skeletal muscle, while the 14C content in fetal liver was significantly low compared to maternal liver. This study demonstrated that 14C-PAP and/or its metabolites traverse the placenta and cumulate preferentially in the amniotic fluid.


Citral is a fragrance and flavor additive used in the consumer products and food industries. Studies conducted on chick embryos have implicated citral as a possible teratogen. Pregnant Sprague-Dawley rats were exposed via inhalation to target citral concentrations of 0, 10, or 35 ppm as vapor, or 85 ppm as an aerosol/vapor mixture, for 6 h/day on gestational days 6 through 15. Preliminary studies showed that an aerosol/vapor mixture was necessary to produce maternal toxicity. Dams were sacrificed on day 20 and fetuses were evaluated for gross, visceral and skeletal malfornations. At the highest exposure level, dams showed significantly reduced (p<0.05) body weight gain and clinical signs of toxicity. No maternal toxicity was noted at the lower exposure levels. There were no exposure-related effects on the number of corpora lutea, implantations or resorptions. Additionally, fetal viability, litter size, sex ratio and fetal body weight were not adversely affected by exposure. No exposure-related fetal malformations were observed. The incidence of hypoplastic bones (lumbar and pubis) was increased slightly over controls, but only at the maternally toxic exposure level. Results of this study indicate that inhalation of citral at concentrations up to maternally toxic levels does not produce teratogenic effects in rats.
MULTIGENERATION STUDIES CONDUCTED AT DOSAGE LEVELS OF 0.05 UP TO 3.6 mg/kg/day indicated that the anthelmintic, ivermectin, was toxic to neonatal rats at doses > 0.4 mg/kg/day, as evidenced by increased postnatal pup mortality up to Day 10 postpartum and decreased pup weights in surviving offspring. A cross-fostering study showed that the neonatal toxicity was not related to in utero exposure but to postnatal exposure via maternal milk. Studies with radiolabeled ivermectin in rats demonstrated significantly higher brain and plasma drug residues in nursing offspring compared to dams. The period of enhanced neonatal sensitivity in rats correlates with increases in the plasma-brain ratios of total radioactivity in offspring from approximately 1 to 3 on Days 4 and 10 postpartum, respectively. To determine human neonatal exposure following a single therapeutic dose of 0.2 mg/kg to lactating women, analysis of milk samples revealed residues which would result in a maximum exposure of 3 µg/kg. A 2-week neonatal toxicity study in 1-week-old rhesus monkeys was conducted at doses up to 100 µg/kg/day. No treatment-related clinical or histological changes were found. These data are consistent with the postnatal completion of rats of the blood-brain barrier, which is formed prenatally in primates.

Recent reports have suggested that soluble nickel salts may affect development, and that this effect may increase with length of exposure and number of litters. In this study female Long Evans rats (FeO) drank NiCl₂ solutions (0.1, 10, 50, 250 ppm) for 11 weeks prior to mating, and then during mating and two successive gestation (G1, G2) and lactation (L1, L2) periods. Pups (F1 a and b) were observed until weaning; breeder males were unexposed. Dams drinking 250 ppm consumed less water than controls during all periods measured. Indices of reproductive performance (percent sperm positive and pregnant, litter size) were comparable among groups. Maternal weight gain was less than controls at 50 and 250 ppm during G1 only. The number of F1a pups dead at postnatal day (pnd) 1 was higher than controls at 250 ppm. F1b pup mortality at pnd 1 was higher than controls at all dose levels of NiCl₂, but the effect at 10 ppm was more pronounced than at 50 ppm. Numbers of pups dying during L1 and L2 were not different across groups. F1a male pup weight at pnd 1 was lower than controls at 250 ppm, and these pups showed a persistent weight decrement until weaning. There were no consistent differences in F1b pup weight across groups at pnd 1 and throughout L2. (Abstract does not necessarily reflect EPA policy).

AMELIORATION OF CADMIUM TERATOGENICITY BY DITHIOCARBAMATES. A Hatori, C.C. Willhite, R P Sharma, W B Howard and M M Jones. Toxicology Program, Utah State University, Logan, UT California Dept. of Health Services, Berkeley, CA and Dept. of Chemistry, Vanderbilt University, Nashville TN.

Dithiocarbamates (DTC) have been suggested as antidotes for acute and chronic cadmium poisoning. Sodium dithiocarbamates, N-benzyl-D-glucamine (I), N-di-(hydroxyethyl) (II), 4-carboxyamidopiperidine (III) and N-methyl-D-glucamine (IV), were used with cadmium doses that caused severe facial and limb malformations. Each DTC compound (2.2 mmol/kg) was given i.p. to hamsters 15 min (group A) or 24 h (group B) before i.v. injection of 10.9 mmol/kg of CdCl₂ at 10 a.m. on day 8 of gestation. On day 15, animals were sacrificed and fetuses were examined for gross external malformations. Treatment with IV alone showed significant increase in terata. All DTC in group A caused a significant decrease in resorptions compared to Cd alone. Abnormal fetuses ranged 0 to 6.9% after DTC protection compared with Cd-only group (97.5%). Fetal body weights in group A were equal to those of controls. In group B, there were no significant changes in the terata due to DTC treatment. Compound IV with Cd showed a higher resorption rate (94.7% vs 45.8% in Cd only). Results indicate that simultaneous treatment with chelators complexed cadmium and prevented its teratogenic effect in vivo. (Supported in part by NIH HD 21399 and ES 02538)

DEVELOPMENTAL EFFECTS ON MICE AFTER PRENATAL AND POSTNATAL EXPOSURE TO URANIUM. J L Domingo, A Ortega, J L Paternain, J M Llobera, and J Corbeila. Laboratory of Toxicology & Biochemistry, School of Medicine, E-43201. Reus. Spain.

There is no information on the developmental toxicity due to uranium ingestion. In the present study, the prenatal and postnatal effects of uranium have been investigated in mice. In a first experiment, pregnant Swiss mice were dosed daily p.o. with solutions of uranyl acetate dihydrate on gestational days 6 through 15, at doses of 0, 5, 10, 25 or 50 mg/kg. In a second experiment, perinatal and postnatal effects were studied in mice given uranyl acetate dihydrate, at daily doses of 0, 0.05, 0.5, 5 or 50 mg/kg from day 13 of pregnancy until weaning of the litters on day 21 postbirth. There was no evidence of embryolethality at any dose level. However, reduced fetal body weight as well as an increased incidence of abnormalities were observed at all dose levels. A significant decrease in the survival indices was observed at the 50 mg/kg/day test level. No other perinatal or postnatal effects were observed. The "No observable effect level" (NOEL) for maternal toxicity and for fetotoxicity including teratogenicity was below 5 mg/kg/day. The NOEL for health hazards on the developing pup was 5 mg/kg/day. However, comparison of these doses with the amounts of uranium usually ingested by men suggests a safety factor of approximately 1000.
Aluminum is consumed therapeutically in large quantities as antacid, or phosphate binder by patients with renal impairment. It is a well-documented fact that small quantities of aluminum are absorbed from a variety of orally administered aluminum compounds. To determine the potential fetotoxicity of Al(OH)₃ (the most consumed aluminum compound), pregnant Swiss mice were given a daily dose of 0, 67, 134 and 268 mg/kg on days 6-15 of gestation. Fetal examinations were performed on day 20. No maternal toxicity was observed during the exposure period. No treatment-related changes were recorded in the number of total implants, resorptions, the number of live and dead fetuses, fetal size parameters, or fetal sex distribution data. Examination of fetuses for external, visceral, and skeletal malformations revealed that Al(OH)₃ did not produce teratogenicity or significant fetotoxicity in mice at doses as high as 268 mg/kg/day. This amount of Al(OH)₃ is equivalent to that consumed by adult humans who ingest 4 g aluminum daily.

ACRL, a widely used industrial chemical with neurotoxic effects, was evaluated for developmental toxicity in male and female SD rats. Distilled water was administered once daily by gavage on gestational days (gd) 6-17 to mice (0, 3, 15, or 45 mg/kg) and on gd 6-20 to rats (0, 2.5, 7.5, or 15 mg/kg). At sacrifice (gd 17, mice; gd 20, rats) fetuses were examined for external, visceral, and skeletal malformations. Maternal toxicity during treatment was observed at the highest dose as reduced body weight gain in both species and hind-limb splaying in 48% of high-dose mice. Weight gain corrected for gravid uterine weight was also reduced in rats at 7.5 and 15 mg/kg/day. Whereas embryo/fetal toxicity was not observed in rats, fetal weight was reduced in mice administered 45 mg/kg/day. No increase in the incidence of malformations was observed in either species; however, the incidence of variations (predominantly extra rib) increased with dose. In summary, administration of ACRL during organogenesis produced maternal and developmental toxicity at 45 mg/kg/day in mice and maternal toxicity, but not developmental toxicity, at doses of 7.5 mg/kg/day in rats. Supported by NTP/NIEHS Contract N01-ES-55060.

Potassium diethylidithiocarbamate is commercially available as an industrial biocide. The product supplied contained 99% active ingredient. Twenty inseminated New Zealand White rabbits per group were gavaged with 25, 75 and 150 mg/kg/day of test articles for 13 consecutive days beginning on gestation day 6. Twenty-eight mated COBS/CD rats per group received 25, 150, and 400 mg/kg/day by gavage during gestation days 6-15. For both species, a concurrent control group received water. Females were observed for clinical signs. Body weight and food consumption were measured and Cesarean section was performed on day 20 (rats) and day 29 (rabbits). Fetuses were weighed, sexed and examined for morphological alterations. Dose-related maternal toxicity was observed in the mid- and high-dose groups for both species. Fetal toxicity occurred at the high-dose level in rats, and at the mid- and high-dose levels in rabbits. The toxicity at 150 mg/kg/day in rabbits proved excessive for a teratology study. A "no-effect level" of 25 mg/kg/day was established for both species.

Perinatal dose study of dibutyl phthalate in rats and mice. J Killinger, A Basaran, L Mezza, R Persing, A Peters, and R Melnick. Battelle, Columbus, OH and NIEHS, RTP, NC.

Dibutyl phthalate (DBP) was administered to pregnant F344 rats and CD57BL/6 mice in feed at concentrations ranging from 0 to 20,000 ppm. Designated weanlings were dosed for an additional 4 weeks. Gestation index in rats and fertility index in mice were reduced at 20,000 ppm. Gestation length was increased in mice. Live born pups and pup survival were lower in rats at 20,000 ppm, and mice at 7500 ppm and higher; rat pup survival at 10,000 ppm was also reduced on lactation day 4. During a 28-day lactation period, pup weight reductions were observed at 2500 ppm and higher in rats, and at 10,000 ppm on lactation day 0 in mice. Reductions in terminal body weights were observed for post-weanling female mice and males of both species. Organ weight changes for rats included increases in liver weights, and decreases in testis weights. Additional dams (rats) received up to 30,000 ppm of DBP or 42,000 ppm of diethylhexyl phthalate (DEHP) during lactation for evaluation of peroxisome proliferation. DEHP, but not DBP, caused increased hepatic palmitoyl-CoA oxidase activity in dams, while treatment with both compounds resulted in slight increases in activity at the high doses for suckling pups. (Supported by Contract No. N01-ES-75184 from NTP).
1096 DEVELOPMENTAL TOXICITY OF TETRAHYDROFURAN IN MICE AND RATS. TJ Mast, RL Rommelm, RJ Weigel, KH Stoney, BA Schwez* and RE Morrissey*. Pacific Northwest Laboratory, Richland, Washington. NIEHS/NTT. Research Triangle Park, NC.

The developmental toxicity of tetrahydrofuran (THF), a 4-carbon cyclic ether, was assessed in Sprague-Dawley rats and Swiss (CD-1) mice exposed to 0, 600, 1800, or 5000 ppm THF vapors, 6 h/day, 7 dy/wk, 6-17 days of gestation (mice; dg) and 6-19 dg (rats). A high purity vapor of THF in nitrogen (150,000 ppm) was generated using a rotary evaporator and delivered to the exposure chambers for further dilution. Mice exhibited varying degrees of narcosis at 1800- and 5000-ppm maternal deaths occurred at 5000-ppm. Sacrifice body weights were reduced for mice at 1800- and 5000-ppm (18 dg), and for rats at 5000-ppm (20 dg). The mean gravid uterine weights for mice were reduced (significant) at 1800- and 5000-ppm, and for rats at 5000-ppm (not significant). The extra-gestational weight gains were reduced for the mice at 1800- and 5000-ppm (significant) and for rats at 5000-ppm (not significant).

There were no effects on the number of implantations or on the fetal sex ratio in rats or mice. In mice, there was a reduction in the percent live pups/litter at 1800- and 5000-ppm with a corresponding increase in the percent resorptions/litter; rats were not affected. Surviving pregnant mice at 5000-ppm had litters with a 95% resorption incidence. Fetal rat weights were significantly reduced at 5000-ppm. There were no significant differences in the incidence of abnormalities for either rats or mice. Mice were more susceptible to THF than were the rats; however, if the conceptus survived, development continued in a normal fashion. Supported by NIEHS/NTT. Contract 1Y01ES70153.

1098 THE EFFECT OF PHENOBARBITAL (PB) ON THE METABOLISM AND EXCRETION OF THYROXINE IN RATS. R M McClain, A A Levin, R Posch, and J C Downing. Department of Toxicology and Pathology, Hoffmann-La Roche Inc., Nutley, NJ.

The effect of PB on thyroid function and the metabolism and biliary excretion of thyroxine was determined in rats. PB (100 mg/kg/day) administered for two weeks resulted in an increase in hepatic and thyroid gland weights, decreased circulating levels of T4, T3 and T3S and increased TSH levels in treated rats. In thyroidectomized rats the plasma clearance of T4 was increased with PB. In bile duct cannulated male PB-treated rats (4-8 weeks at 100 mg/kg) the hepatic uptake of 125I-T4 at 4 hours was markedly increased. Bile flow was increased and the 4 hr cumulative biliary excretion of administered radioactivity was increased by 48%. Most (75%) of this increase was accounted for by an increase in the excretion of T4-glucuronide in PB-treated rats. Hepatic T4-glucurononyltransferase (T4GT) activity in PB-treated rats expressed as pmol/min/mg protein was increased by 40%; enzyme activity per gm of liver was increased by about 2 fold which, when coupled with increased hepatic weight, resulted in about a 3 fold increase in total hepatic T4GT activity in PB-treated rats as compared to controls. It is concluded that the effect of PB on thyroid function in rats is primarily a result of its effects on the hepatic disposition of thyroid hormone. The induction of T4GT appears to play an important role in the increased metabolism and excretion of thyroxine in PB-treated rats.

1097 TWO GENERATION REPRODUCTION STUDY OF SULFUR MUSTARD IN RATS. L B Sasser, J A Cushing, R L Buschbom and J C Dacre. Pacific Northwest Laboratory, Richland, WA and U.S. Army Biomedical Research Development Laboratory, Ft. Detrick, Frederick, MD.

Comprehensive data is not available to evaluate the potential risk to reproduction from long-term exposure to sulfur mustard (HD), [bis(2-chloroethyl)sulfide]. Groups of rats (27 females and 20 males/group/generation) were gavaged with 0, 0.33, 0.1 or 0.4 mg/kg HD for 13 weeks prior to mating, and throughout gestation, parturition and lactation in a 42-week two-generation study. There were no significant effects on reproductive function or pregnancy outcome in either generation. Growth of adult F1 rats of both sexes was reduced by the 0.4 mg/kg exposure. Although not different at birth, growth of the 0.4 mg/kg F1 and F2 offspring was depressed during lactation. A dose-related lesion of the squamous epithelial mucosa of the forestomach was observed in both sexes. The lesion was characterized by thickening of the squamous mucosa with varying degrees of hyperkeratosis. Benign neoplasms of the forestomach were found in about 10% of the intermediate (8/94) and high (10/94) dose groups. The NOEL in this study was 0.03 mg/kg for toxicity and 0.4 mg/kg for reproductive effects. Supported by the U.S. Army Medical Research and Development Command Contract BAP4865 under a Related Services Agreement with the U.S. Department of Energy under Contract DE-AC06-76RLO 1830.

1099 DIFFERENTIAL EFFECTS OF DIETHYLSTILBESTROL AND ESTRADIOL-17β IN COMBINATION WITH TESTOSTERONE ON RAT PROSTATE LOBES. M C Bosland and P Oner, NYU Medical Center, New York, NY and Tufts University School of Medicine, Boston, MA.

The effect of combined treatment with testosterone (T) and estradiol-17β (E2) or diethylstilbestrol (DES) on weight, morphology, and metabolism of 3H-labeled 5α-dihydrotestosterone (DHT) and E2 was examined in dorsolateral (DLP) and ventral prostate (VP) of intact adult Nb rats.Compounds were administered for 16 weeks by Silastic implant. E2 and DES at approximate equi-estrogenic doses, and radioisotopic metabolism was measured in short-term organ culture. Both T+DES and T+DES caused weight increase and epithelial dysplasia in the DLP. In the VP, T+E2 caused mild weight increase and no morphologic changes, but T+DES caused dysplasia-like changes and no weight increase. Both treatments decreased metabolism of E2 and terminal hydroxylation of DHT metabolites in the DLP. In the VP, T+DES, but not T+E2, decreased E2 metabolism and hydroxylation reactions, and thus E2 and 5α-androstan-3β,17β-diol (3β-diol) accumulated. These data suggest that differential effects of these estrogens on T-supported rat prostate are determined by chemical structure and not by estrogen potency, and that induction of dysplasia in rat prostate by testosterone is related to accumulation of E2 and the putative estrogen agonist 3β-diol. (Supported by EPA Grant No. CR813481-01-1).
THE ALTERATION OF SERUM HORMONE LEVELS BY SINGLE AND REPEATED OTHER ANESTHESIA.
S. Roberts, T d’Elia, F Fournier and B Stoll, Dept of Preclinical Safety Assessment, Sandoz Research Institute, E Hanover, NJ.

The need to monitor serum hormones during chronic rodent toxicity studies on xenobiotics required a procedure that would allow daily sampling, obtain a large blood volume and minimally change serum hormones. Sampling from the retro-orbital plexus (ROP) and ether anesthesia were examined for their ability to influence serum hormone levels. Blood was obtained from the ROP of male Sprague-Dawley rats with and without ether anesthesia. Ether anesthesia produced significant increases in LH (65%), prolactin (58%) and ACTH (59%) while corticosterone was unchanged. When compared to hormone levels after decapitation, sampling from the ROP without ether anesthesia had no significant effects on serum hormones. Repeated sampling (Days 1, 11 and 22) from the ROP without ether anesthesia did not significantly alter serum hormones. Repeated ether anesthesia yielded similar changes in serum hormones as was observed with a single exposure. These data demonstrate that obtaining blood samples from the ROP without ether anesthesia can be repeatedly performed with no effects on serum hormones. As a result of the significant hormonal changes produced by ether anesthesia, it could seriously confound studies for which hormonal effects were suspected.

THYROID TOXICITY IN RATS TREATED WITH HISTAMINE RECEPTOR ANTAGONISTS- AN INSIGHT INTO THEIR MECHANISM OF ACTION. A Poole and R Jones, Smith Kline and French Research Ltd., The Frythe, Welwyn, Herts, UK. Sponsor: T Leonard.

Administration of SKF 93944 (H1 receptor antagonist) or SKF 93479 (H2 receptor antagonist) to Wistar rats caused thyroid lesions which were associated with reduced plasma T3 levels. These studies were designed to determine if changes in T3 clearance could account for these effects. Biliary clearance of 125I-T3 (measured for 5 hours after treatment) was increased in rats treated with SKF 93944 (300% of control) or SKF 93479 (250% of control) or phencobarbital (160% of control). Phenobarbital treatment primarily increased the biliary excretion of T3 as the glucuronide conjugate whilst treatment with the histamine antagonists enhanced T3 excretion in the free unconjugated form. Similar studies measuring T3 clearance showed that while phenobarbital treatment caused some slight increase neither of the SKF compounds had any effect in T3 hepatic clearance. None of the compounds appeared to have any effect on the renal clearance of T3, with only SKF 93479 producing a small increase in urinary T3 levels. In conclusion the thyroid lesions produced in rats following treatment with the SKF compounds appears to be associated with an increased hepatic clearance of T3 resulting in increased TSH drive to the thyroid.

INHIBITION OF CHOLESTEROL (CHOL) MOBILIZATION TO CYTOCHROME P-450CC IN TESTES OF 2,3,7,8-TETRA-CHLORODIBENZO-p-DIOXIN (TCDD)-TREATED RATS.
R W Moore and R E Peterson, School of Pharmacy and Env. Toxicol. Ctr., Univ. of Wis., Madison, WI.

Since testicular steroidogenesis is not impaired in TCDD-treated rats prior to cAMP formation or after pregnenolone formation, the two remaining possible mechanisms (reduced cytochrome P-450cc activity and defective substrate (CHOL) mobilization to this mitochondrial enzyme) were examined. Seven days after 100 μg TCDD/kg, maximum cytochrome P-450cc activity (assayed using an exogenous substrate, 20a-OH-CHOL) was 57% of control. The amount of endogenous CHOL readily available in mitochondria as substrate for this enzyme (reactive CHOL) was also measured. During the plateau phase of human chorionic gonadotropin (hcG)-stimulated testosterone secretion, reactive CHOL pools were lower in TCDD-treated rats at all hCG doses. Since substrate buildup was not observed, the reduction in cytochrome P-450cc activity is apparently not severe enough to inhibit steroidogenesis. Reactive CHOL pools were also measured in rats given a cytochrome P-450cc inhibitor (aminoglutethimide), then dosed with hcG and killed 0-40 min later. The rate of CHOL mobilization, which normally limits steroidogenesis, was greatly reduced by TCDD treatment. Together, the results indicate that the key TCDD-induced lesion in the testes is an impairment in the mobilization of CHOL to cytochrome P-450cc. In contrast the key lesion in the rat adrenal is inhibited cytochrome P-450cc activity. (NIH ES 01332)

2,3,7,8-TETRA-CHLORODIBENZO-p-DIOXIN (TCDD) TREATMENT DECREASES PITUITARY RESPONSIVENESS TO GONADOTROPIN-RELEASING HORMONE (GnRH) IN MALE RATS. R C Bookstaff, F Kame1*, R W Moore, and R E Peterson. School of Pharmacy and Dept. of Physiol.*, Univ. of Wisconsin, Madison, WI.

TCDD treatment decreases plasma testosterone (T) without the normal compensatory increase in plasma luteinizing hormone (LH). We now report that inappropriate LH secretion occurs within 1 day of dosing. This appears to be due to decreased pituitary responsiveness to GnRH stimulation, since TCDD-treated rats infused with exogenous GnRH have substantially lower plasma LH than control. Decreased GnRH responsiveness is not due to decreased synthesis of LH, since the pituitary content of LH remains unchanged. Rather, decreased LH secretion appears to be due to a decrease in pituitary GnRH receptor number. Scatchard analysis shows that the number of specific GnRH binding sites was reduced to 33% of control, with no change in Kd, when measured 7 days after 100 μg TCDD/kg. In addition, the dose-response curve for inappropriate plasma LH (ED50 15 μg TCDD/kg) is very similar to that for the decrease in the number of GnRH binding sites. Finally, in the absence of T, TCDD has little or no effect on either GnRH receptors or plasma LH. We conclude that the decrease in GnRH receptor number contributes to reduced pituitary responsiveness to GnRH, and that this decrease is at least part of the mechanism by which TCDD increases the potency of T as a feedback inhibitor of LH secretion. (NIH ES01332.)
Iodine has been proposed for use as a drinking water disinfectant in the manned space station. We have carried out studies to determine the relative effects of 127I and 129I on thyroid function in rats. In a subchronic study, male and female rats were treated with 1, 3, 10, or 100 ppm of 129I and 127I in drinking water for 100 days. Thyroid weights increased in a dose-related manner only in males treated with 129I. Conversely, I129/I127 ratios were increased in animals treated with 129I, but not with 127I. This suggests that 129I interferes with detoxification of 128I in peripheral tissues. In a separate study, male rats were maintained on 127I or 129I-treated drinking water for 7 days, then challenged with 125I-1 and sacrificed 1 hour later. Radioiodide uptake by the thyroid was depressed in a dose-related manner in both groups, but the effect was significantly greater in the 129I group. Depression of radioiodide uptake would explain increased thyroid weights seen with 129I and 127I. (Supported by NASA Grant No. NAG 95226).

DETTDA was administered in the diet to male and female rats for 90 days at 0, 50, 125 or 320 ppm. Dose-related alterations in a number of parameters were found. High dose animals experienced a high mortality rate and severe body weight loss. A high incidence of islet cell vacuolation and diffuse atrophy of the pancreatic acinar cells, bilateral cataractous change in the eyes, renal hydropic change, and atrophy and/or lymphoid depletion in several organs was detected in this group. A minimal to moderate degeneration of pancreatic acinar cells, but no effect on the islet cells, was present in rats in the mid and low dose groups. Based on light microscopy, the initial or primary effect of DETTDA appeared to occur in the pancreas. An apparent trend in the development of pancreatic lesions was observed beginning with focal involvement of acinar cells in the low dose male rats, progression of this change in mid dose male and female rats, and subsequent involvement of both pancreatic acinar and islet cells and other organs in male and female rats of the high dose group.

We previously showed that inhaled T-2 toxin causes adrenal cortical necrosis in female but not male mice. In this study we tested the effect of (1) pre-puberty and post-puberty castration of male mice, (2) reconstitution of castrated male mice with exogenous testosterone and (3) priming of female mice with exogenous testosterone on the induction of adrenal cortical necrosis from T-2 toxicosis. T-2 toxin caused adrenal cortical necrosis in all castrated mice regardless of time of castration. Reconstitution of both pre- and post-puberty castrated male mice with exogenous testosterone prevented the adrenal lesion. Priming female mice with exogenous testosterone also prevented the adrenal lesion in female mice. Since all of the mice exposed to T-2 toxin had a severe lymphocytolysis in the cortex of the thymus (used as a marker to confirm systemic myotoxicosis), we conclude that the role of testosterone in preventing adrenal cortical necrosis is a local effect.

Weanleison is known to exist that differ in their induction upon treatment with various agents such as phenobarbital (PB), pregnenolone-16α-carbonitrile (PCN), clofibric acid (CLO), and 3-methylcholanthrene (3MC). An induction of UDP-GT could increase the elimination of T4 leading to hypothyroxinemia. The purpose of this study was to determine if treatment with UDP-GT inducers can cause hypothyroxinemia with a subsequent stimulation of the thyroid gland. Male rats (250-275g) received PB (1200 ppm), PCN (1000 ppm), CLO (2500 ppm), or 3MC (250 ppm), in the feed for 21 days. On days 3, 7, 14 and 20, blood was collected and serum levels of free and total T4 and T3 were determined by radioimmunoassay. On day 21, following treatment with Na131I, the thyroid gland was removed, weighed and the amount of 131I present in the thyroid was determined. Serum free and total T4 levels were decreased (days 3-20) by all inducers except CLO. Total and free T3 levels were variably, but a general trend toward reduction was seen with PB and PCN. PCN and PB increased thyroid 131I uptake while PCN increased thyroid weight. Thyroid stimulation with PB and PCN, suggests the cause for hypothyroxinemia may not be due to a direct toxic effect on the thyroid gland, but rather an increased elimination of T4. Our results indicate that three of four UDP-GT inducers (PB, 3MC, and PCN), which each induce a specific form of UDP-GT, decrease serum levels of T4. (Supported by USPHS grants ES-03192 and ES-07079).
VASULAR TOXICITY OF ALLYLAMINE (AAM) IN AVIAN AND RODENT SPECIES. V Rameo, Texas Tech University Health Sciences Center, Lubbock, TX.

Subchronic exposure of several animal species to AAM (3-aminopropene) results in the formation of vascular lesions which resemble those found in atheroclerotic vessels. As the intrinsic susceptibility to atherosclerosis differs among several taxonomic groups, the present studies were conducted to compare the toxicity of AAM in the aortae of atherosclerosis-susceptible (Japanese quail) and -resistant (rats) animals. Japanese quails (125-135 g) and Sprague-Dawley rats (175-200 g) were gavaged daily for up to 21 days with AAM (0.7-70 mg/kg) or tap water. Quails were more susceptible than rats to the toxic effects of AAM in vivo as reflected by the survival rates and the severity of structural alterations at all times and doses tested. The occurrence of aortic lesions was not associated with alterations in total or free blood cholesterol levels. In contrast, AAM caused time- and dose-dependent fluctuations in the glutathione content of both avian and rodent vessels. Collectively, these results suggest that Japanese quails are more sensitive than Sprague-Dawley rats to the vascular toxicity of AAM.

PROTECTION AGAINST DOXORUBICIN INDUCED REACTIVE OXYGEN SPECIES BY RUTHENIUM RED AND FRUCTOSE 1,6-DIPHOSPHATE. E Chacon, A A Welder, J Swann, and D Acosta. The University of Texas, Austin, TX.

Doxorubicin (DOX) is an anthracene antibiotic with efficient antineoplastic activity. However, the clinical use of the drug is limited due to its serious cardiotoxic side effects. The cardiotoxicity is believed to be caused by reactive oxygen species generated as a result of potential redox cycling with the quinone moiety. The cardiotoxicity of DOX was assessed in a postnatal primary myocardial cell culture system by assaying for the release of the cytotoxic marker enzyme lactate dehydrogenase (LDH) and the formation of reactive oxygen species utilizing 2',7'-dichlorofluorescin diacetate. Both (2.5-5 mM) fructose 1,6-diphosphate and (4mM) ruthenium red protected against (25-50 uM) DOX induced LDH release. The emission of a fluorescent signal from 2',7'-dichlorofluorescein was monitored to assess the extent of formation of intracellular reactive oxygen species. DOX produced an increase in the fluorescent intensity relative to controls, whereas the concurrent administration of ruthenium red resulted in a significant decrease in the fluorescent intensity. Our data suggest that the reduction of reactive oxygen species may be due in part to the blocking of Ca++ ions through the mitochondrial electrophoretic unipporter by ruthenium red. Additionally, the protection observed by fructose 1,6-diphosphate may be attributed to an enhanced capacity of the hexose monophosphate shunt.

CARDIAC TOXICITY IN F-344 RATS FOLLOWING SUBCHRONIC EXPOSURE TO INHALED ALLYLAMINE (AA) VAPOR. D W Lynch, W J Noorman, T R Lewis, P Stober, R D Hamlin*, and R L Schuler**. MIOSH, Cincinnati, OH; *Dept. Vet. Physiol. and Pharmacol., Ohio State Univ., Columbus, OH; **Research Pathol. Assoc., Inc., Sykeville, MD.

Male and female F-344 rats were exposed at 0, 4, 40, or 80 ppm AA vapor, 6 hr/day, 5 days/wk, for up to 24 weeks in order to assess cardiac and other organ system toxicity. Scheduled sacrifices were conducted following 30, 60, 90 (80 ppm only), and 120 days of exposure. Body weight gain in rats of both sexes exposed to all three AA concentrations was statistically reduced compared to the controls throughout the 24 week period. Cardiac necrosis and fibrosis were found in 36/38 rats of both sexes sacrificed following exposure at 80 ppm AA for up to 90 days, compared to 3/38 control rats. Moderate cardiac necrosis was also found in 21/22 rats which died prior to termination of the 80 ppm AA exposures. Rats of both sexes exposed to the lower AA concentrations showed only a minimal amount of focal cardiac necrosis which was indistinguishable from controls. Heart/body weight ratios were significantly greater in rats of both sexes exposed at 80 ppm AA at all scheduled sacrifices, and in both sexes of rats exposed to 40 ppm AA for 120 days. In summary, histopathologic evidence of cardiac toxicity was confirmed only in rats exposed at 80 ppm AA.

OXIDATIVE CHANGES IN HYPOXIC-REOXYGENATED RAT MYOCARDIUM. Y Park and J P Kehrer. Division of Pharmacology and Toxicology, College of Pharmacy, The University of Texas at Austin, Austin, TX.

Reactive species of oxygen have been suggested to contribute to myocardial hypoxia and reoxygenation injury. Free radicals are generated at the moment oxygen returns to hypoxic or ischemic heart tissue, but it has not been established whether these radicals produce any tissue damage. We evaluated the possibility of oxidative injury in hypoxic and reoxygenated isolated-perfused rat heart. Glutathione (GSH) and glutathione disulfide (GSSG) were analyzed in the myocardium and coronary effluent. In addition, protein-GSH mixed-disulfides, protein bound sulfhydryls, protein carbonyl groups and vitamin E contents were measured in freeze-clamped heart tissue. Treatment groups included 1) 90 min oxygen (control), 2) 30 min oxygen followed by 60 min hypoxia, and 3) 30 min oxygen followed by 60 min hypoxia and a subsequent 4 min of reoxygenation. After 60 min hypoxia, GSH decreased from 886 to 507 and GSSG increased from 11 to 15 nmol/g wet wt. There was further decrease in GSH after 4 min reoxygenation while GSSG returned to control levels. Protein sulfhydryl content (~87 nmol GSH equivalents/mg protein) were unchanged with all treatments. Protein-GSH mixed disulfides increased from 0.23 to 0.45 nmol/mg with hypoxia. There was again no further change with reoxygenation. An identical pattern was also seen with protein carbonyl content. Cardiac α-tocopherol content was unchanged by any of the treatments. There was a small increase in GSH released into the coronary effluent during hypoxia and a massive increase in both GSH and GSSG release at reoxygenation which correlated with cell lysis and the release of intracellular enzymes. These data suggest that oxidative stress occurs during hypoxia when oxygen tensions are reduced to low (but not zero) levels. Few additional oxidative changes develop at reoxygenation. (JPK is the recipient of Research Career Development Award HL01435.)
Previous studies have shown that the acute in vivo cardiotoxicity of SKF 96079 is characterized by a decrease in contractility, heart rate as well as arrhythmias. The present study investigates the mechanism underlying the observed cardiotoxic response. Cardiac myocytes were enzymatically isolated from four adult rat hearts with a simultaneous perfusion technique, followed by gravity sedimentation. The purified preparation yielded 18.0 +/- 0.9 x 10^6 cells/ml, quiescent cells representing 73 +/- 1.2% of the total cell population. The cell suspension was equilibrated in a KREBS buffer, containing 0.1% BSA and either 4.5 (Basal) or 60mM (Activated) KCl. The suspension was pre-treated for 5 minutes with SKF 96079 (or vehicle) and calcium content was determined at 15 minutes after the addition of CaCl_2 (25mM/ml). Basal calcium uptake was reduced by 54% at a dose of 300mM SKF 96079. During activation, calcium content was significantly reduced at 10mM, 30mM, 100mM and 300mM SKF 96079, however, the inhibition was not dose dependent. These data suggest that voltage-dependent calcium uptake in the cardiac myocyte is sensitive to SKF 96079.

The previously reported cardiotoxicity of bemi- tradine, a triazolopyrimidine diuretic, was investigated in young female CD rats. We established that 400 and 700 mg/kg given twice daily (separated by 6 hr) for 3 days caused myocardial necrosis and inflammation in significant numbers of rats. This regimen (10 to 20 rats per treatment group with appropriate controls) was used in the 3 experiments described herein. 1) When rats were pretreated with phenobarbital (80 mg/kg ip for five days prior to the start of the bemitradine regimen) the incidence and severity of myocardial damage were decreased at 400 mg/kg bemitradine. 2) When rats were treated with propranolol (10 mg/kg po given 0.5 hour prior to each dose of bemitradine) there were decreases in bemitradine related lethality as well as the incidence and severity of myocardial damage. Hence, bemitradine induced cardiotoxicity may not be due to a metabolite, but may be directly mediated or controlled through the A2 adrenergic receptor.

Repeated exposure of rats to TCP produces diffuse, myocardial necrosis (MN). Cardiopathologic effects of TCP were further studied and possible hepatotoxic effects of TCP were examined. Male rats were orally gavaged with TCP in corn oil at varying doses and exposures (1-10 days). Pretreatment with 6-hydroxydopamine (6HD) was given ip with saline as control. Serum transaminases and histopathology of heart and liver were evaluated. Tissue TCP levels were analyzed. Acute TCP treatment at 1.6-2.8 mmole/kg resulted in increasing hepatotoxicity without MN. Repeated TCP exposures were restricted to 1 mmole/kg/day since larger doses were eventually lethal. Ten day exposure of rats (n=10) to 0.2, 0.4, 0.6 and 0.8 mmole TCP/kg/day produced a MN incidence of 0%, 0%, 10% and 80%, respectively, in absence of hepatotoxicity. Remaining work involved a cardiotoxic dose of 0.8 mmole/kg/day. MN was first observed after 6 days of TCP exposure. TCP tissue levels were markedly elevated after 10 days compared to 1 day exposure, suggesting bioaccumulation. Peripheral catecholamine depletion with 6HD reduced MN from moderate to minimal severity. Results of this study suggest that dose and time of tcp exposure determine the appearance of heart or liver toxicity. Further, cardiopathic effects of TCP may involve bioaccumulation and sympathoadrenergic factors. (Abstraction does not necessarily reflect EPA policy).
Tricyclic antidepressants (TCAs) are currently used in the treatment of mental depression and nocturnal enuresis. When given in therapeutic dosages, they can alter myocardial function, induce arrhythmias, and produce heart block in individuals with a normal cardiovascular history. The present study was undertaken to establish a culture system of spontaneously contracting adult primary myocardial cells for toxicological testing and to examine their contractility, morphology, and lactate dehydrogenase (LDH) release after treatment with the most cardiotoxic TCA, amitriptyline. Primary myocardial cell cultures were obtained from approximately 60-day-old Sprague-Dawley rats. After the cells had been grown in culture for 11 days, they were treated with amitriptyline (1 x 10^{-6} M and 1 x 10^{-5} M) for 2 to 24 hr. Some beating activity was still evident after 2 hr exposure of the cultures. Low level (LL) Pb may cause essential hypertension (EH). We examined the effect of Pb on blood pressure in rats. 0% Pb in drinking water on blood pressure (BP) & VR to norepinephrine (NE) & to a low molecular weight human endogenous plasma NKA inhibitor (EPI) in tail artery segments in paired SD rats. Compared to controls, HL rats at 1,3,6 & 9 mo. revealed no difference in BP. VR was markedly increased only at high doses of NE (6 & 9 mo. HL rats.) in contrast, 1 mo. LL rats showed a marked increase in VR at all doses of NE, despite an absence of BP change. However, BP increased significantly in 3 mo. LL rats without change in VR to NE but with increase in VR to EPI. Kinetic studies indicated Pb & EPI had a synergistic inhibitory effect on NKA. The results suggest that, at appropriate amounts, they may promote vascular contraction via inhibition of Na & Ca fluxes. The discrepancy between HL & LL remains to be explained.

The purpose of this study was to determine if differences in the cardiovascular response to vasodilators and vasoconstrictors exist in two strains of minipig. The study utilized five Hormel-Hanford and five Yucatan minipigs. The pigs were fitted with a catheter in the vena cava, then placed in a Panepistone sling. The physiological measurements made were heart rate, systolic and diastolic blood pressures, and mean arterial blood pressure. Three sessions were used to acclimate the animals to the sling. A fourth session involved I.V. administration of a vasodilator (angiotensin II), and a fifth session involved I.V. administration of a vasoconstricter (vasopressin). A rest period of several weeks was allowed between administrations of angiotensin and vasopressin. Angiotensin produced an immediate increase in blood pressure followed by a decrease and return to normal; the heart rate was unchanged in the Hormel-Hanford and slightly increased in the Yucatan strain. Vasopressin reduced the blood pressure slightly and elevated the heart rate in both strains of minipig. Since no statistically significant difference was evident in physiological parameters measured for the two strains, no advantage exists for using one strain of pig over the other for physiological measurements.
The cardiovascular effects of the gastric K⁺/H⁺ ATPase inhibitor, SK&F 96079, were evaluated. Cardiovascular parameters were assessed in the anesthetized rat, pithed rat and the isolated, working rat heart preparations. A 20 μmol/kg IV bolus injection of SK&F 96079 produced a hypertensive response of approximately 50 mmHg, a bradycardia of 186 bpm, and arrhythmias. The hypertensive response plateaued at about 40 sec. In the pithed rat, the hypertensive and bradycardic responses were retained indicating the lack of a central nervous system component. In addition, pretreatment with the α-adrenergic antagonist, phentolamine (10 mg/kg), did not alter the response to SK&F 96079. However, pretreatment with nifedipine (3 mg/kg, i.a.) in the anesthetized rat, abolished the hypertensive response, leaving the bradycardia and arrhythmias intact. In the isolated working rat heart preparation, SK&F 96079 (10 μM) reduced contractility by 42% and spontaneous rate by 38%. These data indicate that 1) the cardiovascular responses to SK&F 96079 observed in the rat are the result of a direct effect on the heart and vasculature, and 2) the depressor response to SK&F 96079 is mediated by a calcium dependent mechanism in the vasculature.

SAFETY ASSESSMENT OF WIN 54,177-4: AN ANTIARRHYTHMIC WITH A UNIQUE PHARMACOLOGIC PROFILE. D A Mayes, T A Barboit, A M Ezrin, D Rosi, C C Eames, D J Bradley and Y Greener. Sterling-Winthrop Research Institute, Rensselaer, N.Y.

Win 54,177-4 (N-(13-Diethylamino)propyl)-4,5-diphenyl-1H-pyrazol-1-acetimidazo is a potent antiarrhythmic compound that possesses activity against both simple spontaneous ectopic arrhythmias and complex reentrant arrhythmias. Win 54,177-4 is an orally active antiarrhythmic agent which prolongs ventricular refractoriness (Ezrin et al., FASEB 1988). One-month oral toxicity studies in rats and dogs have been completed. Sprague-Dawley rats (12/sex/group) administered dosages of 0 (water), 20, 80 and 320 mg base/kg exhibited toxic signs at the two higher dosages which included hypersalivation, decreased body weight and food consumption, increased relative liver weight, duodenal villous atrophy and hepatic centrolobular hypertrophy. Male rats were most severely affected. Beagle dogs (4/sex/group) were administered dosages of 0 (water), 3, 10, and 30 mg base/kg. The principal effects, hypersalivation and emesis, were frequent only at the high dosage where plasma levels were 7-fold greater than required for therapeutic efficacy in dogs. Electrocardiographic evaluation of all dogs during the study did not indicate any abnormalities attributable to Win 54,177-4. These studies indicated that therapeutically relevant dosages are well-tolerated and may offer an improved therapeutic index 2-7 times greater than currently available drugs with similar indications.

SUBCHRONIC TOXICITY STUDY OF A COMBINATION OF Terazosin and Enduron® IN DOGS. C L Yang, S Tekef, P Cusick, and R Patterson. Drug Safety Evaluation Division, Abbott Laboratories, Abbott Park, IL.

Terazosin is an alpha-adrenergic blocking antihypertensive drug candidate. Enduron® is a currently marketed diuretic antihypertensive agent. A six-month toxicity study was performed in which Terazosin and Enduron® mixture was administered orally to dogs at dosage levels of 0.6/0.3, 3/1.5, 17/8.5 and 34/17 mg/kg/day. The lowest dose was approximately three times the maximum intended clinical dose of 10/5 mg/50 kg person/day. Blood samples collected at 2 and 24 hours after dosing indicated serum drug concentrations were approximately proportional to the dosage administered. Dose-related prostatic and relaxed third eyelid were observed in all drug-treated groups. Decreased activity, dehydration and emesis occurred in dogs at 17/8.5 and 34/17 mg/kg/day. Decreased serum potassium levels and sinus tachycardia occurred in dogs at 34/17 mg/kg/day. These signs were considered to result from pharmacologic effects of the test compounds. No treatment-related morphologic lesions were observed. No significant changes were seen in body weight. Therefore, a dosage of 34/17 mg/kg/day for a combination of Terazosin and Enduron® was considered to be the no-toxic-effect dose in this study.

CARDIOVASCULAR EFFECTS OF EXCITATORY AMINO ACIDS (EAA) IN RATS AT PROCONVULSIVE AND BEHAVIORALLY ACTIVE DOSES. T J Shetler, D L Moellin, D R Helton, and P D Williams. Toxicology Division, Lilly Research Laboratories, Eli Lilly & Company, Greenfield, IN.

The potential cardiovascular (CV) effects of N-methyl-d-aspartic acid (NMDA) and L-glutamic acid (GLU) were evaluated in the conscious rat at doses which result in both proconvulsive and proconvulsive changes in mice (Pharmacologist 1988: Abstract #107). The putative NMDA antagonist 2-amino-4-phosphonobutyric acid (AP-4) was also examined at a dose which antagonizes these behaviors. NMDA (3.125, 12.5, and 25 mg/kg), GLU (500, 1000, and 2000 mg/kg), and AP-4 (3.125 and 12.5 mg/kg) were administered intraperitoneally. CV measurements (heart rate (HR) and arterial blood pressure, (ABP)) were obtained by an indwelling catheter placed in the femoral artery of male, Sprague-Dawley rats. No significant changes in HR and ABP were seen as a result of GLU or AP-4 administration. NMDA produced a significant reduction in HR and a slight decrease in ABP when compared to pre-dose values at 25 mg/kg. These data indicate that behavioral data are not likely to be confounded by non-selective peripheral CV effects of EAA since NMDA, GLU, and AP-4 did not significantly alter CV function at doses which disrupt normal CNS activity.
CROSSLINGING OF PROTEINS IN VITRO BY REACTION WITH TRANS,TRANS-MUCONALDEHYDE, A RING-OPENED METABOLITE OF BENZENE. V B Mylavarapu, S Madra, B D Goldstein, and C Witz. UMDNJ-Robert Wood Johnson Medical School, Piscataway, N.J.

Trans,trans-muconaldehyde (MUC) is an alpha,beta-unsaturated six carbon diene dialdehyde identified in our laboratory as a microsomal hematoxic metabolite of benzene. In its structure, MUC may be able to exert its toxicity in part by crosslinking proteins, nucleic acids and other cellular constituents via Schiff-base formation involving the two aldehyde functional groups and/or Michael addition reactions at the two activated double bonds. The ability of MUC to react with amino groups of proteins to form crosslinked products was investigated by incubating lysine-rich histones (10^{-4} M) with MUC (10^{-5} - 5x10^{-3} M) for 1.4 and 24 hr at 37 °C. The reaction of MUC with histone amino groups exhibited complex concentration and time relationships. At 10^{-4} M MUC and 24 hr incubation and 10^{-3} M MUC and 1 hr incubation, there was a 38% and 33% decrease in available amino groups, respectively, as measured by the fluorescamine assay. All histone samples incubated with MUC exhibited crosslinking as determined by SDS polyacrylamide gel electrophoresis. The chemical nature of the observed crosslinking and the role of crosslinking in general in the mechanism of MUC toxicity remain to be elucidated. This work is supported by NIH grant #ES02559.

RELATIONSHIP BETWEEN BENZENE TOXICITY AND URINARY MUCONIC ACID LEVELS. P Hu and R Snyder. Joint Graduate Program in Toxicology, Rutgers University and UMDNJ/Robert Wood Johnson Medical School, Piscataway, NJ

Benzene toxicity was measured by the [59Fe] uptake method of Lee et al. (Env. Hlth. Persp.28, 1981) and the appearance of muconic acid in the urine was measured by the method of Gad-F1 Karim (Xenobiotica 15,221,1985). A linear relationship was demonstrated between the depression of [59Fe] uptake and the appearance of muconic acid in the urine of benzene treated Swiss Webster female mice (single dose of 88, 440, 880, 2200 mg/kg, sc). In mice given a combination of phenol (100 mg/kg) and hydroquinone (100 mg/kg), ip, in three doses of 59 36 hours after each dose, a depression in [59Fe] uptake was observed without an increase in muconic acid in urine. Instead a new urinary metabolite was observed which has yet to be identified.

In animals given the benzene doses cited above coadministered with toluene at 175, 800, 1750, and 4400 mg/kg, respectively, benzene toxicity and muconic acid production was reduced in the three higher dose ranges but not in the lowest dose range. (Supported by ES02931)

HYDROQUINONE METABOLISM IN HUMAN MYELOPEROXIDASE IS STIMULATED BY PHENOL AND SEVERAL OTHER COMPOUNDS. K L Steinmetz, D A Eastmond and M T Smith. School of Public Health, University of California, Berkeley, CA.

Phenol and hydroquinone are two principal metabolites of benzene. The repeated co-administration of these two metabolites to B6C3F1 mice produces myelotoxicity similar to that caused by benzene (Tox. Appl. Pharmac. 91, 85, 1987). We have previously demonstrated that phenol will stimulate the HO2-dependent oxidation of hydroquinone to the highly toxic p-benzoquinone catalyzed by horseradish peroxidase. Here we report that relatively low concentrations of phenol (20-100 μM) will enhance up to 4-fold the metabolism of hydroquinone (1-10 μM) by purified human myeloperoxidase (MPO), the major peroxidase enzyme present in the blood and bone marrow. This phenol-induced stimulation shows a clear dose-response and an apparent threshold. Several other phenolic and amine compounds, including aniline and guaiacol, have also been found to stimulate the MPO-mediated metabolism of hydroquinone. The potentiation of MPO-mediated hydroquinone oxidation by phenolic and amine compounds could therefore play a role in both hydroquinone and benzene toxicity and carcinogenesis. Moreover, interactions between phenolic and amine compounds during peroxidase-catalyzed activation could have important toxicological implications.

Supported by NIH grant P42 ES04705 and the University of California Toxic Substances Program.

HEMOLLOBIN ADDUCTS REFLECT EXPOSURE NUT NOT TOXICITY IN RODENTS TREATED WITH BUTADIENE, ISOPRENE, OR BENZENE. R P Henderson, J D San, A R Dehl, P J Sebourin, L A Bond, G Lucier*, and L S Hinrichsen. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM and NIH, ETS, NC.

Hemoglobin (Hb) adducts have been proposed as markers of exposure. Such adducts might also reflect the blood levels of reactive metabolites, and therefore, the potential toxicity of the compounds in studies with 14C-1,3-butadiene and 14C-isoprene the formation of Hb-adducts was found to be linearly related to the i.p. injected dose up to 100 μmol/kg (butadiene) or 500 μmol/kg (isoprene) in F344 rats and B6C3F1 mice. The efficiency of Hb-adduct formation (adducts formed per unit of retained dose) from butadiene was greater in the rats than in the mice (0.407±0.019 vs. 0.177±0.007 μmol adduct/mg globin)/(μmol/kg retained dose). This is in contrast to the higher concentration of reactive epoxide metabolites reported in the blood of butadiene-exposed B6C3F1 mice versus F344 rats and the greater sensitivity of the mice in long-term bioassay studies. Efficiency of formation of Hb-adducts from isoprene-exposed rats was lower than that from butadiene-exposed rats, despite earlier studies showing 2 to 6 times higher blood levels of reactive metabolites in isoprene- versus butadiene-exposed rats. Hb-adduct formation was equally efficient in rats and mice exposed to 14C-benzene, despite the fact that mice metabolized more of the benzene to reactive metabolites than rats, and mice were more sensitive to benzene toxicity in bioassay tests. Based on these data, Hb-adducts appear to have potential for markers of exposure, but not as markers of toxicity. (Supported by NIH through IAA ES-20002, U.S. DOE/GER contract No. DE-AC04-76EV01013.)
EFFECT OF REPEATED BENZENE INHALATION EXPOSURES ON SUBSEQUENT METABOLISM OF BENZENE. P. J. Sabourin, J. D. Sutphen, L. S. Birnbaum, G. Lucier, and K. E. Henderson. Lawrence Radiation Laboratory, University of California, Livermore, CA.

Benzene is a known human leukemogen and animal carcinogen. To aid in risk assessments for benzene exposure, one must determine if repeated benzene inhalation exposures would affect the metabolism of benzene. F344 rats and B6C3F1 mice were exposed nose-only to 600 ppm benzene or air (control) for 6 hr/day, 5 days/ wk for 3 weeks. On the last day, benzene pre-treated and control animals were exposed to 600 ppm [14]Cbenzene for 6 hr. Results indicated that benzene pre-treatment did not significantly affect respiratory parameters or total metabolites in 24 hr urine samples.

Analysis of individual metabolites by HPLC indicated that benzene pre-treatment had no effect on the metabolite profiles except for an increase in hydroquinone glucuronide (HQG) in rat urine and a slight shift from glucuronidation to sulfation in mouse urine. Benzene pre-treatment had no effect on formation of 14C-labeled hemoglobin adducts from [14]Cbenzene in either species. Interestingly, mice and rats had similar levels of hemoglobin adduct binding despite the much higher metabolism of benzene by mice. These results indicate that repeated exposures at 600 ppm do not affect the total metabolism of benzene. However, conversion of benzene to specific metabolites (i.e., HQG in rats) may be induced by repeated exposures to 600 ppm benzene. The risk to man from repeated use will depend upon both the metabolic pathways of benzene in man as well as the degree of induction of specific pathways at the low exposure concentrations likely to be encountered in the work place.

LIVER SLICES: AN IN VITRO SYSTEM FOR THE STUDY OF SPECIES DIFFERENCES IN HEPATIC DRUG METABOLISM. J. Barr, M. Shalla, K. Brendel, and L. G. Sipos. Dept. of Pharmacology & Toxicology, College of Pharmacy, Univ. of Arizona, Tucson, AZ.

The oxidative o-deethylation of 7-ethoxycoumarin (7-EC) to 7-hydroxycoumarin (7-HC) and the subsequent conjugation with glucuronic acid or sulfate has been studied using liver slices (300 μm thick, 1 cm diameter) from different species. Hepatic tissue from F-344 rats, dogs (beagles and greyhounds), pigs and humans was used. Liver slices were incubated for 6 hours with 25 μM 7-EC (a non-cytotoxic concentration) and the media analyzed for the presence of 7-EC and of the sulfate and glucuronide conjugates. The relative activity of the liver slices to o-deethylation of 7-EC was 13, 5, 2 and 1 nmol 7-EC/hr for dog, rat, pig and human, respectively. Retention of the integrated phase I and phase II metabolism was maintained. The ratio of the sulfate to glucuronide conjugates of 7-EC determined were 5-7, 2-7, 9-6 and 8-6 for rat, dog, pig and human, respectively. The preferential formation of the sulfate conjugate was observed in rat liver slices incubated in the presence of a wide range of 7-EC concentrations (25-400 μM). These results predict the in vivo situation, where the urinary metabolites over a 24 hr period after oral doses of 2.25, or 250 mg/Kg 7-EC were >80% sulfate conjugates. The results suggest that liver slices may be applied to the study of species differences in the hepatic metabolism of xenobiotics. The liver slice system may be useful in predicting whole animal metabolism for compounds subject to hepatic biotransformation. (Supported by NIH No. 1RO1-ES-55112)

THE USE OF HUMAN CRYOPRESERVED PRECISION-CUT LIVER SLICES IN HEPATOTOXICITY STUDIES. R. L. Fisher, A. J. Gandolfi, C. L. Krumbieck, and K. Brendel. Departments of Pharmacology, U of Arizona, Tucson, AZ and Department of Nutrition Science, U of Alabama, Birmingham, AL.

Precision-cut liver slices from human organ donors were cryopreserved in fetal calf serum containing 10% dimethyl sulfoxide by lowering the temperature -1°C/min to -70°C and then at >1000°C/min to -196°C. Slices were stored in liquid nitrogen for one day and then thawed in fetal calf serum at a rate of approximately +10,000°C/min to 37°C. Fresh and cryopreserved slices were compared with regard to their ability to respond to allyl alcohol intoxication during 8 hr in culture. Viability parameters measured were K+ retention and protein synthesis. The relative toxicity for cryopreserved and fresh slices at the different allyl alcohol concentrations was similar for both parameters. For example in one experiment 125 μM allyl alcohol caused an inhibition of K+ retention by 28% in fresh slices as compared to 30% in cryopreserved slices. The analogous numbers for 250 μM allyl alcohol are 40% and 47% respectively. The ability to cryopreserve human liver slices will facilitate the potential use in a broad spectrum of human in vitro hepatotoxicity studies. (Supported by NIH GM 38290)


The effects of storage at -196°C on the biotransformation of 7-hydroxycoumarin, 7-ethoxycoumarin, and phenacetin by cryopreserved human precision cut liver slices in culture was examined. Human liver slices were cryopreserved by the method of vitrification and stored for 4 and 8 wk in liquid nitrogen. After rapid thawing the slices were cultured up to 6 hr with a non-toxic concentration of the test compound. The culture medium of the cryopreserved slices was analyzed for metabolite production and parent substrate disappearance and compared to the biotransformation rate of the fresh liver slices at the time of acquisition. Slices stored for 4 and 8 wk retained at least 70% of their biotransformation capability. More importantly, time of storage seems not to have any influence on the recovery of metabolic activity. Also phase I and II reactions remained coupled upon storage. Thus human liver slices can be successfully cryopreserved and stored for subsequent drug screening and metabolism experiments. Future work will investigate long term storage and the effects of toxicants on vitrified human liver slices. (NIH NOES 55112)

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We have reported the use of precision cut liver slices for xenobiotic metabolism experimentation. The availability of human tissue is restricted to samples obtained during surgery, non-transplanted organ donations, and potentially from puncture biopsies. The use of the extremely small amounts of tissue typically available from human liver puncture biopsies requires scaling down the present methodology to work with slices cut from tissue plugs 1 - 2 mm in diameter. We have developed equipment which makes both cutting and organ culture of hepatic microslices possible. This methodology also enables isolation of microslices from specific regions of the liver (i.e. perportal and pericentral). We report biotransformation experiments with 7-ethoxycoumarin, pentoxyresorufin, and the fluorinated compounds from isolated microslices taken from phenobarbital and B-naphthoflavone induced rats and non - pretreated domestic pigs. Microslices were exposed for several hours in culture to the test compounds and metabolism reflecting homogenous and heterogenous (i.e. regio specific) tissue distribution could be clearly demonstrated. Linear increases of metabolites over time were observed in most experiments. (NIH GM 38290).

BIOTRANSFORMATION OF HALOTHANE IN GUINEA PIG LIVER SLICES. J Fernado, RN Ghantous, A J Gandolfi, K Brendel, Dept Anesthesiology, University of Arizona, Tucson, AZ.

The role that biotransformation plays in the hepatotoxicity of halothane in guinea pigs is unclear. Studies with liver slices allows one to study this interaction. Hartley male guinea pig liver slices (250 - 300 μm) were incubated in sealed roller vials containing Krebs-Henseleit buffer (vitamins, amino acids, glutamine) at 37°C under different O₂ tensions (2.5 - 95%). After a 1 hr pre-incubation, halothane was vaporized in the vial producing a 1.9 μM media concentration. Halothane metabolites [Br-, trifluoroacetic acid (TFA), F⁻] were measured at 2.4, and 6 hr. Decreasing O₂ tensions (95 + 2.5%) caused a decrease in intracellular K⁺ (48%). Regardless, the production of metabolites was linear over the first 4 hr. Halothane was more readily defluorinated (15% + 2.7 pmoles/mg/hr) at low O₂ tension (2.5%). Dehalogenation increased with increasing O₂ tension to undetectable levels under 95% O₂. Production of the oxidative metabolite, TFA, was highest at 95% O₂ (193.2 + 30.1 pmoles/mg/hr). TFA production decrease with decreasing O₂ tension. Br⁻ production, reflecting total halothane metabolism, remained constant. Since guinea pig liver slices can biotransform halothane (oxidative/reductive), this system appears suitable for correlating the role of biotransformation in halothane hepatotoxicity. (NIH DK 16715)

COMPARISON OF THE METABOLISM OF AMPHETAMINE IN FREELY ISOLATED AND CRYOPRESERVED HEPATOCYTES FROM SEVERAL SPECIES. J J Lorettz, A P Li, R D Holm, and A G E Wilson. Monsanto Environmental Health Laboratory, St. Louis, MO.

In this study, freshly isolated and cryopreserved hepatocytes from rat, rabbit, Rhesus monkey, and human have been used to study the metabolism of amphetamine. In good agreement with reported in vivo differences, the major amphetamine metabolite found in rat hepatocytes was p-hydroxymethamphetamine (pHA), while in rabbit hepatocytes the predominant metabolite was benzoyl acid (BA). Human and Rhesus monkey hepatocytes formed approximately 2-3 times as much BA as pHA. The rate of metabolism of amphetamine was fastest in rabbit, followed in decreasing order by rat, monkey, and human. Following a 4 hour incubation with 10μm amphetamine, the percent of amphetamine remaining in each culture was rabbit, 0%; rat, 65%; and monkey, 89%. After a 24 hour incubation, the amount of unchanged amphetamine in human cultures was 84%. The metabolism of amphetamine by cryopreserved rat and monkey hepatocytes was compared to metabolism in freshly isolated cells. The profile of amphetamine metabolites and the rate of formation did not change significantly following cryopreservation. The ability to cryopreserve intact hepatocytes with retention of metabolic activity will be useful in providing a continuous supply of hepatocytes from human or other species for which sources of liver tissue are limited. Cryopreservation will also help overcome the limitation imposed by the time-dependent decline in drug metabolism enzyme activity in cultured hepatocytes.

COMPARISON OF PAPAVERINE METABOLISM IN CULTURE SYSTEMS OF RAT HEPATOCYTES AND FUNGAL CELLS. D Acosta, G C Hsieh, J C Davila, G D Reddy, and P J Davis. College of Pharmacy, University of Texas, Austin, TX.

To evaluate the suitability and relevancy of fungal cells as alternative testing systems for investigating the metabolism and toxicity of xenobiotics, cell suspensions of the fungus Cunninghamella echinulata and primary cultures of rat hepatocytes were used to compare the biotransformation of papaverine, a widely-used smooth muscle relaxant and a reputed hepatotoxin. Both cell types effectively transformed this compound into 6-desmethyl metabolites, but papaverine was metabolized at a slower rate by the hepatocyte cultures when compared to the fungal cells. The major metabolites were characterized by comparison of their chromatographic, mass, and NMR-spectral properties. The major organic solvent-extractable compounds found in the culture media of the hepatocyte cultures were 4'- and 6-desmethylpapaverine, plus the parent compound, papaverine. Smaller amounts of these compounds were extracted from the cells. The metabolism of papaverine was similar in the fungal cell suspensions, although an additional 3'-desmethyl metabolite was found. These results suggest that fungal cells may prove to be useful models for metabolism and toxicity studies of hepatotoxic compounds.
1136 SPECIES DIFFERENCES IN THE METABOLISM OF ACRYLONITRILE (ACN) TO 2-CYANOETHYLENE OXIDE IN LIVER AND LUNG OF THE F-344 RAT AND THE B6C3F1 MOUSE. A E Roberts, M J Turner and J Swenberg, Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

ACN is carcinogenic in rats but no other species has been studied. The oxidative metabolism of ACN to 2-cyanoethylene oxide (ANO), an epoxide which can alkylate DNA, has been suggested to be involved in carcinogenesis. Kinetic values were determined using gas chromatography/high resolution mass spectrometry to monitor ANO formation in liver and lung microsomes of F-344 rats and B6C3F1 mice. In mouse liver microsomes, the Vmax (pmol ANO formed/min/mg protein) and the second order rate constant for binding and catalysis, V/K (pmol ANO formed/min/mg/μM), were 4 times greater than Vmax and V/K in rat liver microsomes. The apparent Km (μM) for ANO formation in rat and mouse liver microsomes was similar. In mouse lung microsomes, Vmax and V/K were 12 and 15 times greater than Vmax and V/K in rat lung microsomes, respectively. The apparent Km for lung was similar for both species. The species difference in kinetics was due, in part, to the presence of higher concentrations of cytochrome P-450/mg tissue in mouse lung and liver relative to rat lung and liver. The comparison of ANO formation across species may provide an important end point for interspecies extrapolations of carcinogenic risk.

1138 METABOLISM OF INHALED BUTADIENE IN MONKEYS: COMPARISON TO RODENTS. J D Sun, A E Dehl, J A Bond, L S Birnbaum* and E P Henderson, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM; *Mees, Research Triangle Park, NC.

1,3-Butadiene (BD), an intermediate in rubber manufacturing, is metabolized to mutagenic epoxide and diol epoxide metabolites, and causes cancer in rodents. To gain insight into the human carcinogenic potential of BD, the metabolic profile of inhaled BD was studied in monkeys and compared to that previously found in rodents. Male cynomolgus monkeys (Macaca fascicularis) were exposed by inhalation to 10, 300, or 8000 ppm 13C-BD (n=3) for 2 hr. Exhaled air, urine, feces and blood samples were collected during and/or after exposures. Tentative identification of 13C-BD metabolites was made by vacuum distillation and HPLC methods. At the 10 ppm level, the major route of metabolite elimination was exhaled 13CO2 and 13C2 in urine (31% and 39% of the 13C-BD excreted metabolites, respectively). Epoxide (4%), and dioloxide/diol (DEP/D) (12%) metabolites were also found in expired air. The 8000 ppm level, elimination of exhaled 13CO2 and EP decreased to 4.7% and 0.3%, respectively, while exhaled DEP/D increased to 6%. Blood levels of EP and DEP/D ranged from 1.6 and 1.9 μmol/ml at the 10 ppm level to 1100 and 470 μmol/ml at the 8000 ppm level, respectively. Previously, mice exposed to 330 ppm 13C-BD showed blood levels of EP and DEP/D that were 3 and 30 times lower, respectively. After exposures to 330 ppm 13C-BD, mice had roughly comparable levels of these blood metabolites. Thus, the carcinogenic potential of inhaled BD for monkeys may not be as great as that for rodents at low exposures, but may be comparable at higher exposures. (Research supported by NIHES through UAA ES-20062, U.S. DOE/DOE Contract DE-AC04-76EV03015).

1137 EFFECT OF CYTOCHROME P-450 INHIBITORS AND ETANOL ON THE ACUTE TOXICITY OF ACRYLONITRILE AND ITS IN VIVO METABOLISM TO CYANIDE. D E Nerland, P W Benz, C Babuk, and W M Pierce. Department of Pharmacology and Toxicology, University of Louisville, Louisville, KY and BP America, Inc., Cleveland, OH.

The symptoms observed after administration of an acutely toxic dose of acrylonitrile (AN) include motor stimulation, cholinomimetic signs, tremors, seizures, hypothermia, asphyxial signs, depression and death. AN is metabolized in vivo to cyanide. Inhibiting the metabolism of AN to cyanide would allow the toxic effects of the parent molecule to be dissociated from the effects produced by cyanide. Male Sprague-Dawley rats were pretreated with either SKF-525A, metyrapone (M), benzylisadazole (B) or ethanol (E). An LD90 dose of AN was then administered SC and the symptomatology, time to convulsions, time to death and blood cyanide concentration at death were determined. M had no effect and SKF-525A only slightly reduced blood cyanide levels. However, both B and E dramatically reduced blood cyanide, altered symptomatology, increased the time to seizures and the time to death. E also significantly reduced the incidence of seizures. Neither agent affected the incidence of AAI-induced mortality. These data suggest that the initial seizures seen with AN are cyanide induced but even in the absence of cyanide production, the parent AN molecule is acutely toxic.

1139 SEX AND SPECIES DIFFERENCES IN METABOLISM OF DIBASIC ESTERS BY NASAL CARBOXYLESTERASE. C R Kee*, M S Bogdanoff*, C M Keenan*, K P Keenan*, and J F Resau*. 'E I du Pont de Nemours & Co, Inc, Haskell Laboratory for Toxicology and Industrial Medicine, Newark, DE and University of Maryland Dept. of Pathology, Baltimore, MD.

Inhalation exposure of rats to dibasic esters (DBE), a solvent mixture of dimethyl-succinate (DMS), -glutarate (DME), and - adipate (DMA), causes degeneration of the olfactory mucosa (OLF). The mechanism of action may be related to formation of toxic acid metabolites of the esters within OLF. Since female rats appear to be more sensitive to DBE-induced olfactory toxicity than males, it was of interest to measure the rate of hydrolysis of DBEs in male and female nasal mucosal homogenates and compare these values to those derived from human nasal tissue obtained at autopsy. For both male and female rats, Vmax/Km values (followed the order DMA > DME > DMS) paralleled carbon chain length. The Vmax/Km values for female OLF using DME or DMS as substrate were twice or one half the value for male OLF, respectively. Hydrolysis of DBEs was detectable in only 3 of 6 human samples. Activity values that were measurable were 2 or 3 orders of magnitude greater than that of rat respiratory or olfactory mucosa, respectively. These data suggest the rate of conversion of DBEs to acid metabolites in nasal tissue is less significant in humans than in rats and that the rat may be more sensitive than man to the effects of DBEs on nasal mucosa.

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1,3-Dinitrobenzene (DNB), but not 1,2- or 1,4-DNB, is a testicular toxicant in rats, causing Sertoli cell damage and condensation of pachytene spermatocytes in vivo and in vitro. To determine if testicular metabolism accounts for this isomer-specific toxicity, studies were conducted using a seminiferous tubule segment culture system in which metabolism and toxicity of 14C-DNB could be determined. 1,2- and 1,4-DNB were extensively metabolized in 24 hours yielding glutathione conjugates and nitroanilines (NA). Metabolism of 1,3-DNB was not detected. Only 1,3-DNB produced any toxicity (evident as condensation of pachytene spermatocyte nuclei in live tubule squashes) at concentrations equivalent to testicular levels measured following a toxic in vivo dose (1 x 10^{-5} M). Toxicity was also absent following incubation with the various reduced metabolites of the three isomers (NA, hydroxyaminonitrobenzenes or nitrosonitrobenzenes). Thus, metabolism does not appear to be required for the induction of testicular toxicity following 1,3-DNB, but it may play a protective role after administration of the other isomers.

1142 BROMOBENZENE-GLUTATHIONE EXCRETION INTO RAT BILE REFLECTS TOXIC ACTIVATION OF BROMOBENZENE. C. Madhu and C.D. Klaassen. Univ. of Kansas Medical Center, Kansas City, KS.

Bromobenzene (BB) is biotransformed by cytochrome P-450 in liver to a reactive metabolite which is detoxified by conjugation with glutathione (GSH). Because GSH conjugates are mainly excreted into bile, the rate of biliary excretion of BB-GSH may reflect the toxic activation of BB. In order to examine this hypothesis, the effect of chemicals (i.e., P-450 inducers and inhibitors) thought to influence activation of BB was studied in rats by quantitating the biliary excretion of BB-GSH. BB-GSH was the major BB metabolite in bile. A linear relationship was observed between the dosage of BB administered and BB-GSH excreted into bile, up to a dose of 0.25 mmol/kg of BB. This dosed of BB decreased liver GSH (22%). Of the inducers tested, phenobarbital dramatically increased the rate of biliary excretion of BB-GSH over that in control animals, whereas it was decreased by 3-methylcholanthrene (30%) and pregnenolone-16α-carbonitrile (26%). Inhibitors of P-450 such as SKF-525A and piperonyl butoxide decreased the biliary excretion of BB-GSH. These findings are in agreement with the concept that specific forms of cytochrome P-450 are responsible for the activation of BB to the toxic intermediate. These results support the hypothesis that quantitating the rate of biliary excretion of BB-GSH can be used to determine factors that are important in altering the activation and toxicity of BB. Quantitative biliary excretion rate of GSH conjugates of reactive metabolites of other xenobiotics may be a general method of assessing species differences and chemical interactions. (Supported by USPHS Grant ES-03192).

1141 EFFECT OF GLUTATHIONE (GSH) DEPLETION ON COVALENT BINDING (CB) OF DINITROBENZENE (DNB) ISOMERS IN ISOLATED HEPATOCYTES. S F McEuen and M G Miller. Department of Environmental Toxicology, University of California, Davis. Sponsor: L Shell.

Previous investigators have suggested that the metabolic clearance of DNB isomers plays an important role in their testicular toxicity. 1,2-DNB is rapidly metabolized via GSH conjugation and is relatively nontoxic, whereas 1,3-DNB is less rapidly metabolized and apparently does not form GSH conjugates but causes testicular toxicity. 1,4-DNB represents an intermediate situation. The present studies have examined the CB of all three isomers in freshly isolated hepatocytes prepared from control and diethylnlelestatetreated GSH-depleted rats. Radiolabeled 1,2-DNB and 1,4-DNB were prepared by oxidation of their 14C-nitroaniline derivatives in the presence of perthiobarbituric acid. 14C-1,3-DNB was commercially available. Purity was checked by HPLC and >99.5%. Studies were carried out at 25, 50 and 100 μM for 15, 30 and 60 min for the 3 isomers. CB of 1,2-DNB and 1,4-DNB was markedly greater in viable cells which were not incubated and in dead cells, suggesting that the parent compound was partly responsible for the CB reaction. In contrast, 1,3-DNB CB was at background levels in the 0 time incubations and with dead cells. CB induced by 1,3-DNB was both time and concentration dependent. Further studies are examining the relationship between metabolism and CB and the nature of the DNB binding species.

1143 BILIARY EXCRETION OF 1,2-DICHLOROETHANE (DCE) METABOLITES IN THE RAT. D.H. Marchand, K Tulip, and D.J. Reed. Oregon State University, Corvallis, OR.

Two metabolic pathways have been proposed for the bioactivation of DCE: one pathway involves oxidation to 2-haloacetaldehydes; the other pathway involves conjugation of glutathione (GSH) leading to the formation of an episulfoxonium ring compound. Neither 2-haloacetaldehyde nor the episulfoxonium ring compound have been identified in animals treated with DCE. We attempted to isolate the putative precursor of the episulfoxonium ring compound, S-(2-chloroethyl)GSH (CEG) from the bile of rats treated intravenously with l-bromo-2-chloroethene (BCE; 75 mg/kg) or DCE (50 mg/kg). The bile was examined by reverse-phase HPLC in conjunction with fluorescent detection. A rapid and sensitive precolumn procedure for derivatizing CEG using o-aminophthaldehyde and 2-mercaptoethanol was developed. Analysis of the bile indicated the presence of a peak corresponding to CEG in rats treated with BCE but not in rats treated with DCE. Additional studies involving the intravenous administration of radiolabeled DCE to bile-cannulated rats revealed that radioactivity was present in the bile but that none of the radioactivity was due to the presence as CEG or its predicted product S-(2-hydroxyethyl)GSH. Subsequent to the administration of radiolabeled DCE, the amount of radioactivity in the bile peaked at 2 hours. Over a 5 hour period, a total of 34% (n=4) and 7% (n=3) of the radiolabel was excreted in the bile and urine, respectively. Further HPLC analysis indicated that the majority of the radioactivity in the bile was present as S-(2-carboxymethyl)GSH. This finding suggested that the appearance of biliary metabolites of DCE was, in part, due to oxidative metabolism; however, preliminary studies involving the coadministration of the P450 monooxygenase inhibitor piperonyl butoxide (1000 mg/kg), have resulted only in small changes in the biliary excretion of DCE metabolites. (Supported by PHS #ES 00040).
THE ROLE OF GLUTATHIONE IN THE HEPATIC MICROSOMAL METABOLISM OF SENECONINE, R L Reed, C L Miranda, M C Henderson, and D E Ruble. Toxicology Program and Dept. of Agric. Chem., Oregon State Univ., Corvallis, OR.

Seneconine (SN), a pyrrolizidine alkaloid found in Senecio jacobaeae, is known to be bioactivated by the liver to reactive pyrrololic metabolites. One such metabolite is 1-dehydro-2-pyrroline (DHP), which has been shown to be toxic to DNA, cyssteine and glutathione (GSH). In the present study, we investigated the role of GSH as a trapping agent for the reactive metabolites of SN formed by phenobarbital-induced rat liver microsomes. Adding increasing concentrations of GSH (0-150 mM) to the incubations caused the progressive loss of free DHP and appearance of a 1:1 adduct of GSH and DHP, as determined by mass spectrometry. GSH added after the reaction was terminated only partially eliminated the DHP peak and less DHP-GSH adduct was formed. The pH optima for the microsomal production of DHP and for DHP-GSH conjugate formation are both pH 8, but non-

enzymic formation of conjugate is greater at acidic pH (such as 6.5). In addition, GSH (10-50 mM) increases approximately two-fold the production of pyrrololic metabolite(s), virtually all of which binds to GSH as DHP-GSH. These findings provide evidence for the microsomal formation of an unstable metabolite of SN which is more reactive than DHP towards GSH, and its rate of formation is modulated by GSH itself. (Supported by NIH grants: CA22524 and ES00210).

SOMAN DOES NOT INHIBIT MONOAMINE OXIDASE (MAO) OR CATECHOL-O-

METHYLTTRANSFERASE (COMT) IN RABBIT TISSUES IN VITRO. C H Hsu, C Y Hu, and C P Robinson. College of Pharmacy, University of Oklahoma, Health Sciences Center, Oklahoma City, OK.

Previous studies showed that the daily administration of 5 mg/kg of soman for 7 days reduced MAO and COMT activity of several rabbit tissues. These studies were carried out to determine if soman applied in vitro would also inhibit these enzymes. Samples of rabbit renal, pulmonary, mesenteric and central ear artery, and aorta, liver, lung, cerebrum, cerebellum, brain stem and diaphragm were frozen at -70°C until assayed for enzyme activities. The MAO activity was determined by the method of Wurtman and Axelrod, and COMT activity by the method of Axelrod. Enzyme activities were determined in the presence of 10 μM soman or no soman as a control. All tissues had detectable MAO and COMT concentrations. Neither MAO activity (n = 6) nor COMT activity (n = 6) were significantly altered (P > 0.05) in any of the tissues by soman exposure. Therefore, acute soman administration, unlike repeated soman administration, does not inhibit MAO or COMT activity. Supported by DOD Contract DAMD17-85-C-5114.

CONJUGATION OF CHLOROACETIC ACID WITH CHELOLER-OL. H K Bhat and G A S Anjari. Divisions of Biochemistry and Chemical Pathology, University of Texas Medical Branch, Galveston, TX.

Chloroacetic acid (CA) is used as a herbicide, in the synthesis of organic chemicals and is a metabolite of some halogenated compounds. CA is neurotoxic and causes blood-brain barrier damage. It is highly corrosive to tissues and can cause death by systemic exposure. We have studied the interaction of CA with microsomes using [14C]-CA under in vivo and in vitro conditions. Rats were fed CA orally (8.75 mg/kg 30 μCi in 0.9% saline) and sacrificed after 6 hrs. The livers were removed, extracted with CHCl3: MeOH (2:1, v/v) and separated into neutral lipids and phospholipids by seph-pak column chromatography. Neutral lipid fraction was subjected to preparative thin layer chromatography on silica gel using hexane: ethyl acetate (19:1, v/v). The region corresponding to cholesterol chlo-roacetate was scraped, eluted and subjected to reverse phase high performance liquid chromatography using hexane: methanol (3:97, v/v) at a flow rate of 1 ml/min. The fraction having a retention time of 40 min (standard cholesterol chlo-roacetate had a retention time of 20 min.) was collected, concentrated and subjected to ammonia and chloroform isionization mass spectrometry (CIMS) which gave pseudo molecular ion at m/z 486/482 (ratio 3:1) and the fragmentation pattern was similar to that of the standard. To study the in vitro esterification of cholesterol by CA, rat liver microsomes were incubated with [14C]-CA in the presence of CoA and ATP. The incubation mixture was processed as described above. CIMS gave pseudo molecular ion peaks at m/z 480 and 482 (3:1) thus confirming the formation of cholesterol chlo-roacetate. These studies indicate that CA forms cholesterol ester. The effect of such conjugation reaction on the cell membrane and its contribution to the toxicity of CA is presently unknown. (Supported by NIH grant ES04815).

4, 6-DIMETHYLENE-BIS(2-CHLOROANILINE) [MOCA]:

COMPARISON OF MACROMOLECULAR ADDUCT FORMATION AFTER ORAL OR DERMAL ADMINISTRATION IN THE RAT. K L Cheever, D E Richards, W W Weigel, K E Begley, D C DeFord, T F Swearengin and R E Savage Jr. NIOSH, DBBS, EBT, TMS, CINCINNATI, OH.

The macromolecular binding of MOCA, a suspect human carcinogen, was studied in the adult male Sprague-Dawley rat after both oral and dermal administration. Rats were sacrificed 1, 3, 7, 14, 21 and 29 days after a single 75 mg/kg by dose of 14C-OMCA (oral = 213 μCi/kg, dermal = 904 μCi/kg). DNA from various tissues and hemoglobin were isolated for determination of the time-course of MOCA macromolecular binding. After oral administration adduct formation was rapid with maximum levels appearing at 24 hr. The 24-hr covalent binding associated with the globin was 3.6 pmol/mg globin (t1/2 = 14.3 day). More extensive 24-hr covalent binding was detected for liver DNA with 22.5 pmol/mg DNA (t1/2 = 11.1 day). After dermal administration of MOCA the major portion of the dose, 86.2%, remained at the application site throughout the study. For these rats the 24-hr covalent binding determined for liver DNA was 0.4 pmol/mg DNA (t1/2 = 15.6 day). Although lower levels were detected after dermal application, similar stability of MOCA-DNA adducts indicates that quantification of such MOCA adducts may be useful for the long-term industrial biomonitoring of MOCA exposure and for the evaluation of DNA MOCA-adduct formation, a lesion thought to be associated with the production of cancer.
The Amersham RAS1000 image analysis system was evaluated for the quantitation of tissue concentrations of radiocarbon from whole-body autoradiograms. Initially, the self-absorption of beta radiation was investigated by exposing non-radioactive, whole-body sections between a homogeneous radioactive source and X-ray film. Tissues appeared to have similar absorption characteristics (4%) with the exception of bone (34%), fat (16%), and eyes (0%), thus validating the use of a common calibration curve for most tissues. Blood, liver, and brain tissues from rats dosed orally with 14C-glucose were used to calibrate 14C-microscales purchased from Amersham and a comparison was made to the 14C-labeled brain tissue calibration published by Amersham. Comparisons between these two calibration curves and tissue concentrations determined by direct scintillation counting were made using rats dosed intravenously with 14C-acetylsalicylic acid. Results indicated that concentrations obtained by both methods were similar.

In preclinical safety studies, the antiarrhythmic enantiomers, U-76,541E and U-76,542E, induced leukopenia in dogs administered 48 mg/kg/day orally for 30 days. The leukopenia was more pronounced in animals treated with U-76,541E. The compounds were evaluated for their potential to inhibit cell proliferation in three nonlymphocytic cell lines (Chang: human liver, SIRC; rabbit cornea, and L929: mouse connective tissue) and in the natural killer-like YT cell line. Cells were treated with 3.75 to 120 ug/ml for 4 hrs in serum-free medium. Cell proliferation was analyzed at various times following treatment. No significant, drug-dependent effects were observed on the cell proliferation of any nonlymphocytic cell lines. YT cells treated with 120 ug/ml of U-76,541E and U-76,542E showed 32% and 4% decreases, respectively, in cell numbers when compared to controls. The approximate doubling times (hrs) were as follows: SIRC: 19, L929: 23, YT: 31, and Chang: 33. The fact that the proliferation of the lymphocytic YT line was inhibited, but that of faster dividing nonlymphocytic lines (e.g., SIRC and L929) was not, suggests a mechanism based on cell type specificity rather than an innate capacity of these compounds to inhibit proliferation of rapidly dividing cell types in vivo.

Urethane is present in fermented foods and beverages and its metabolism is inhibited in mice by ethanol or DMSO. Massive doses of urethane produce tumors in rodents although there is no evidence of its carcinogenicity to humans. Mice were administered i.p. either: A. 5 g/kg ethanol; B. 250 mg/kg paraldehyde; C. 400 mg/kg acetaldehyde; D. 2.25 g/kg Na acetate. 125 μmol/kg urethane was given orally in water immediately following the pretreatment compound. Blood collected from the orbital sinus was applied to a solid-phase column (Chem Elut 1000 M). Ethyl (13-NH2)-carbamate was added and the column was eluted with methylene chloride (2x3 ml). The eluate was concentrated to 50 μl, and 5 μl was injected onto a DB-WAX capillary column (0.17 mm X 20 m) with temperature held at 110°C (2 min), then programmed at 32°C/min to 200°C. The retention time of urethane was 9.6 min. The unlabeled/labeled ratio for the principal ions (m/z 62/64) allowed quantification. Mice without pretreatment or with Na acetate pretreatment metabolized urethane to undetectable levels within 4 hrs. Ethanol, acetaldehyde or paraldehyde prolonged the presence of urethane in blood. (Supported by Distilled Spirits Council of the United States, Inc. and Pharmaco Research Foundation, Inc.)
Rapid intravenous administration of the glycopeptide antibiotic, vancomycin, may cause a hypotensive reaction which can usually be prevented by infusing vancomycin in dilute solutions. The release of histamine from circulating cells such as basophils has been implicated in the hypotensive reactions since the effects can be prevented by antihistamine pretreatment. The effects of vancomycin on histamine levels in RBL-1 cells were investigated. RBL-1 cells were exposed to vancomycin in suspension (10⁵ cells/ml) at concentrations infused clinically (2.28-4.56 mg/ml) for 30-60 minutes. A time- and dose-dependent release of histamine into the suspension media was observed (max 150% of control). In addition, residual releasable histamine in the cellular pellet fraction was higher in vancomycin-treated cells (max 210% of control). These data indicate that vancomycin causes histamine release from RBL-1 cells and also may cause an increased mobilization or mobilization of cellular histamine stores. RBL-1 cells may be a useful model to evaluate the potential of investigational agents to release histamine and to study mechanisms of histamine release.

Hemolysis in Rats after 28-Day Oral Administration of 4-Nitro-N-Methyl-pentamidine (4-NPI). K R Goshenoff, L W Smith, and S L Yurasevac. Hazleton Labs, Vienna, VA; Occidental Chemical, Niagara Falls, NY; GE Plastics, Pittsfield, MA.

4-NPI is a chemical intermediate with demonstrated teratogenicity in rats and rabbits. A 28-day feeding study suggested hemolysis at doses as low as 100 mg/kg. The toxicity of 4-NPI was further investigated following oral administration to Sprague-Dawley rats at dose levels of 0, 10, 100, 500, or 1000 mg/kg/day. Mortality occurred and food consumption was depressed in animals given 1000 mg/kg. Mean body weight and weight gains were significantly depressed at 500 and 1000 mg/kg. There were significant depressions in red cell mass and bone marrow M:E ratios and significant increases in the WBC and reticulocyte counts in animals administered 100 mg/kg or more. The WBC effect was an artifact of electronic cell counting due to large polychromatophilic RBCs which are resistant to lysis in the Coulter Counter. Gross necropsy (and organ weight data) revealed enlarged and darkly discolored spleen (100 mg/kg) and liver and kidney (500 mg/kg). Histomorphologic evaluation confirmed the presence of hemosiderin in the macrophages of the splenic red pulp, Kupffer cells, and renal proximal tubules. Thus, oral administration of 4-NPI, at 100 mg/kg or more caused hemolysis; 10 mg/kg was the apparent no-effect level.

HEMATOLOGIC EFFECTS OF 2-BUTOXYETHANOL (BE) IN VIVO AND ITS EFFECTS ON THE MORPHOLOGY OF RAT ERYTHROCYTES: S Ward, P C Blair, and B I Ghanayem. NIH/NIAMS, Bethesda, MD.

Earlier reports indicated that BE causes acute hemolytic anemia in rats as evident by a time- and dose-dependent decrease in the number of RBC's, HGB concentration, and packed cell volume (hematocrit or HCT). In these studies, we utilized an Ortho ELT-8/ds hematologist analyzer. In more recent in vitro studies, we found that hemolysis was preceded by erythrocyte swelling as evident by increased HCT. Since an increased HCT was not originally observed in vivo, the in vivo effects of BE were repeated using a Coulter S-Plus IV hematologist analyzer. Results obtained using the Coulter analyzer and confirmed using the span HCT indicated early dose- and time-dependent increase in HCT and Mean Corpuscular Volume (MCV). In contrast, analysis of the same blood samples using the Ortho analyzer indicated a decreased HCT with no change in MCV. Therefore, it appears that the Ortho analyzer is unable to detect increases in cell volume and its use has resulted in spurious results. As part of this study, smears from these same blood samples were concomitantly prepared. Microscopic examination of these blood smears showed enlarged erythrocytes accompanied by a time- and dose-dependent appearance of stomatocytes (erythrocytes with slit-like hypochromia) and schistocytes or debris from massive erythrocytes destruction. Stomatocytes are usually seen in rare forms of hemolytic anemia and in hepatic disease/toxic stimuli.
CYANIDE (CN) INHIBITS THE FORMATION OF NITROSYLHEMOGLOBIN (HbNO) FROM DEOXYHEMOGLOBIN (Hb) AND SODIUM NITROPRUSSIDE (SNP). R P Smith, D W Wilcox*, H Kruszyna, R Kruszyna, Dept. Pharmacol/Toxicol, Dartmouth Medical School & *Dept. Chem., Dartmouth College, Hanover, NH.

Under anaerobic conditions, 1.5 mM Na2 Fe(CN)5 FeNO (SNP) and 1.1 mM pure reduced Hb were mixed in 0.1M phosphate buffer (pH 7.4); samples were removed at various times, frozen in liquid nitrogen, and analyzed by electron spin resonance (ESR). Initially ESR signals of HbNO and the one electron reduced nitroprusside ion, (CN)5 Fe NO-3 were observed. After one hour the dominant signal was that of HbNO. Addition of 1.5 mM sodium cyanide to this reaction initially resulted in the formation of (CN)5 FeNO-3 but not HbNO. Only after one hour could a small amount of HbNO be detected. Thus, CN prevents the formation of HbNO by SNP but not the one electron reduction of SNP by Hb. However, controlled electroreduction of SNP resulted in formation of two reduced nitroprusside species, (CN)5FeNO-3 and (CN)4FeNO-2 (formed by loss of CN trans to the nitroso), as indicated by their characteristic ESR signals. This suggests that in the SNP reaction with Hb, excess CN prevents formation of (CN)4FeNO-2, which is the SNP species with labile NO responsible for formation of HbNO.

Glutathione (GSH) can also reduce SNP and this hypothesis has been checked in analogous SNP reactions with GSH. (Supported by NIH Grant, HL14127).

ENHANCED PRODUCTION OF TUMOR NECROSIS FACTOR (TNF) BY STROMAL MACROPHAGES FOLLOWING BENZENE TREATMENT OF MICE. L MacEachern, R Snyder and D L Laskin, Joint Graduate Program in Toxicology, Rutgers University, Piscataway, NJ.

Treatment of male Balb/c mice with benzene (B) or a combination of hydroquinone (HQ) and phenol (P), activates bone marrow stromal macrophages. These cells display enhanced hydrogen peroxide production in response to stimulation with phorbol myristate acetate. In the present studies, we determined if B or HQ-P activated stromal cells to produce TNF, a macrophage derived cytokine with potent cytolytic activity. Mice were injected with B (100 mg/kg), HQ-P (50 mg/kg) or control (corn oil or saline) 1-2 times/day for 1-4 days. Stromal cells were isolated and analyzed for production of TNF using a L929 cytotoxicity assay. We found stromal cells from B or HQ-P treated mice produced 40-50% more TNF than did cells from control mice. Release of TNF was time dependent reaching a maximum 4-6 hr after cell isolation. These results support our hypothesis that bone marrow stromal macrophages are a target for benzene and suggest that these cells may contribute to hematotoxicity induced by this solvent. Supported by NTH Grant ES02931.

POTENTIAL ROLE OF INTERLEUKIN-1 IN BENZENE INDUCED BONE MARROW TOXICITY. F M Robertson, L MacEachern, J B Liesch, R Snyder and D L Laskin, Joint Grad Prog Toxicol, UMDNJ-R W Johnson Medical School/ Rutgers Univ, Piscataway, NJ.

Hematopoiesis is regulated by cytokines such as colony stimulating factors and interleukin-1 (IL-1) released by bone marrow stromal cells. Since benzene is a potent bone marrow toxicant, we determined if this solvent modulates the production of IL-1 by stromal cells. Female Balb/c mice were treated with benzene (880 mg/kg), a combination of hydroquinone (HQ) and phenol (P) (50 mg/kg) or control (corn oil or saline) once/day for 3 days. Stromal cells were then isolated from the femur and tibia bones, inoculated into culture dishes and incubated with lipopolysaccharide. IL-1 activity in macrophage culture supernatants was quantified 48 hr later using a thymocyte proliferation assay. We found that stromal cells from benzene or HQ-P treated mice produced 2-3 times more IL-1 than did cells from control mice. Furthermore, production of IL-1 was dependent on the number of adherent cells in the cultures. We hypothesize that enhanced IL-1 production by stromal cells may modulate stem cell development contributing to benzene induced hematotoxicity. Supported by NIH ES 02981.

QUINONE REDUCTASE (QR) AS A DETERMINANT OF STROMAL CELL SUSCEPTIBILITY TO HYDROQUINONE (HQ). L E Twerdok and M A Trush, Johns Hopkins University, Baltimore, MD.

DBA mice have been reported to be more susceptible than C57 mice to the bone marrow toxic effects of two quinone-generating chemicals, benzo[a]pyrene and benzene. In this study we have investigated the possible role of QR, a quinone detoxifying enzyme, as a determinant of the toxic effect of quinone compounds on bone marrow-derived stromal cells. Stromal cells cultured from DBA mice had a lower basal level of QR activity compared to those of C57 mice and as such exhibited a greater sensitivity to the toxic effects of tert-butyl hydroquinone or HQ, a metabolite of benzene. HQ toxicity to stromal cells was enhanced in the presence of horseradish peroxidase (HRP) and H2O2. Prior induction of QR protected against the toxicity of HQ in the absence and presence of HRP and H2O2. Thus, a lower basal level of QR coupled with our previous demonstration that bone marrow-derived neutrophilic cells from DBA exhibit a greater chemical activation than C57 mice identifies two biochemical conditions which could contribute to the susceptibility of DBA mice to quinone-generating bone marrow toxins.

Supported by ES 03760, ES 07141, OH 02632 and American Cancer Society S10-3.
Bone marrow stroma consists primarily of two cell types: macrophages (MFs) and fibroblastoid stromal cells (FSC) which interact via the production of soluble mediators to regulate myelopoiesis. MFs are more sensitive as FSC to the toxic effects of the benzene metabolite hydroquinone (HQ). The goal of this study was to determine if differential bioactivation of HQ by MFs represented a potential explanation for this selective toxicity. A fibroblastoid stromal cell line (LTF), derived from long term bone marrow cultures, and MP cultures, derived from B6C3F1 mouse bone marrow cells, were used. The cultures were incubated with 10 μM 14C-HQ and, after 24 hours, the amount of 14C covalently bound to protein was determined. MFs had 16 times more covalently bound 14C than did LTF cells. Peroxide (H2O2)-dependent bioactivation in MP and LTF cell homogenates was also examined. H2O2 could support bioactivation of HQ to protein binding species in MP homogenates but not in LTF homogenates. This suggests that a peroxide-mediated event may be responsible for MP selective toxicity. Supported by NIH grants ES04808-04 (D.W., M.J.R.) and ES04112 (D.R.), NIKA/WIGM Training grant T32GM07039 (D.J.T.) and an SOT/Moffen-LaRoche graduate student fellowship (D.J.T.).


Mineral fibers (MF) cause human pulmonary diseases including fibrosis and cancer. While the mechanisms responsible for fiber-induced disease states remains poorly understood, the alveolar macrophage (AM0) appears to play a critical role. We have investigated the role that activation of these cells plays in the response to MF. By treating macrophages in vitro with various "priming" signals such as serum (FBS) and interferon (IFN), as well as "trigger" signals such as lipopolysaccharide (LPS), populations of rat AM0 in various stages of differentiation termed resident, responsive, primed and activated were obtained. Interleukin 1 (IL-1) secretion is significantly stimulated upon exposure to chrysotile and crocidolite asbestos but not to the non-fibrogenic particulate, wollastonite. This was observed in responsive (FCS) and primed (IFN) cells while resident AM0 (non-stimulated) remained refractory to MF induced IL-1 modulation. In contrast, the production of tumor necrosis factor (TNF) is stimulated by MF in AM0 in all stages of activation. Furthermore, we observed that AM0 pretreated with fibrogenic MFs become hypersensitive to very low concentrations of LPS, and, in this manner the fibers appear to act as priming agents themselves. MF-induced modulation of H2O2 production is not significantly altered until macrophages mature into the primed and activated stages and appears to be a function of any particulate. Taken together, these data indicated that the activation stage is an important determinant of the ultimate immunologic response of AM0 to MFs and may help explain why certain individuals (e.g. smokers) are particularly susceptible to the adverse effects of such particulates.

IMMUNOMODULATION BY A RECOMBINANT HUMAN ALPHA INTERFERON - EVIDENCE FOR DIVERGENT EFFECTS ON IMMUNE EFFECTOR CELL FUNCTION. R P Stranahan, M Fort, D R Germolec, M Ackermann, P Blair, K J Schwab, M Luster, and G J Rosenthal. NIH, NEHS, NT, RTP, NC.

Alpha-interferons consist of a group of naturally occurring cytokines that mediate numerous biological effects and have demonstrated therapeutic potential in a variety of malignancies. Recent use of α-IFN in AIDS therapy has made it particularly important to understand the interaction between α-IFN and components of the immune system. A recombinant hybrid of alpha subtypes of human IFN, HIFN-α/A/D, has demonstrated antiviral activity in murine cells in vivo and in vitro. This study examines the effect of HIFN-α/A/D on the immune functions of C57Bl/6J mouse following both acute and subchronic exposure. α-IFN was administered i.p. at 0.1, 1,000, 10,000, and 100,000 units/day for either one day or ten consecutive days. Many of the known effects in humans were demonstrated in our murine system, including increased hepatic transaminases, leukopenia, anemia, thrombocytopenia, and increased NK activity. While the majority of these responses were observed following multiple exposure, marked leukopenia and thrombocytopenia were also observed following acute dosing. Multiple exposure to α-IFN resulted in significant suppression of the lymphoproliferative capacity of both B and T lymphocytes. However, an equivalent amount of IFN given in a single dose did not produce the same effect, suggesting that for α-IFN the frequency of administration may be more important toxicologically than the dose. In contrast to the suppressive effects on B cell proliferation, a clear stimulation was seen in the AFD response to SRBCs. The effect of α-IFN on bone marrow cellular targets was also examined. Cells programmed to differentiate into granulocytes (CFU-G) were suppressed at the high dose following multiple exposure, while macrophage progenitors (CFU-M) were stimulated in a dose-related fashion under both exposure regimes. Taken together, these data suggest that long term administration of α-IFN can have adverse effects on immune function, and this system may be important to monitor in future clinical trials.

EFFECT OF 7,12-DIMETHYLBENZ(A)ANTHRACENE (DMBA) ON INTERLEUKIN-2 (IL-2) RESPONSIVENESS IN MURINE SPLEENOCYTE CULTURES. M J Pallardy, R V House and J H Dean. Dept. of Cellular and Molecular Toxicology, Chemical Industry Institute of Toxicology, Research Triangle Park, NC.

Previous studies in this laboratory have shown that exposure of mice to the carcinogenic polycyclic aromatic hydrocarbon DMBA results in suppression of cell-mediated immunity. The purpose of the present study was to evaluate IL-2 responsiveness using long-term cultured splenocytes from B6C3F1 mice exposed in vitro to DMBA. Splenocytes were exposed to DMBA (0-40 μM) for one hour and IL-2 (0-50 units/ml) was added for an additional 24 hours. Proliferation as measured by [3H]-thymidine incorporation was inhibited dose-dependently at all DMBA concentrations examined. High-affinity IL-2 receptor expression measured by a radiolabeled binding assay was likewise decreased in a dose-dependent manner. In contrast, no change was noted in expression of the P55 (low affinity) subunit of the IL-2 receptor as determined by labeling with the monoclonal antibody 7D4 and subsequent cytofluorography. These results demonstrate that in vitro exposure to DMBA alters the IL-2 responsiveness of long-term splenocyte cultures, which may be of potential significance in the immunotoxicity produced by this chemical.
A SUBACUTE INTRAVENOUS TOXICITY STUDY OF MACROPHAGE-COLONY STIMULATING FACTOR (M-CSF) IN THE RAT. [P] Nachman, H L Moon, J R Kopplin, Cetus Corporation, Emeryville, CA and R Cinprich, Cimprich Associates, Elmer, NJ.

Recombinant human macrophage colony stimulating factor (M-CSF) is a potent stimulator of macrophage production in vivo and in vitro. CD rats were given daily intravenous doses of M-CSF at 1, 3, or 10 mg/kg for 10 days (5/sex/dose). One female high dose animal died on study day 5. Mainly in the top dose group, M-CSF treatment produced: (1) increased white cells (3-fold), absolute neutrophils (6-fold), and absolute lymphocytes (3-fold); (2) decreased red cell and platelet counts and (3) altered some serum enzymes. Macroscopic findings included enlarged spleen, liver, and lymph nodes. Microscopic findings were extramedullary hematopoiesis and macrophage infiltration of lymph nodes, spleen, and liver. Bone marrow smears showed decreased M:E ratio and megakaryocytosis, indicating a responsive anemia. Based upon this study, the maximum tolerated dose of M-CSF was 3 mg/kg/day.

DEVELOPMENT OF TOLERANCE TO RECOMBINANT HUMAN INTERLEUKIN-1 ALPHA IN A TWO-WEEK IV TOXICITY STUDY IN MICE. D Lucas, W Benjamin, TD Anderson, and TD Hayes, Departments of Toxicology and Pathology, and Immunopharmacology, Hoffmann-La Roche Inc., Nutley, NJ.

In two-week toxicity studies in mice with recombinant human interleukin-1 (rIL-1) toxicity was evident during week 1 of treatment but mice showed partial tolerance to the effects of rIL-1 during week 2. To examine the role of antibody formation in tolerance development, we treated CD-1 mice with 5-1000 ug/kg/day for 2 weeks and monitored clinical signs, hematologic and clinical chemical changes, and anti-rIL-1 antibody formation by ELSA after 6, 8, and 15 days of rIL-1 administration. On days 6 and 8 there was clinical evidence of toxicity (decreased body weight and unkemptness). In addition, there were decreases in alanine aminotransferase and alkaline phosphatase, slight anemia, and a profound granulocytosis (6-17 fold). All changes were decreased in magnitude or back to normal on day 15. Anti-rIL-1 antibodies were not present on day 6, but were present on days 8 and 15. These studies show that antibodies to rIL-1 develop in mice after repeated IV administration and that tolerance to rIL-1 develops with continued administration. The role of factors other than antibody in inducing tolerance to rIL-1 remains to be determined.

EFFECT OF RECOMBINANT ERYTHROPOIETIN (rHuEPO) ADMINISTRATION IN MONKEYS. A M Dempster, I L Smith and G J Davis. Research Laboratories, Ortho Pharmaceutical Corporation, Raritan, N J.

To determine if the progenitor cell population of the monkey becomes refractory to exaggerated doses of rHuEPO, Cynomolgus monkeys were intravenously injected with 0, 100, 500, or 1000 U/kg rHuEPO daily for 13 weeks. Peripheral blood parameters including hematologic and serum biochemical indices were evaluated at pretest, weeks 8 and 13, and 8 and 13 weeks postdose. At both 8 and 13 weeks, red blood cell count, hemoglobin concentration and packed cell volume were significantly increased in all rHuEPO-treated monkeys. Five hundred and 1000 U/kg rHuEPO produced a 50-60% increase in red blood cell count whereas 100 U/kg rHuEPO resulted in a 30-40% increase when compared to controls. Erythropoiesis appeared to be maximally stimulated in that no significant increases in red blood cell parameters were noted at week 13 compared to week 8. Serum EPO profiles were determined on days 5 and 89; no changes in the kinetics were observed. All red blood cell indices returned to baseline levels by 8 weeks postdose. In conclusion, the progenitor cell population of the monkey was dramatically increased and was never refractory throughout the entire dosing period but returned to baseline levels once dosing had ended.


Recombinant interleukin-2 (rIL-2) is a novel protein drug working through the host's immune system to stimulate cell-mediated anticancer activity. The mouse, rat, rabbit, and sheep treated with rIL-2 exhibited similar constellations of toxicities which were reversible and generally demonstrated an "exuberant pharmacological" response. Dose dependent deaths; clinical signs of hepatotoxicity and respiratory distress; laboratory findings of anemia, thrombocytopenia, lymphocytosis, eosinophilia, and elevated liver enzymes; postmortem findings of hepatosplenomegaly, subcutaneous edema, pulmonary edema, and lymph node enlargement; and histopathological findings of extramedullary hematopoiesis, pleocellular infiltration, lymphoid infiltration, lymphoid hyperplasia, and reticuloendothelial cell hyperplasia were observed variously in one or more of the species. Increased lung-lymph flow, fever, and decreased peripheral vascular resistance were measured in the sheep (200 ug/kg/day by intravenous bolus). Mouse lymphoid organs demonstrate null cell (Thy', Ly-1-Ly-2, L3T4) hyperplasia. At doses in excess of 1 mg/kg/day by intravenous bolus for ten days, the cause of death in the rat is hepatic necrosis due to severe extramedullary hematopoiesis.
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