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Abstracts of the 1984 Annual Meeting
Preface

This issue of the Toxicologist is devoted to the abstracts of the presentations for the platform and poster sessions of the 23nd Annual Meeting of the Society of Toxicology, held at the Atlanta Hilton Hotel, Atlanta, GA, March 12-16, 1984.

An alphabetical Author Index, cross-referencing the corresponding abstract number(s), appears on pages 257-264.

The issue also contains a Keyword Index (by subject or chemical) to the titles of all the presentations, beginning on page 195. The Keyword Index was prepared by Elton R. Homan, Bushy Run Research Center.

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Male and female B6C3F1 mice were exposed to methyl bromide at either 0, 12, 25, 50, 100, or 200 ppm 6 hr/day, 5 d/wk for 10 days or, in a separate study, to 0, 10, 20, 40, 80, or 120 ppm 6 hr/d, 5 d/wk for 13 weeks. Mice were evaluated for cytogenetic (SCR and micronuclei), reproductive, hematologic, neurobehavioral and histopathologic effects. In the 10 day study minimal mortality was seen only at the highest dose (6/10F and 9/10M). Neurobehavioral effects consisting of tremors, increased jumping and paralysis were seen primarily in the two highest dose groups. Histologic changes were minimal. In the 13 week study little exposure-related mortality was noted. The most prominent effects reflected during exposure were marked neurobehavioral changes. After six weeks, the males exposed to 120 ppm exhibited pronounced curling and crossing of the hind limbs with difficulty in locomotion. These symptoms appeared to decrease throughout the remainder of the study. Results of cytogenetic, reproductive, hematologic, neurobehavioral and histologic studies will be presented.

Supported by the NTP under Interagency Agreement No. 3 Y01-ES-10087 and by the USDOE Contract No. DE-AC02-76CH00016.

2. PULMONARY RESPONSES TO INHALED TERBIUM PEROXIDE (Tb4O7). A. Jacobs, H. Reiter, J. F. Renfro, and P. Moraw. University of Rochester Medical Center, Department of Radiation Biology and Biophysics, Toxicology Division, Rochester, NY

Terbium peroxide (Tb4O7) is the most stable oxide of terbium in distinction to other rare earth oxides which have the general formula, M2O3. Terbium is regarded as a transitional lanthanoid, i.e., having properties of the light lanthanoids, no biological studies of Tb4O7 have been performed and no inhalation studies of any Tb compound have been reported. Beagles were exposed to a neutron-activated 99.9 percent Tb4O7 aerosol for up to 2 hours via an endotracheal tube while anesthetized.

Initial deposition ranged from 5.2 to 1423 µg 160Tb4O7 when exposed to a 2.4 µm MMAD (log = 1.9 aerosol) having an initial specific activity of 3.7 x 1026 bq µg-1. Pulmonary retention measurements were carried out in vivo and after sacrifice (78 to 116 days post-exposure) by counting the 0.78 - 1.56 MeV gamma photons. In five studies, the mean effective half-life for 160Tb4O7 in the dog lung was 50.5 days (S.D. 6.0) inferring a mean biological half-life of 164 days. Histopathologic investigation of the lung at the time of sacrifice revealed a minimal macrophage response and a major association of retained TbO7 with the pulmonic lymphatics. A number of pathologic changes were also noted, including small alveolar epithelial metaplasia, focal emphysema, and smooth muscle hypertrophy.

3. PROTECTION OF PULMONARY ENDOTHELIAL CELLS FROM OXYGEN RADICAL DAMAGE AND MAINTENANCE OF NAD/NADH BY SOD, NICOTINAMIDE OR NICOTINAMIDE B1, J. L. Thies and A. P. Autor. Department of Pharmacology, University of Iowa, Iowa City, IA 52242, USA

Oxygen radical damage in the lung is associated with hyperoxia and oxygen radical-producing leukocytes, which increase and adhere to lung microvasculature during chronic inflammation. The initial site of damage in the lung is the endothelium. To study the effects of oxygen radicals, a model system of endothelial cells was derived from the bovine pulmonary artery and grown to confluent monolayers in primary culture. Autooxidizing dihydroxyfumarate (DHF) was used as a controlled source of oxygen radicals. Toxicity to the cells was assessed by measuring release of cytosolic lactate dehydrogenase into the medium. Previous studies showed that superoxide dismutase (SOD), 10 mM nicotinamide (NA) and 10 mM picolinate (PA), when added to the monolayers at the same time as DHF, provided 100%, 72% and 77%, respectively, protection against DHF-induced toxicity. SOD did not provide protection when added > 15 min after DHF, whereas 10 mM NA provided protection up to 2 hr after DHF. The ability of SOD or the poly ADP-ribose synthetase inhibitors, NA and PA, to prevent NAD+ NADH (total NAD) depletion was assessed by HPLC 30 min after the various interventions. Total NAD levels were 10%, 62%, 41% and 38% of control cells after incubation in DHF alone, DHF+SOD, DHF+10 mM NA and DHF+10 mM PA, respectively. Supported by NIH grants GM12675 and training grant GM07065.

4. DIFFERENCES IN NASAL CAVITY TOXICITY BETWEEN RATS AND MICE EXPOSED TO ACIDIC ACID VAPOR. L. A. Buckley, R. A. James, and C. S. Barrow. Chemical Industry Institute of Toxicology, Res. Tri. Pk., NC

Inhalation exposure of F344 rats and B6C3F1 mice to 5, 25, or 75 ppm acetic acid (AA) produced pathologic lesions confined to the nasal passages, and revealed that mice were more susceptible than rats to AA toxicity (Miller et al., Fund. Appl. Toxic., 1:271, 1981). This study was designed 1) to compare the nasal "dose" of AA in F344 rats and B6C3F1 mice and 2) to quantitate the sensory irritation potency of AA in both species. Groups of rats and mice were pre-treated to 75 ppm AA (6 hr/day, 4 days) and on the 5th day respiratory rate and tidal volume were recorded during exposure (75 ppm, 6 hr). From these, minute volume was calculated, and the "dose" of AA (µg/min/cm²) was derived. For sensory irritation studies, respiratory rate depression was measured during exposure of animals to various concentrations of AA. The concentrations required to reduce respiratory rate by 50% (RD50) and 10% (RD10) were 685 and 47 ppm in mice and 213 and 48 ppm in rats. Minute volume changes during the 6th hour, 75 ppm exposures were also similar, with each species showing a decrease of 20 to 30%. However, after normalization of the dosimetry to nasal surface area, mice received a nearly two-fold increase in calculated "dose" compared to rats. These results demonstrate the need for quantifying minute ventilation to better understand species differences in nasal toxicity from inhaled sensory irritants.
Certainly graphite fibers may have fibrogenic activity as demonstrated by cytotoxicity in the RAM test. This current study determined the cytotoxicity of metal electroplated graphite fibers and bare PAN type graphite fiber in the RAM test. Alveolar macrophages were incubated in triplicate with graphite fibers (1.1 - 1.5 mm) at concentrations of 0.01 to 1.0 mg/ml. After macrophages were incubated for twenty hours, cell viability and ATP content were determined to assess cytotoxicity. The positive control for these tests, crystalline silica, produced a 91% and 97% decrease in cell viability (EC50 = 0.2 mg/ml) at silica concentrations of 0.3 and 1.0 mg/ml, respectively. Nickel on graphite, silver over nickel on graphite, and bare graphite fibers had EC50 values greater than 1.0 mg/ml. None of the graphite fibers had a significant effect on ATP content at levels up to 1.0 mg/ml. Since the degree of toxicity is considered non-detectable if the EC50 value is greater than 1.0 mg/ml, the graphite fibers were determined to have no detectable cytotoxicity in the RAM test.


An aerosol generator was designed, built and characterized. The generator consisted of a vertical column which is closed off at both ends with dental rubber dams. A loudspeaker, driven with 10 Volts at 60 Hz delivers the energy into the column. The energy, in the form of sound waves, agitates the bulk cotton dust which is placed inside the column. This agitation shakes loose small cotton dust particles from the fibrous bulk material. These particles are carried away by an airstream which passes through the column. The output can be conveniently connected to the animal exposure chamber.

Thirty grams of bulk dust, agitated for 1/2 hours, will provide enough particles to maintain a constant aerosol concentration of about 80 mg/m^3 at a flow rate of 5 L/min. Lower concentrations can be achieved by dilution. Aerodynamic mass median diameter was about 3 μm with a geometric standard deviation of 1.7.

The generator is easy to build, reliable and easy to maintain.

Supported by USDA Cooperative Agreement.


Inhalation of cotton dust has been associated with the respiratory syndrome hyssinosia. Symptoms include dyspnea, chest tightness and difficulty breathing upon exertion. An animal model for this disease is under development. Guinea pigs were exposed to cotton dust for 6 hours per day, 5 days per week for a 6 week period. Chamber atmospheres contained 20 mg/m^3 cotton dust. Inhalation of cotton dust resulted in increased respiratory frequency and decreased tidal volume. Respiratory parameters were measured both when animals were breathing room air, and air enriched with 10% CO2. Maximum response was noted 18 hr following the initial exposure. Subsequent exposure to cotton dust on weekdays resulted in minimal respiratory response. However, every Monday, a heightened respiratory reaction was observed. Control animals maintained in identical plethysmographs and exposed to ambient air gave no respiratory response. Similarly, no response was observed when guinea pigs were exposed to 20 mg/m^3 cellulose dust. The pattern of response in animals mimics that described for cotton workers. Further development of the animal model should allow comparison between dusts of potency and permit recommendation of cotton dust safe exposure levels for man. Supported by USDA, Cooperative Agreement and NIEHS 1 R01-ES02747.


Symptoms of hyssinosia, resulting from exposure to cotton dust, include chest tightness, dyspnea, and difficulty breathing upon exertion. An animal model for the disease is under development in this laboratory. Exposure of guinea pigs to 20 mg/m^3 cotton dust resulted in increased respiratory frequency and decreased tidal volume. Reactions were monitored when animals were breathing room air or air containing 10% CO2, 20% O2 and 70% N2 (CO2-driven response). The presence of a concentration-response relationship between the concentration of dust and respiratory response was examined. Guinea pigs were exposed to dust concentrations ranging from 1 to 27 mg/m^3. Exposure was for 6 hr. Concentration-response data were generated for respiratory frequency, tidal volume, and SpO2 (oxygen content in the blood) caused by animal respiration) at 6 and 18 hr. Each function was measured when animals were breathing room air and when breathing the CO2-enriched air. Highly significant concentration-response correlations were obtained. Recognition of the concentration-dependent relationship for the acute respiratory response to cotton dust will permit comparison between potencies of various grades and sources of dust, as well as between various dust washing treatments. Supported by USDA Coop. Agrnt. and NIEHS 1 R01-ES02747.
9 BACTERIAL AND ENDOTOXIN CONTENT OF COTTON DUST CAUSING ACUTE RESPIRATORY REACTIONS IN GUINEA PIGS

Exposure of guinea pigs to respirable particles of cotton resulted in an acute "Monday morning" respiratory response. The response was typified by increased respiratory rate and decreased tidal volume following a 6 hour exposure to cotton dust. A similar response was observed following inhalation of purified E. coli endotoxin. Controversy exists concerning the etiologic agent responsible for the cotton dust response. An investigation was undertaken of the bacterial and endotoxin content of the dust. Cotton dust atmospheres were created by resuspending dust collected from the condenser waste system of a textile mill. The microbiological content of the dust in the animal chambers was examined and compared with that of dust before the resuspension process. Both sources yielded 10^5 gram negative bacteria per g dust. The most prevalent species were Enterobacter agglomerans and Klebsiella pneumonae. Thermophilic bacteria and fungal counts were typical of those found in card room dust. The endotoxin content of dust averaged 400 μg endotoxin per gram dust, a value typical of that found in cardroom dust. It was concluded that the regeneration system for exposure of animals to cotton dust had not caused a change in the microbial flora of the resuspended cotton dust. Supported by USDA Cooperative Agreement.


Fischer-344 rats were exposed to 0, 2, 10 or 20 mg/m^3 of crystalline quartz (Mn-U-511 S) for 6 hr/d, 5d/wk to study the development of fibrosis. Lung tissue from each exposure group was analyzed for water, protein, DNA, elastin, and collagen content after 3 and 6 months of exposure and 6 months following 6 months of exposure. After 3 months the connective tissue content was slightly elevated in all of the silica exposed groups. Six months of exposure resulted in well-defined dose-dependent increases in both elastin (6.9, 7.4, 8.2, and 9.0 mg/lung) and collagen (2.5, 2.9, 3.2, and 3.4 mg/lung) in the 0, 2, 10, and 20 mg groups. Similar dose-dependent changes were observed in total lung elastin and collagen following the 6 month recovery period. However, when the lung components in this final group were expressed as a function of dry weight significant decreases were observed in the 20 mg/m^3 group, indicating an increase in an unmeasured tissue component. An increased lipid content is hypothesized because lipo-proteinosis has been demonstrated in some silica-exposed animals. Preliminary findings using proton activation analysis have indicated limited clearance of silica from the lungs of the 20 mg/m^3 group during the 6 month recovery period. Supported by the NTP under Interagency Agreement 222-YO1-ES-9-0043 and USDOE No. DE-AC02-76CH00016

11 MULTIPLE ASSESSMENT OF FIBROSIS IN THE RAT AFTER SUBCHRONIC INHALATION OF CdCl₂ AEROSOL.

Cadmium chloride-induced lung lesions in the rat were used to compare measures of pulmonary mechanics and connective tissue composition to conventional morphological assessment of disease. Groups of 24 male, F-344 rats (SPF) inhale CdCl₂ aerosol (VMD 0.7 μm, 3g 1.17) at 0, 0.2, or 1.0 mg/m^3 for 6 h/d, 5 d/wk for 62 days. One week post-exposure, each rat underwent a series of lung function tests, after which the lungs were prepared for histopathology and connective tissue analysis. The predominant morphologic changes were in the end- Airways. The hyperplasia and flattening of Type II cells, the inflammation, and the proliferation of fibroblasts were dose-dependent. Compositional alterations due to Cd were generally in parallel with increased lung weights, except for hydroxyproline and elastin which were disproportionately high at 1.0 mg/m^3. The restrictive functional lesion at 0.2 mg/m^3 appeared to be primarily interstitial with reduced lung compliance and diffusion capacity and slightly augmented flow. At 1.0 mg/m^3, pronounced obstruction to airflow was probably due to the enlarged airway-associated foci of fibrosis observed morphologically. Only the functional indices of parenchymal disorder correlated well with the subjective scoring of pathology and relative concentrations of connective tissue. USDOE # DE-AC02-76CH00016; NTP # 222-YO1-ES-9-0043

12 FUNCTIONAL CHARACTERISTICS OF ALVEOLAR MACROPHAGES FOLLOWING THE DEPOSITION OF IRON OXIDE IN THE LUNG. B.E. Lehner* and P.E. Morrow, Los Alamos National Laboratory, Los Alamos, NM, University of Rochester, Rochester, NY.

Rats were exposed to aerosols of iron oxide (MMAD=1.6 μm, σg=3.0) at a nominal concentration of 20 mg/m^3 for 2 hr in order to determine how the deposition of a low lung burden (=30 μg) of innocuous particles affects the size of the lavageable alveolar macrophage (AM) pool, and the subsequent functional status of AM as assessed in vitro by their abilities to: 1) exclude trypan blue, 2) adhere to plastic substrate, and 3) phagocytize and bind sheep erythrocytes opsonized with immunoglobulin G (SRBC-IgG). Iron oxide deposition did not bring about significant changes in: 1) cell types or numbers of AM lavaged, 2) AM viabilities, or 3) the plastic substrate adherence characteristics of AM. As of 1 day postexposure, however, the ability of AM to phagocytize SRBC-IgG was increased. Phagocytosis was maximally enhanced 3-7 days postexposure, and returned to pre-exposure levels by 20 days after exposure. The increase in phagocytic activity correlated with an increase in AM avidities for SRBC-IgG. The kinetics of the phagocytic response did not parallel the alveolar clearance rate of the deposited particles (τ/2 biog = 54 days). The results of these studies show the deposition of a low lung burden of iron oxide transiently enhances Fc receptor-mediated particle binding and phagocytosis by AM.

Occupational exposure to asbestos clearly is associated with fibrotic lung disease. Pulmonary macrophages have been proposed as mediators in the pathogenesis of this disease. We have shown in rats that lung macrophages migrate to sites of asbestos deposition to form a component of an early lesion. We sought the mechanism(s) by which these cells accumulate at alveolar duct bifurcations. Our results show that chrysotile asbestos fibers activate serum complement in vitro to stimulate pulmonary macrophage chemotaxis. In addition, protein lavaged from the lungs of asbestos-exposed rats produced a significantly enhanced chemotactic response compared to sham-exposed controls. The MW of the chemotactic factor was in the 14-18,000 range. Furthermore, the chemotactic activity of sham-lavaged protein was potentiated following in vitro incubation with chrysotile fibers. To determine the nature of the factor, congenic strains of C57 sufficient (C57) and deficient (C57/-) mice, as well as normal and cobra venom factor (CVF) treated rats were exposed to chrysotile for 3 hrs. The numbers of macrophages at sites of asbestos deposition were depressed (p<0.01) in the C57 and CVF-treated animals compared to controls. To conclude that inhaled chrysotile fibers activate complement-derived chemotactic factors for macrophages on alveolar surfaces.

14 EFFECT OF VAPOR CONCENTRATION ON THE DISPOSITION OF INHALED 2,3-DICHLOROPROPENE IN FISCHER-344 RATS. J.S. Dutcher, N.A. Medinsky, J.A. Bond, Y.S. Cheng, M.B. Snipes and R.F. Henderson. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM; L.S. Birnbaum, NIHES, Research Triangle Park, NC.

The mutagen, 2,3-dichloropropene (DCP), has high human exposure potential due to its industrial and agricultural use. Elimination of 14C was studied in rats after nose-only inhalation of 1.3, 27 or 180 µg of 14C-DCP vapor/L of air for 6 hr. Urine, feces, and expired air were collected for 60 hr. Rats were then sacrificed and tissues, carcass, excreta, and expired air analyzed for 14C. Excretion patterns were independent of dose, with < 50% of the 14C excreted in urine, 15% in feces, 10% as CO2, and < 1% as DCP. Although 14C was generally distributed throughout tissues and carcass (25%), somewhat higher concentrations were found in the respiratory and GI tract and tissues with high metabolic capacity. The excretion pattern resembled that after po or ip administration of DCP except that more 14C was excreted in the urine and little remained in the body after po or ip administration. Results indicate DCP metabolism and excretion rates are relatively constant throughout the dose range used and results from more detailed pharmacokinetic studies (and possibly toxicity studies) at one DCP concentration may be extrapolated to other concentrations within this range. (Supported by NIHES through interagency Agreement with U.S. DOE Contract No. DE-AC04-76EV01013.)


Primary cultures of intact, functional hepatocytes were used to compare the relative toxicity of four well-known anti-inflammatory agents (indomethacin, benoxaprofen, aspirin, and ibuprofen) with that of orpanoxin. We evaluated the relative toxicity of these compounds on the basis of LDH leakage, total area (an indicator of a liver specific function), cellular viability (based on dye exclusion), and morphology after a 12-hour exposure to doses ranging from 0 to 1000 µM. Comparison of data generated from the first three parameters showed that these compounds were toxic in the following order: indomethacin > benoxaprofen > ibuprofen > aspirin > orpanoxin. Morphological evaluations of hepatocytes exposed to these agents under these conditions supported these results. Ultrastructurally, indomethacin-exposed cells at all doses were severely damaged as evidenced by marked cellular necrosis, nuclear pleomorphism, margination, swollen mitochondria, reductions in the number of microvilli, smooth endoplasmic reticulum proliferation, and cytoplasmic vacuolation. In comparison, exposure of hepatocytes to only the highest dose of orpanoxin resulted in increased vacuolation and pyknosis in a number of cells, as well as a moderate increase in cellular necrosis. Supported by Norwich Eaton project #725.59.00-PC.


The development of a transplantation system by which rat hepatocytes can be implanted and remain viable in the interscapular fat pads of two-thirds hepatotomized syngeneic hosts, enabled us to determine that such transplanted hepatocytes retain the capacity to recognize and respond to the peroxisome proliferators cipofibrate, a hypolipidemic compound and 1,2-(2-ethylhexyl)phthalate (DEHP), an industrial plasticizer. In the present study we have utilized the xenotransplantation system: isolated hepatocytes obtained from cat, dog and human liver were heterotransplanted into the interscapular fat pads of partially hepatotomized athymic nude mice. Nude mice with transplanted hepatocytes were fed either cipofibrate (0.03%) or DEHP (2%) containing diets for 10-16 days. Transplanted hepatocytes, as well as pieces of homotopic (host) liver were processed for electron microscopy. Morphometric analysis revealed that both cipofibrate and DEHP increased the volume density of peroxisomes in dog and cat hepatocytes transplanted in nude mice. Transplanted human hepatocytes also responded to the peroxisome proliferative effect of cipofibrate. The results suggest that hepatocytes of humans, primates and other species heterotransplanted into nude mice should provide a valuable model system for toxicologic evaluation.
APPLICATION OF ACUTE FOOD CONSUMPTORY BEHAVIOR FOR TOXICOLOGIC INVESTIGATIONS IN RATS: I. EFFECTS OF ANTIAPPETITE DRUGS. L. R. Weiss, Food and Drug Admin., Washington, DC 20204.

Food consummatory measurements (FCM) are mainstays for prolonged adimix drug and chemical evaluations. Acute FCM has been generally ignored as an index of toxicity or adverse drug effects. We have developed a rat model to study the antiappetite actions of drugs that may be useful for studies unrelated to this action. Adult rats can be rapidly trained to consume their daily food in 4 hrs., 5x wk followed by an ad lib two day off period to maintain body weight and well-being. Drugs are given by gavage (5 ml/kg) 30 min prior to adding preweighed feeders to cages on Day 3. Day 1 is used as a "washout" period. Day 2 is the pretreatment day. Day 4 and 5 are post-treatment residual days. Significant effects on acute FCM can be differentiated between treatment and vehicle on Day 3 and by comparing FCM of each animal on pre and post days with Day 3. Using this method, OTC antiappetite drug, phenylpropanolamine (PPA) was compared with ephedrine (EPH), dextroamphetamine (DAM), and p-chloroamphetamine (PCA). The results show that aminergic PCA at 3.1 mg/kg, inhibits FCM on Day 3 and 4 and the adrenergic DAM caused inhibition at 6.2 mg/kg on Day 3. PPA and EPH were effective on Day 3 at 12.5 mg/kg. The data suggest acute FCM provides an objective tool to judge certain pharmacologic and toxicologic potency profiles.

TOLERANCE OF REPEATED INTRACISTERNAL INJECTIONS OF IODEXOL IN MONKEYS. E. J. Fabian, B. A. Mayes, T. A. Barbolt and H. P. Drobeck.

Iodexol is a new, nonionic contrast medium for myelography. To assess its potential for producing CNS and systemic toxicity, it was administered to cynomolgus monkeys intracisternally in a variety of experimental designs. Having determined the safety of two injections, given 9 days apart, the effect of an exaggerated regimen of five injections given once per week for five weeks was evaluated. The results revealed CNS inflammation, as evidenced by elevations in WBC in the cerebrospinal fluid (CSF) and mild subarachnoiditis. Assessments of the effects of vehicle, isotonic and hypertonic saline and the trouse of repeated injections into the subarachnoid space have shown that these control procedures induced similar increases in WBC in the CSF and mild inflammatory response in the CNS.

The results of these studies indicated that subarachnoid injections of Iodexol produced no greater changes than comparable injections of vehicle or saline.

RATE OF METHEMOGLOBIN FORMATION OF SEVERAL 8-AMINOQUINOLINES IN VITRO IN CANINE HEMOLYSATES. C.M. Link, A.D. Theocharides, and H. Chung. Department of Pharmacology, Walter Reed Army Institute of Research, Washington, DC 20037

A rapid and reproducible assay which measures the initial rate of methemoglobin (Mhb) formation induced by exogenous chemicals has been developed. The assay utilizes RBC hemolysates in which the pyridine nucleotide dependent reductases are removed. With this assay, the rates of Mhb formation of several 8-aminoquinoline compounds and postulated primaquine metabolites were determined.

Primaquine (N4-[6-methoxy-8-quinolinyl]-1,4-pentanediamine) and WR 6026-2HCl (N,N-diethyl-N'-(6-methoxy-4-methyl-8-quinolinyl)-1,6-hexanediamine) do not significantly stimulate the rate of Mhb formation, whereas WR 242,511 (N4-[5-hexyloxy]-6-methoxy-4-methyl-8-quinolinyl)-1,6-hexanediamine, WR 238,605 (N4[2,6-dimethoxy-4-methyl-5-(3-trifluoromethyl) phenoxo]-8-quinolinyl]-1,4-pentanediamine) and WR 225,448 (N4-[6-methoxy-4-methyl-5-(3-trifluoromethyl) phenoxo-8-quinolinyl]-1,4-pentanediamine) all significantly stimulate the rate of Mhb formation with descending rates respectively. Studies with postulated metabolites of primaquine, i.e., 5-hydroxy-6-desmethyly derivatives and 5-hydroxy derivatives indicate that 5-hydroxy-6-desmethyl derivatives are the most potent Mhb forming 8-aminoquinoline compounds discovered thus far in this assay.


Nolinium bromide (NB) has gastric antisecretory-antiulcerogenic and intestinal spasmodic activity in experimental animals. Preclinical toxicopatliology results indicated the dog as an oversensitive species to the effects of the drug. In an attempt to modify overt responses, NB was given po as coated (C) tablets to dogs at 30, 60, 120 and 240 mg/kg/day for 6 weeks. Comparable dosage groups (except 30 mg/kg/day) received uncoated (U) tablets. Signs of toxicosis appeared sooner with greater severity after administration of the UNB than the CNB. Time-dosage related effects ranged from minimal body weight loss, moribundity and death (time to initial necropsy was 12 days for U vs. 24 days for C with 240 mg/kg/day); mildly depressed hemograms to frank anemia; increased hepatic enzyme activities indicative of varying degrees of pericholangitis; and inhibition of GI motility that led to stasis, which indirectly caused mucosal cysts, necrosis and ulcers. Lymphanitis, pancreatitis, thymic and lymphoid atrophy, neutrophilia, pycnoliphesis, and adrenalsitis appeared in a similar dose-time relationship among the dogs.
21 ACUTE AND SUBACUTE TOXICITY EVALUATION OF A NOVEL ANTICHOLINERGIC BRONCHODILATOR

The orally active anticholinergic bronchodilator 3-[2-(ethylamino)propyl]-1,2,3,4-tetrahydro-5H-[1]benzopyrano[3,4-c]pyridin-5-one (CI-923) was tested for toxicity in rodents and dogs. LD50 values in mice and rats following single oral doses were approximately 300 mg/kg for both sexes. Approximated maximum tolerated dose in dogs was 350 mg/kg. Intolerance was manifest as emesis, tremors, ataxia and diarrhea. Rats tolerated up to 300 mg/kg/day for 2 weeks and were devoid of clinical biochemistry, hematology, urinalysis or pathology findings. Dogs treated with 12.5 mg/kg/day for 2 weeks showed no changes; 25 and 40 mg/kg induced emesis; and 50 mg/kg caused ataxia, tremors, ptosis of eyelids and electrocardiograms showed prolongation of QRS complexes. Hyperplasia of lymphoid tissue in the intestinal tract was noted at all dose levels. Even though there were exaggerated pharmacologic effects, the magnitude of the significant cardiovascular toxicity.


The novel cardiotonic agent 4,5-dihydro-6-(1H-imidazol-1-yl)phenyl]-3(2H)-pyridazinone hydrochloride (CI-914) was evaluated following iv administration to mice, rats, dogs and monkeys. After single iv dosing, LD50 values were 226 and 228 mg/kg for male and female mice, and 136 and 98 mg/kg for male and female rats, respectively. In a 4 week study, 10 rats/sex/group received 15, 30 and 60 mg/kg/day; 15 mg/kg/day revealed no overt toxicity. Levels of 30 and 60 mg/kg/day increased salivation with swelling of cervical region and increase in BUN. Body weights were decreased in rats at 60 mg/kg. Non-specific myocarditis was found in the 30 and 60 mg/kg groups. Escalating dose studies in dogs (0.05 to 25.6 mg/kg over 14 days) and in monkeys (0.05 to 51.2 mg/kg over 13 days) evoked emesis, reduced activity, anorexia, weight loss, and pallor of mucosae at levels above 6.4 mg/kg. Heart and liver were target organs of toxicity in monkeys and dogs; the gastrointestinal tract and the vascular system were also involved in dogs. A 4 week study in monkeys at 0.5, 1.0 and 2.0 mg/kg elicited no clinical or pathologic drug-related toxicity, establishing adequate margins of safety for clinical studies.


The toxicity of pirmenol HCl, a new antiarrhythmic drug, was studied in dogs and rats for long-term chronic effects. Previous safety evaluation was based on preclinical subacute studies (Schardin et al., Tox. Appl. Pharmacol. 56:294, 1981). Treatment was for 52 consecutive weeks; dogs were given po 10, 20 and 30 mg/kg/day and rats received diet mixes yielding 25, 50 and 100 mg/kg/day. In dogs, electrocardiograms showed non-dose related increased heart rates with sinus rhythm and no wave irregularities. Transient responses represented pharmacologic reactions, such as dry oral mucosae and sporadic emesis. Rats showed weight gain suppression and lower food consumption; slightly reduced serum glucose was seen at 50 and 100 mg/kg. There were no overt drug-related aberrations seen in the remaining parameters, indicating that pirmenol HCl does not possess detrimental effects on experimental animals on a long term basis.


KJ, an antibiotic produced by Actinomadura sp., has been shown to be a potent P. aeroxus lipase inhibitor. Acute oral LD50’s were 92000 mg/kg in rats and mice and >960 mg/kg in dogs. Acute intraperitoneal LD50’s were approximately 125 mg/kg in rats and 170 mg/kg in mice; at necropsy, signs indicative of peritonitis were observed in both species. Daily oral administration of KJ for two weeks at doses up to 60 mg/kg in rats and dogs resulted in no evidence of compound-related toxicity. The absence of microbiological activity in dog serum suggested the lack of or incomplete absorption following oral dosing. Dermal sensitization testing of KJ in guinea pigs by the Maximization Test (Magnusson, B. and Klignman, A.M.: J. Invest. Derm. 52:268-276, 1979) indicated the compound to be a potentially strong (grade IV) sensitizer.
25 ORAL TOXICITY EVALUATION OF A NOVEL CARDIOTONIC

A novel cardiotonic agent, 4,5-dihydro-6-[4-(1H-imidazol-1-yl) phenyl]-2(3H)-pyridazinone HCl (CI-914), was evaluated in rodents, dogs and monkeys. Following single oral doses, LD50 values were 250 mg/kg and 125 mg/kg in mice and rats, respectively. In multiple dose studies, 5 to 75 mg/kg/day given to rats for 2 weeks were non-toxic, except for lower body weight gain in females at 25 mg/kg and males at 50 mg/kg or above. Plasma protein, HbC, hemoglobin and hematocrit were elevated at 25 mg/kg without lesions. Doses of 5 to 30 mg/kg/day to rats for 13 weeks reduced body weights in females only. Cholesterol, LDL and CPK were increased in males and females, and alkaline phosphatase in males only. Liver showed increased weights and microscopic vacuolation of hepatocytes. Administration of 2.5 to 10 mg/kg/day to dogs for 2 weeks resulted in emesis, anorexia, depression and tachycardia. Electrocadiography, clinical laboratory and pathology established the heart as target organ. Monkeys dosed with 5 to 10 mg/kg/day for 2 weeks showed myocardial lesions. In a 13 week study, monkeys given 1-8 mg/kg/day showed no significant toxicity, with an adequate margin of safety.


Carcinogenicity and chronic toxicity potential of meclofenamate sodium, a new non-steroidal antiinflammatory agent, was evaluated in CF1 mice. Groups of 65 mice /sex received meclofenamate sodium as dietary admixture at 2, 4, and 6 mg/kg/day for 92 weeks. Intercurrent mortality was observed at 6 and 6 mg/kg, although survival rates in all groups at study completion were adequate for statistical analysis of tumor data. Clinical or ophthalmic examinations revealed no drug-related findings. Body weights and food consumptions were comparable in all groups. Drug related intercurrent deaths were due to gut lesions comprising ulceration with peritonitis, an expected finding with this class of drug. Most frequent neoplasms in either treated and untreated female mice were alveologenic carcinoma, ovary cystadenoma, uterine polyps, and lymphoid tumors; in males most common tumors were liver cell adenoma and carcinoma, alveologenic carcinoma and lymphoma. Onset, latency, incidence rates and tumor types from treated groups were not significantly different from controls and there was no evidence of dose relationships. Thus, the chronic administration of meclofenamate sodium to mice did not evoke a positive tumorigenic potential.

26 COMPARATIVE CARDIOTOXICITY OF NYSTATIN IN CULTURED RAT MYOCYES AND CELL MEMBRANES, ISOLATED RAT HEARTS AND INTACT RATS. A. Szabo*, A. Panescu, E.F. Reynaldo, G.C. Yang, and A.W. El-Hage*. Department of Toxicology, Drug Biolog and 5Chemistry and Physics, Food and Drug Administration, Washington, D.C. 20020.

Sponsor: S. Green.

The action of nystatin, a polyene antibiotic, was studied in primary cultures of neonatal rat myocytes, isolated rat hearts, and intact rats. Arrhythmias were induced by 10 and 25 µg nystatin/ml within minutes of addition to 7-day cultures of beating myocytes. The myocytes stopped beating after 24 hr without other morphological indications of cytotoxicity. The arrhythmic state could be minimized by elevated concentrations of K+ and Mg2+ or reversed by washing the cells. Similarly, the isolated heart responded to 100 µg nystatin/ml with arrhythmias that could be mollified by addition of K+ and Mg2+. The iv injection of the drug caused heart failure in intact animals at the 4 mg/kg dose level. At the subcellular level, nystatin caused the myocyte membranes to become more rigid, as measured by electron spin resonance spectrometry. These findings indicate a parallel between physicochemical changes caused by nystatin in the myocyte membrane and the biological changes caused by this drug in cultured myocytes, isolated heart, and heart of the intact animal.

28 TOXICOLOGIC ISSUES IN AN EPIDEMIOLOGIC STUDY OF HUMAN CANCER AND EXPOSURE TO DIOXIN-CONTAINING CHEMICALS. J.S. Woods, Battelle Research Center and L. Polissar and R.K. Severson, Fred Hutchinson Cancer Research Center, Seattle, WA

Reports of increased incidence of soft tissue sarcomas (STS) and other tumors in human populations working with phenoxy herbicides and other dioxin (TCDD)-containing chemicals (DCC) have given rise to serious concern regarding the possible risk of malignant lymphomas. This case-control study is evaluating the incidence of STS and malignant lymphomas relative to past DCC exposure among 1,000 men in a part of WA state where DCC have been used for over 30 years. Exposure status among study subjects is established by detailed occupational and residential history acquired by personal interview. Toxicologic issues being studied in relation to DCC exposure include possible alteration mechanisms of cancer induction, dose-response and latency, and the utility of chloroform, skin lesions and porphyria in the diagnostic assessment of past DCC exposure. Interactions of other chemicals and environmental factors are also being studied. It is anticipated that careful assessment of toxicologic manifestations of DCC among study subjects will provide both for improved indices of DCC exposure as well as for a clearer interpretation of potential cancer and other health risks. (Supported by CA 29900)
The disposition of CH₂O was studied in A/J mice to determine the elimination of CH₂O and to assess its accumulation in tissues. Mice were dosed i.p. with 6 mg/kg or 100 mg/kg of ¹³C₁₂H₂O. Most of the dose, 70-75%, was excreted as ¹³CO₂ within 4 hr but an additional 10% of the dose was eliminated as ¹³CO₂ in 24 hr. The rate of ¹³CO₂ excretion in mice given CH₂O was slower than the rate of ¹³CO₂ excretion in mice given an equivalent dose (100 mg/kg) of formate (HCOOH), the obligatory intermediate in CH₂O oxidation to CO₂. These results suggested that CH₂O might accumulate in tissues. To assess this possibility, whole body levels of ¹³C₁₂H₂O were determined by the dinitrophenyl precipitation method following a dose of 100 mg/kg. The elimination t₁/₂ of CH₂O was calculated to be 100 min with a rate constant of 0.42/hr. However, multiple rate constants were observed such that 80% of the dose was recovered as HCOOH at 30 min. At 2 hr, the level of ¹³C₁₂H₂O in the plasma was 1.07±0.25 µg/mL and the liver level was 1.74±0.87 µg/g. ¹³C₁₂H₂O levels in other tissues were similar to the liver level. This level of ¹³C₁₂H₂O is at least 50% lower than the endogenous level of CH₂O which has been reported. These results suggest that there is more than a single CH₂O pool in mice but that, nevertheless, CH₂O does not accumulate in tissues at levels which are significant relative to the endogenous tissue CH₂O level. (Supported in part by NIHES Grant ES07090).

LACK OF INHIBITION OF DRUG METABOLISM BY ETHANOL IN CULTURED HEPATOCYTES. J. Sinclair, L. Zaitlin, L. Smith, P. Sinclair, and H. Bondovsky, VA Hospital, White River Jct., VT and Depts. of Biochemistry and Medicine, Dartmouth Med Sch, Hanover, NH, USA.

In rats and humans, simultaneous administration of ethanol and various drugs, including amphetamine, results in decreased clearance of the drug. Since ethanol is a substrate for cytochrome P450 in vitro, it has been proposed that inhibition of drug metabolism in vivo after acute ingestion of alcoholic beverages results from competitive binding to cytochrome P450 by ethanol. Simultaneous exposure of cultured chick embryo hepatocytes to ethanol (200 mM) and amphetamine (0.65 mM) did not decrease 4-aminoantipyrine (AAP) formation in either control cells (3.2 moles AAP/mg protein/4h) or in cells preincubated for P450 with propylisopropylacetamide (PIMA), a phenobarbital-like inducer (17.2 moles AAP/mg protein/4h). In contrast, hexobarbital (1.5 mM) and testosterone (0.25 mM), other substrates of P450, inhibited amphetamine metabolism 57 and 59%, respectively, with no cell toxicity. Ethanol binds to P450 in microsomes isolated from these cells, as shown by: 1) a reverse type I spectral change (Kₘ=100 mM); 2) competitive inhibition of amphetamine demethylation (K_i=160 mM). CONCLUSIONS: Acute exposure to ethanol does not inhibit amphetamine metabolism in cultured chick hepatocytes. However, ethanol competitively inhibited amphetamine demethylation in chick hepatocyte microsomes. Our results suggest that ethanol does not inhibit drug metabolism in the liver by competitive binding to cytochrome P450.

HEPATIC ADENOSINE 3',5'-PHOSPHATE 5'-PHOSPHOSULFATE (PAPS) AND UDP-GLUCURONIC ACID (UDPGA) CONCENTRATIONS DECREASE AFTER ACUTE ACETAMINOPHEN ADMINISTRATION. J. J. Hjelle, G. A. Hazleton and C. D. Klaassen, Dept. of Pharmacol., Toxicol. and Therupt., Univ. of Kansas Medical Center, Kansas City, KS.

Acetaminophen (AA) sulfation and glucuronidation exhibit capacity-limited pharmacokinetics. Possible AA-induced cosubstrate depletion was examined by measuring the concentration of PAPS and UDPGA in rat liver after administration of various doses of AA (2 hrs after 25, 75, 150, 300 and 600 mg/kg, ip or after different intervals (0.5, 1, 2 and 4 hrs) following 150 or 600 mg AA/kg. AA produced a dose-dependent decrease in PAPS concentration in liver 2 hrs after injection of 150, 300 and 600 mg AA/kg (51% of control after the highest dose). Further, PAPS levels were lowered (40-60% of control) 0.5, 1, 2 and 4 hrs after administration of 600 mg AA/kg. Serum sulfate concentrations were also significantly lowered following AA. A decrease in PAPS concentrations occurred only when serum sulfate was 0.1 mM or lower. Significant decreases in UDPGA concentration were also observed. UDPGA levels declined rapidly to 10, 23, 42 and 83% of control values 0.5, 1, 2 and 4 hrs after 600 mg AA/kg, respectively. These findings demonstrate that AA decreases PAPS and UDPGA concentrations and suggest that cosubstrate depletion is the mechanism for capacity-limited AA sulfation and glucuronidation. (Supported by USPHS Grants ES 07079 and ES 03192).


MDA produces acute cholestatic liver toxicity in rats but not in rabbits, and its disposition was examined to determine what metabolic differences paralleled these findings. A single i.p. dose of ¹³C-MDA was administered to 4 Sprague-Dawley rats (30 mg/kg, 35 µCi), and 2 New Zealand white rabbits (50 mg/kg, 65 µCi). Urine and feces collected daily for 4 days showed that rats excreted 91% of the administered dose, 56% recovered from the feces, while rabbits excreted 97%, with 16% in the feces. Urine specimens (0-48 hrs) subjected to several hydrolysio techniques showed that both species excreted approximately 60% of the total urinary radioactivity as unconjugated metabolites, with another 20% recovered from the rabbit as N-glucuronide or N-sulfate conjugates. Using synthetic standards, the structure of the extractable metabolites was determined by TLC/autoradiography, HPLC, and GC/MS. MDA (45%), N-acetyl MDA (13%), and N,N-diacetyl-4',4'-diaminobenzeno (6%) were the major metabolites isolated from rabbit urine, with small quantities of azoxy, benzophenone and glycolamide metabolites; while the diacetylated benzylidene (42%) was the main metabolite from rat urine, with small amounts of acetylated phenols, benzophenones, and MDA derivatives detected. Thus there are differences in the routes of excretion and metabolism that may influence the susceptibility to MDA hepatotoxicity. (Supported in part by a Monsanto Fund Fellowship and GM 27028).

N-Acetyl-p-benzoquinoneimine (NAPQI) has been proposed as the toxic metabolite of acetaminophen (APAP) for the past 10 years. We report for the first time 1) direct detection of NAPQI formed as an oxidation product of APAP by cytochrome P-450 and cumene hydroperoxide, and 2) indirect evidence that is compelling for NAPQI formation from APAP by P-450, NADPH, and NADPH-cytochrome P-450 reductase. NAPQI was monitored by HPLC using electrochemical and radiochemical detection. The concentration of NAPQI was 2.6x10^-7 M in this system. However, NAPQI was not detectable when purified P-450 oxidation of APAP was supported by NADPH and NADPH-cytochrome P-450 reductase or in mouse liver microsomes combined with an NADPH regenerating system. Evidence is provided for the rapid reduction of NAPQI back to APAP by NADPH and NADPH-cytochrome P-450 reductase such that levels of NAPQI were below our detection limits.

In mouse liver microsomal incubations, radioabeled analogs of both NAPQI and APAP bound covalently to microsomal protein and the binding was decreased by GSH with formation of 3-gluta-thionylacetaminophen. Binding was decreased by ascobic acid and NADPH with reduction of NAPQI to APAP. Partitioning experiments show that NAPQI is a major metabolite of APAP, but most is rapidly reduced back to APAP.

35 THE EFFECT OF REPRODUCTIVE STATE AND VEHICLE ON THE DISPOSITION OF 14C-2,4,5,2',4',5'-HEXACHLOROBIPHENYL (6-CP) FOLLOWING IV ADMINISTRATION. M.J. Vodicnik and J.J. Leech. Medical College of Wisconsin, Milwaukee, WI

The disposition of 6-CP was examined in virgin, pregnant and lactating mice following its IV administration in either Emulphor:saline (E, 0.5 μl) or in association with human very low density lipoproteins (VLDL, 0.35 μl). Mice were sacrificed after 2, 5, 30, 60 and 720 min. [6-CP] between adipose tissue (AT) and mammary gland (MG) did not differ in virgins at any sacrifice time regardless of vehicle (1 h: AT = 0.47 ± 0.09 μg/g, MG = 0.47 ± 0.07 μg/g; VLDL: AT = 0.26 ± 0.07 μg/g, MG = 0.19 ± 0.02 μg/g). Using E as vehicle, [6-CP] was not different between AT and MG in pregnant animals at any time (1 h: AT = 0.32 ± 0.10 μg/g, MG = 0.36 ± 0.04 μg/g). When injected in association with VLDL, [6-CP] in MG of pregnant animals was consistently 2-fold higher than that in AT (1 h: AT = 0.14 ± 0.01 μg/g, MG = 0.29 ± 0.01 μg/g). In postpartum mice [6-CP] was 2-3-fold higher in MG than AT regardless of vehicle (1 h: AT = 0.32 ± 0.05 μg/g, MG = 0.65 ± 0.1 μg/g; VLDL: AT = 0.26 ± 0.02 μg/g, MG = 0.47 ± 0.02 μg/g). These data indicate that IV 6-CP distributes differently depending on reproductive state. Furthermore, association of the compound with VLDL may direct its disposition in the late pregnant mouse. (Supported by HD 13591 and MOD 15-9).

34 FORMATION OF MUCONALDEHYDE FROM BENZENE IN A MODEL HYDROXY RADICAL GENERATING SYSTEM.


It has been proposed that muconaldehyde (MUC), a six carbon α,β-unsaturated diene dialdehyde, may be a hematoxic metabolite of benzene. The present studies suggest that this compound is derived from benzene in vitro in a model hydroxy radical (-OH) generating system containing ascorbate, ferrous sulphate and ethylenediaminetetraacetic acid in phosphate buffer, pH 6.7. Pure trans,trans-MUC was previously found by us to react with thioarbituric acid (TBA) to form an adduct with a 495 nm absorption maximum and 510 nm emission maximum. MUC formed from benzene was identified as the TBA adduct by spectrophotometry and fluorimetry using the known adduct as standard. Purification was accomplished using a Baker-10 solid phase extraction system utilizing a C-18 reverse phase column, rinsing with phosphate buffer and eluting with methanol. The role of -OH radicals in the reaction was investigated using DMSO and mannitol as -OH scavengers. DMSO and mannitol were found to cause a dose-dependent decrease in MUC formation. Work by others indicates that microsomal cytochrome P-450 1-dependent oxidation of benzene is mediated by hydroxyl radicals. The present work suggests that in a model -OH generating system reactive species are generated with enough energy to cause benzene ring-opening. An analogous process may occur in vivo. Supported by NIH Grant ES02558.


The translocation of 6-CP from hepatic tissue to circulating buffer components was examined during in situ liver perfusion in the female rat. Animals were pretreated with 2 μg 6-CP (0.25 mg/kg) 48 h prior to perfusion. The recirculating perfusate consisted of Krebs-Henseleit buffer, 4% BSA, 10% RBCs and human very low density lipoproteins (VLDL) at either 0, 100, or 400 mg/dl. In the absence of exogenous VLDL, livers released 10% of their total 6-CP content into the perfusate after 2 h. This 6-CP was completely associated with the protein fraction (0.014 ± 0.007 ng/mg) even though livers were found to synthesize tricacylglycerol (TG) (89.63 ± 16.20 mg/dl). Addition of 100 mg/dl VLDL did not influence the percentage of 6-CP released from livers (10.3% ± 2.1%). Fractionation again showed virtually complete association of 6-CP with protein (99%, 0.020 ± 0.003 mg/mg). In the absence of BSA but the presence of 100 mg/dl VLDL, 4% of 6-CP was released from the liver and partitioned to VLDL (0.85 ± 0.17 mg/mg TG), indicating saturation of TG with 6-CP had not occurred previously. A primary determinant for 6-CP distribution among plasma fractions may be the TG:protein ratio. (Supported by HD 13591 and MOD 15-9).
Waterborne contaminants are of particular concern in the Great Lakes region because consumption of sport-caught fish containing lipophilic chemicals such as PCB represents an easily accessible route of human exposure. A cohort of 600 persons who consume greater than 24 pounds of sport-caught fish per year had a median serum PCB concentration of 214 parts per billion (ppb) and a range of 3 to 400 ppb. These levels were significantly greater than those measured in 500 comparison persons from the same communities. The pattern of PCB isomers found in the sera of exposed humans was similar to that observed in cooked trout and salmon. Chromatographic peaks containing 18 specific PCB congeners were identified and quantitated. Congeners with 5 and 6 chlorine atoms constituted the majority of forms present. Among these, 2,3,4,2',4',5' and 2,4,5,2',4',5', hexachlorobiphenyl had the greatest concentration. Other congeners of toxicologic interest such as 2,4,5,3',4',4', 2,3,4,3',4',4', and 2,3,4,5,3',4', were present at lower concentrations.

Humans exposed to environmental sources of PCB appear to retain congeners which have cytotoxic or tumor promotion characteristics.

The fate of picloram (4-amino-3,5,6-trichloropicolinic acid), a herbicide used to control broadleaf weeds and woody plants, was defined in 6 male volunteers following single oral doses of 5.0 and 0.5 mg/kg, and a dermal dose of 2.0 mg/kg. Picloram was given orally as the sodium salt in grape juice, and the dermal dose was applied to the volunteers' back as the free acid dissolved in ethanol. The data indicate that picloram was rapidly absorbed from the gut (t \(1/2 = 0.5\)) and actively excreted as unchanged picloram by the kidney. Over 90% of the oral dose was recovered in the urine as unchanged picloram; most of this (>75%) was excreted during the first 6 hr and the remainder was eliminated with an average half life of 27 hr. By comparison picloram was slowly absorbed through the skin (t \(1/2 = 12\) hr) and, based on the quantity of picloram found in the urine only 0.2% of the picloram applied to the skin was absorbed. These data demonstrate that picloram is rapidly excreted and thus has a low potential to accumulate during prolonged or repeated exposures. In addition, picloram is poorly absorbed through human skin and it is unlikely that acutely toxic quantities will be absorbed by this route.
42 DISPOSITION OF 14C-MIREX IN CLASSES OF CD1 MOUSE HEPATOCYTES. A.K. Charles and R. Abraham. Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, NY.

The disposition of 14C-mirex was examined in classes of hepatocytes, liver, kidney, heart and brain of CD1 mice. Adult mice were given a single ip dose of 14C-mirex (30 μCi/kg) in corn oil (CO) or dimethylsulfoxide (DMSO). At 24 and 72 hr post-injection, mice were killed and various tissues removed. Hepatocytes were isolated following collagenase perfusion (72 hr) and separated into diploid and polyplody classes on 1-4% Ficoll gradient. Portions of livers were subjected to subcellular fractionation. More than 50% of radioactivity recovered was detected in polyplody hepatocytes compared to diploid hepatocytes. Mitochondria contained highest levels of mirex followed by microsomes and soluble fraction with no detectable 14C-mirex in nuclei. Irrespective of vehicle of injection used, liver contained significantly higher amounts of mirex than other tissues. Among the brain regions, cortex, cerebellum and brainstem showed no differences in mirex levels. DMSO-induced influx of mirex into brain was markedly different from that of CO group. In keeping with our previous studies, the preferential and selective uptake of mirex by polyplody hepatocytes may be indicative of their susceptibility and involvement in the tumorigenic process in rodent livers. Supported by NIEHS Grant No. 5T32ES07058.

43 INVESTIGATION OF THE MECHANISM OF TOXICITY OF HYMENOXON IN THE MOUSE: J.C. Merrill, H.L. Kim, Haake, J.E. and S. Safe, Dept. of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX 77843.

Field cases of hymenoxon poisoning are observed in grazing animals and experimental poisonings can be produced in sheep, goats, rabbits, and mice. Mice were treated with hymenoxon (20 mg/kg, i.p. in corn oil/DMSO) and examined 1, 4, 8, 12 and 72 hours after pretreatment. Several previous reports indicate that glutathione and reduced thiols protect mice from hymenoxon toxicity, however, only minimal changes were observed in hepatic glutathionine levels at 1, 4, 8, 12 and 72 hours after administration. The effects of hymenoxon on hepatic drug-metabolizing enzymes including several cytochrome P-450-dependent microsomal monooxygenases, glutathione transferase, glutathione peroxidase and glutathione reductase were determined. The data indicated that hymenoxon elicited minimal effects on these enzymes since only dimethylaminoantipyrine N-demethylase was induced 72 hours post exposure. These results suggest that the hepatic drug-metabolizing enzymes and glutathione may not play a major role in mediating the effects of this toxic acrylamide lactone. (Texas Agricultural Experiment Station Grant No. 6255 and 6376).


Ochratoxin A (OA), a naturally occurring nephrotoxic mycotoxin, is an inhibitor of protein synthesis, competing with L-β-phenylalanine (Phe) at the enzyme phenylalanine-tRNA aminotransferase. Previous investigators have reported decreased OA toxicity in bacteria, yeast, cultured cells, and mice by administering Phe simultaneously. This current study was designed to examine the effects of OA and dietary Phe in rats. Surgical pretreatment resulted in partially-nephrectomized (PN) and sham-operated control (SO) rats. Two levels of OA (0.5 and 2.0 mg/kg body weight) were injected ip daily and two dietary levels of Phe (1% and 5% of dry ration weight) were fed ad libitum over a 6-day duration. Animals were acclimatized to their diets for 4 days prior to OA administration. OA toxicity was enhanced in PN rats. A cumulative 6-day OA dose of 12.0 mg/kg produced 50% mortality in PN animals compared to 0% in SO rats. There was no observed protection from ochratoxicosis by dietary Phe supplementation in both PN and SO rats with regard to daily weight gain, feed consumption, water intake, urine output, and final organ weights. These results suggest that OA toxicity may result from phenylalanine-independent mechanisms in the rat. (Supported by CCM 18820-4 and TAES H6215.)
45 TOXICITY OF OCHRATOXIN A IN FISCHER 344 RATS.
K.L. Pavkov, A.C. Peters, R.L. Persing, and
M.B. Powers. Battelle, Columbus Laboratories,
Columbus, OH 43201 and National Toxicology
Program, NIEHS, Bethesda, MD 20205

Ochratoxin A is a major metabolite of Aspergillus
and Penicillium molds, and is found as a contami-
nant in cereal feeds, beans and peanuts. Spontan-
eous nephropathies have been reported in swine
and poultry ingesting contaminated feeds.

Groups of eighty rats per sex are receiving
gavage doses of 210, 70, 21 µg/kg or the vehicle
(corn oil) for 2 years (5d/wk) to study the toxic-
ity from chronic exposure to Ochratoxin A. Body
weight gain, hematology, serum analyses, urine
analyses and urine concentrating ability are
being evaluated at intervals. After 9 months,
organ weights and histopathology were assessed
in 15 rats/sex/dose. Weight gains in the high
dose groups were significantly reduced (112 M
and 122 F) compared to controls. HGB, RBC count,
WBC count, and bone marrow cellularity were
decreased in the high dose groups. Urine volume
was increased at 6 and 9 months (M and F) at
the high dose level, and urine creatinine and Sp. Cr.
were decreased. Urine concentrating ability
progressively decreased in the two highest dose
groups (M and F) and at 9 mo. the kidney weights
were significantly decreased in a dose related
manner. Histopathology showed that the epithe-
lium of the renal tubules was the target tissue.
Supported by NTP Contract No. N01-ES-95653.

46 TOXICITY OF ANGUDINE IN MICE. J. deCamargo, F.M.
Newberne, A.E. Rogers, P. Punyarat, S. Riegroppo-
itak, and M.W. Conner. Department of Nutrition
and Food Science, Mass Institute of Technology,
Cambridge, MA

The epoxycyclohexene mycotoxins, including
angudine, have been implicated in diseases of man
and animals. Angudine has cytotoxicic properties
but its extreme radiomimetic toxicity has limited
its usefulness as a chemotherapeutic agent.

We have characterized the acute toxicity of angudine
in mice in order to establish a model for eval-
uating the efficacy of compounds which may protect
against its toxicity. The LD50 of angudine in
CD-1 mice was 20 mg/kg by intraperitoneal injec-
tion, 15.5 mg/kg by gastric gavage and 11.3 mg/kg
by inhalation. Intraperitoneal injection of 10
mg/kg of angudine induces a leukocytosis that
peaks at 8h followed by leukopenia and anemia at
2 days. During the first day after injection
there is severe necrosis and cell depletion of the
thymus, spleen and bone marrow. Lymph nodes are
less severely affected. All lymphopoietic and
hematopoietic organs appear normal histologically
by 3 days. Intraperitoneal injection of 15-25 mg/
kg of angudine caused epithelial nerosis of the
small intestine and bronchi. The lesion distribu-
tion was the same in animals exposed to angudine
by inhalation.
(Supported by Contract DAMD 17-82-C-2235, from The
Department of Defense).

47 HEPATOTOXICITY OF THE BLUE-GREEN ALGAE (CYANOBAC-
TERIA) MICROCYSTIS AERUGINOSA. W. C. Theiss,1
W. W. Carmichael,2 M. M. Mullins,2 R. H.
Bruner,1 1Dept. of Biological Sciences, 2Dept.
of Physiology, Wright State Univ., Dayton, OH
and 3AFML, Wright-Patterson AFB, OH.
Sponsor: R. W. Gardier

Rats and mice injected with Microcystis toxin
die within 4-3 hours, showing enlarged livers
engorged with blood and exsanguination of the
remainder of the body. Livers show severe hemor-
rhagic centrilobular necrosis with loss of sinu-
soidal epithelia and disruption of normal hepatic
plate architecture. Lungs show mild edema with
occasional patches of debris. Other organs are
pale but otherwise normal. Phenobarbital in-
duction or SKF-525A inhibition of 2-450 does not
affect mouse survival time. Measurement of sys-
temic arterial, jugular, and hepatic portal pres-
ures plus blood lactic acid levels show a pattern
typical of hemorrhagic shock with no evidence of
systemic venous congestion. Tests of blood and
urine of subcutely dosed rats indicated no renal
effects. These results show that the liver dam-
ger is a direct effect rather than secondary to
heart failure, venocclusion, disseminated intra-
vascular coagulation, pulmonary congestion, or
systemic vasodilation. The actual cause of death
in acutely dosed animals appears to be hemorrhagic
shock.

48 INCREASED MUTAGEN FORMATION DURING HUMIC ACID
CHLORINATION IN THE PRESENCE OF BROMIDE. J.R.
Toxicology and Microbiology Division, HERL,
USEPA, Cincinnati, OH 45268. Sponsor: L. Condle

We have proposed the use of humic acid as a model
substrate for studying mutagenic and carcino-
genic by-products that result from water chlorina-
tion, and have previously demonstrated the formation
of mutagenic activity following humic acid chlorina-
tion. The present study was conducted to deter-
mine whether the addition of bromide to the humic
acid chlorination reaction would have any influ-
ence on the degree of mutagen formation. A
commercially obtained humic acid was reacted with
"chlorine" (NaOCl/HOCl) at pH 7 using a humic
acid concentration of 1g total organic carbon/
liter and a carbon to chlorine molar ratio of 1:1.
Potassium bromide was added at bromide to chlorine
molar ratios ranging from 0.01:1 to 1:1. Mutagen-
ic activity was monitored with the Salmonella/
microsome assay using strains TA98 and TA100.
The results with both strains indicate that mutagenic
activity increases with increasing bromide con-
centration up to a Br:Cl ratio of 0.1:1, then
gradually and decreases at higher bromide levels.
The activity at all Br levels is direct acting and
99 reduces part of the activity to levels which
parallel the pattern of the direct mutagen-
icity. The implication of these results is that
the concentration of bromide in water may
have an important impact on levels of mutagen-
eci ty found after chlorination. (This abstract
does not necessarily reflect EPA policy.)

Previous studies of aflatoxin B1 (AFB1) penetration through the skin of rats and rabbits in vivo have resulted in contradictory conclusions. Because agricultural workers in the Southern United States may be frequently exposed to AFB1, the potential for percutaneous absorption needed to be assessed. Simple Teflon diffusion cells (6 cells/experiment) were used to study the in vitro penetration of [3H]AFB1 and [14C]AFB1 through isolated human epidermis. The radiochemical purity of the dose was determined by thin layer chromatography (TLC) prior to dosing. Each 3.1 cm² skin disc was dosed with 10-30 nmol of AFB1. The chemical nature of radioactive penetrates was determined by TLC of chloroform extracts of the receptor fluid. Based solely on the accumulation of radioactivity in the receptor fluid, 3H penetrated the epidermis 30-60 times faster than 14C. However, 99.7% of the 3H label was associated with water. This tritium-exchange process was not heat labile but appeared to result from exchange within the epidermis. When [14C]AFB1 was used, the major radiochemical penetrant recovered from the receptor fluid was AFB1. Movement of AFB1 through non-occluded epidermis was very slow (0.34 pmol/hr). Movement through epidermis hydrated by occlusion was 30 times greater (9.9 pmol/hr).

The results indicate that AFB1 can penetrate human epidermis.


Ingestion of STX-contaminated shellfish can result in rapid neuromuscular paralysis and, in some cases, death. We have tested anti-STX rabbit serum as a possible therapeutic agent for STX poisoning using the mouse as the experimental model. In vitro mixing of antiserum and STX followed by mouse bioassay revealed that the antiserum neutralized 34 nmol STX/ml serum. Mice were injected with 100 µl antiserum (1:8) i.p. followed 1, 6, or 24 hrs later by an s.c. injection of 16.5 µg STX/kg (an LD100 dose). These mice showed 20, 0, and 0% mortality, respectively, indicating that the antiserum can effectively neutralize STX in vivo. In another experiment, mice were injected s.c. with 16.5 µg STX/kg followed immediately by an i.m., i.p., or i.v. injection of 100 µl antiserum (1:8). Antiserum injected by the i.p. and i.m. routes caused no significant decrease in STX-induced mortality. However, antiserum injected by the i.v. route decreased mortality to 0%. These results indicate that anti-STX serum can be an effective therapeutic agent for STX poisoning if the antiserum is injected directly into the bloodstream and, therefore, disseminated rapidly in the STX victim.
53 THE ACUTE EFFECTS OF T-2 MYCOTOXIN ON THE CARDIO-
VASCULAR SYSTEM IN THE RAT. G.W. Parker, Jr.,
DVM. US Army Medical Research Institute of
Infectious Diseases, Ft. Detrick, Frederick, MD.
Sponsor: R.M. Mhatre

Electrocardiographic and hemodynamic indices, in-
cluding heart rate, mean arterial blood pressure,
and cardiac output were evaluated in 8 awake,
freely moving catheterized rats before and after
time after intravenous administration of a lethal
dose (7 mg/kg) T-2 mycotoxin and in 8 rats re-
ceiving propylene glycol (toxin vehicle). T-2
mycotoxin resulted in a mean survival time of
10.8 hours. There was a significant (pc.01) de-
crease in cardiac output from 34.73 ± 1.72 ml/
min/100g (mean ± S.E.) to 27.54 ± 2.98 ml/min/
100g by the second hour with a continued decrease
to 14.31 ± 1.76 ml/min/100g by six hours. Heart
rate increased significantly (pc.01) from 380
6.31 to 440 ± 5.86 beats/min by the second hour
and remained elevated through 4 hours, but was
followed by a significant (pc.01) decrease to
328 ± 13.34 and 293 ± 12.9 beats/min at 8 and 10
hours respectively. Mean arterial blood pressure
remained normal through 8 hours and decreased
from 120 ± 3.48 to 101 ± 15.38 mm of Hg (pc.05)
at 10 hours. Electrocardiographic alterations
consisted of QRS interval prolongation (pc.01)
and increased T-wave amplitude (pc.01) by the
second hour. P-R interval prolongations and
widening of the QRS complexes occurred 6 hours
postintoxication. There were no significant
changes in animals receiving propylene glycol.

54 EFFECT OF T-2 MYCOTOXIN ON GLUTATHIONE LEVELS IN
THE MUHSE LIVER. R.F. Frick, L. Keeling, and
B. Beauchamp. US Army Medical Research Institute
of Infectious Diseases, Ft. Detrick, Frederick,
MD. Sponsor: R.M. Mhatre

T-2 mycotoxin (T-2), a naturally occurring fungal
metabolite, is a potent non-protein hepatotoxic
agent. Because glutathione (GSH) has been impli-
cated in the detoxification of T-2, the levels of
hepatic GSH were measured in control (CON) and
T-2 treated mice. At various times after an
LD50 dose of T-2 (4 mg/kg s.c.), livers were re-
moved and assayed for GSH. T-2 treated mice
showed a progressive decline in GSH levels reach-
ing minimum levels at 6-8 hours. Since exposure
to T-2 causes anorexia, which has been shown to
decrease hepatic GSH levels, it is possible that
fasting, rather than T-2 caused the observed GSH
changes. To test this possibility, T-2 (4 mg/kg
s.c.) was given to either fed or fasted mice and
hepatic levels of GSH (mean ± S.E. in μmol/g
Tissue) measured 6 hours later. Mice were either
fed or fasted prior to or after exposure to
T-2. GSH levels for fed mice were 9.01 ± 0.66
(CON) and 4.26 ± 0.41 (T-2). For mice fed prior
to T-2, but fasted post-exposure the levels were
7.8 ± 0.26 (CON) and 3.76 ± 0.65 (T-2). For
mice fasted throughout the entire experiment,
levels were 4.45 ± 0.39 (CON) and 2.45 ± 0.26
(T-2). In conclusion, T-2 treated mice had he-
patic GSH levels significantly lower than the
control. This may be in part contributive to the
hepatotoxicity of T-2 toxin.

55 ELECTROCARDIOGRAPHIC EFFECTS OF HELENALIN AND
RELATED SESQUITERPENE LACTONES IN THE CANINE:
A.C. Anderson, H.L. Kin and L.P. Jones. Texas
A&M University, College Station, Texas. Sponsor:
E.J. Camp

The effects of four sesquiterpene lactones on the
genesis of electrocardiographic pro files, and the
augmentation of characteristic electrophys-
iological, respiratory and vasotensive changes
associated with helenalin or tenulin by preexpo-
sure to furosemide, diethyl maleate, and aceprom-
mazine in dogs were studied. Acute toxicosis
with helenalin, mexicanin-E, helenalin propionate
and tenulin, produce qualitatively identical
electrocardiographic changes, including; AV dis-
sociation, SA nodal block and bundle branch blocks. Characteristic wave forms produced by toxicity
are a large U wave, and elevation of the J junc-
tion. Pathologic changes in the myocardium in-
clude ischemia and pericarditis; vascular changes
include; pericardial edema, subendocardial and
petechial hemorrhages in many organs particularly
the gall bladder. Depletion of endogenous sul-
phdrlyte increases the toxicity of tenulin, and
preexposure to furosemide or acepromazine in-
creases the toxicity of helenalin.

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56 DEVELOPMENT OF BIOASSAY TECHNIQUES FOR AN AFRICAN
ARROW POISON. C.S. Tsai and R.F. Ochillo: Labora-
tories of Pharmacology and Toxicology, Xavier
University of Louisiana, New Orleans, La 70125.

In our continuing search for identifying the ac-
tive component(s) of an African arrow poison, we
previously reported methods of separating the ac-
tive component of the toxin by Thin Layer and Li-
quid Chromatography (J.Liq.Chr. 4,944-557 and 4:
1847-1894, 1981). However, the isolation may alter
the efficacy and toxicity when compared with the
original crude arrow poison. Therefore, we have
developed several bioassay techniques to be used
to test the potency of the toxin and its isolated.
The techniques are the effects of the toxin and/or
the fraction(s) on the 1) contractility of iso-
lated frog heart and 2) cardiac output of frog
heart in situ. The arrow poison dose-dependently
decreased the cardiac contractility of frog heart
within the dose range of 1 to 10 μg/ml. The re-
lation between the concentration of the poison
and the decrease in cardiac contractility was
linear. The infusion of the poison through in situ
heart preparation increased the cardiac output
dose-dependently at low concentration (0.2 to 20 μg
/ml) and decreased the cardiac output at high con-
centration (>200 μg/ml) in a dose dependent fa-
sion. The initial increase in cardiac output can-
not be explained on the basis of our data. The two
bioassay techniques appear to be suitable in fur-
ther investigation of the toxin. (Supported in part
by Grant RR08008-11 from NIH).
Tolerance to DFP-induced antinociception: lack of cross-tolerance to morphine. L.G. Costa and S.D. Murphy, Dept. of Environmental Health, Univ. of Washington, Seattle, WA.

The irreversibility of acetylcholinesterase inhibitor diisopropylfluorophosphate (DFP) has been shown to induce an antinociceptive effect in mice which is antagonized by naloxone, suggesting an involvement of endogenous opiates (Zorn et al., Toxicologist, 1983). Moreover, acute administration of DFP to mice increases met-enkephalin content in the striatum (Zorn et al., Toxicologist, 1984). In the present study we investigated if tolerance develops to DFP antinociception and if cross-tolerance to morphine occurs. Male mice were administered DFP for 14 days (2 mg/kg/day). At the end of the treatment mice were found to be tolerant to the antinociception induced by DFP and by the cholinergic agonists oxotremorine and nicotine. Brain muscarinic and nicotinic receptors were significantly decreased in brain from DFP-pretreated mice. There was no cross-tolerance to morphine and binding of [3H]-dihydromorphine to brain opiate receptors was unchanged. Mice made tolerant to the antinociceptive effect of morphine were not cross-tolerant to DFP, oxotremorine, or nicotine-induced antinociception and muscarinic, nicotinic and opiate receptors were unchanged. Thus, although DFP-induced antinociception appears to involve endogenous opiates, the lack of cross-tolerance with morphine suggests that different neuronal mechanisms may underlie DFP- and morphine-induced antinociception. (Supported in part by grant ES-01831 from NIEHS).

ASSSESSMENT OF SMOOTH MUSCLE FUNCTION IN SESBANIA DRUMMONDI TOXICOSIS IN GALLUS DOMESTICUS.
C.S. Venugopalan, W. Flory, T.A. Tucker, C.D. Hebert and G.M. Strain, Department of Veterinary Physiology, Pharmacology and Toxicology, School of Veterinary Medicine, Louisiana State University, Baton Rouge, LA (Sponsor: C.B. Short).

This investigation was an in vitro assessment of smooth muscle activity in chickens experimentally poisoned with sesbania. The responsiveness of lung parenchymal strips and ilial segments to cumulative concentrations of histamine and carbachol was compared between control groups and groups treated orally with sesbania extract for three consecutive days. Two dosage levels of sesbania (0.25% and 0.5% body weight) were used. Responses (isometric contractions) of both intestinal and lung strips in the higher dose group were decreased significantly whereas in the lower dose group only lung strips showed a significant decrease due to sesbania treatment. This indicates that sesbania treatment decreases responses of smooth muscles to their common agonists. The involvement of lung tissues after oral poisoning suggests the existence of a toxic systemic principle in the extract. The assessment of smooth muscle activity used in this study was very effective in detecting changes in tissues from poisoned birds which did not always show clinical signs.


Subchronic (28 days) daily oral administration of nBM (200 mg/kg), a metabolite of S,S,S-tri-n-Butyl phosphorothioate (DEP), induced a persistent acute anemia in hens and roosters. The early signs of anemia were weakness, sedation, loss of appetite, slight salivation, dyspnea, and both the comb and wattle became cyanotic. In addition a sharp decline (40-60%) in red blood cell (RBC) count, packed cell volume, and hemoglobin content and increase in Heinz bodies and hemichromes were noted. Deformed RBCs having sickled-cell-like shape were seen in the peripheral blood, spleen, and bone marrow cells. Similar observations were seen with rats treated with nBM (200 mg/kg/day). Signs of acute anemia persisted for 28 days with daily oral dosing with nBM. A significant weight loss was noticeable only after 21 days of daily dosing. Spleen weight was normal. The hematobiotic system was hyperactive. Signs of recovery were seen upon ceasing nBM administration. It is demonstrated that oral DEP toxicity may not be solely attributed to its metabolite nBM.
61 ANTICHOLINESTERASE EFFECT OF BIS(TRICHLOROMETHYL)SULFONE (N-1386® Biocide) IN RATS. G. L. Sprague, L. L. Sandvik, and T. R. Castles, Stauffer Chemical Company, Toxicology Department, Richmond, California 94804

Bis(trichloromethyl) sulfone (N-1386® Biocide) was found to be a potent in vitro inhibitor of rat plasma cholinesterase and brain acetylcholinesterase. IC50's for these 2 enzymes were 18 μM and 9 μM, respectively. The inhibition was competitive and reduced by added substrate. The oral LD50 for bis(trichloromethyl)sulfone in male rats was 691 mg/kg. Deaths occurred 1-4 days after treatment and adverse signs suggestive of an anticholinesterase effect were noted. Plasma cholinesterase or brain acetylcholinesterase was not inhibited 2, 4 or 24 hours after single, oral dose of 500 mg/kg. Atropine (300 mg/kg, s.c.) or scopolamine (670 mg/kg, s.c.) pretreatments did not affect the acute, oral LD50 for bis(trichloromethyl)sulfone. However, cholinergic signs were alleviated by these pretreatments. In summary, cholinesterase inhibition by bis(trichloromethyl)sulfone was responsible for clinical signs of toxicity but it did not appear to be the mechanism for mortality produced after oral administration. (N-1386® Biocide is a registered trademark of Stauffer Chemical Company).

62 MECHANISMS OF DELAYED DEATH PRODUCED BY 0,5,5-TRIMETHYL PHOSPHORODITHIOATE (OSS) IN RATS. N. A. Kono, Y. Hirokuto, and T. Imamura. Division of Toxicology and Physiology, University of California, Riverside, CA 92521

Oral administration of OSS, an immunity present in widely used organophosphorus insecticides, causes delayed death in rats (oral 24 day LD50: 50 mg/kg). The delayed toxic signs include body weight loss, red staining around the nose, mouth and eyes, and pulmonary edema. Pretreatment of rats with piperonyl butoxide or small multiple doses of OSS protected against OSS-induced delayed toxicity. Effect of OSS was studied on pulmonary and hepatic microsomal enzymes in rats. Activity of aryl hydrocarbon hydroxylase (AHH) in lung was decreased by OSS treatment (40, 100, 160 mg/kg, 24 hr), dose dependently, whereas no such decrease was observed in liver. Pulmonary and hepatic microsomal malathion carboxylesterase was inhibited dose dependently by OSS. Time course effects (0, 12, 24 and 72 hr) studied at 40 mg/kg OSS also demonstrated selective inhibition of pulmonary AHH activity, which correlated with pulmonary edema. Although scanning electron microscopic observation showed no change in morphology of rat bronchial Clara cells, a major locus of pulmonary P-450, the ability of OSS to inhibit pulmonary AHH and to cause pulmonary edema may suggest that the site of action of OSS resides in the lung. (Supported by U.S. PHS grant ES 02225)

63 EXPOSURE OF RATS TO RESPIRABLE AEROSOLS OF PARAQUAT: LUNG PATHOLOGY AND DISPOSITION OF PARAQUAT. L. L. Smith, Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK, and C. J. Hardy, Huntington Research Centre, Huntingdon, Cambridgeshire, UK. Sponsor: I.F.H. Purchase.

Although paraquat (PQ) is not respirable when sprayed for agricultural use, concern has been expressed that small amounts present in the lung may cause damage. We have used experimentally generated respirable aerosols (<1 μm) PQ to determine the relationship between the concentration of PQ in the lung and histological evidence of damage.

Following exposure to 0.1 μg respirable PQ/1 for 6 h/day, 5 days/week for 3 weeks, the concentration of PQ in the lung reached a steady state level of 1 μg/g within a few days. The half-life of loss of PQ from the lung was ~2 days. Histopathological examination of lungs showed there was no evidence of lung damage. When rats were exposed to 0.5 μg PQ/1 for the levels in the lung were maximal after 4 exposures (~5 μg/g) and decreased within a few days to ~2 μg/g. The lungs of these animals were not damaged and if it is possible this caused the fall in the lung PQ levels. In conclusion the rat lung can tolerate 1 μg PQ/g for at least 3 weeks without sustaining damage and there appears to be a critical threshold concentration of PQ below which the lung is not damaged.

64 ACUTE AND SUBCHRONIC INHALATION TOXICITY OF CHLORSULFURON IN RATS. D. P. Kelly, N. C. Chomyow and R. L. Ferenz. E. I. du Pont de Nemours and Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, P. O. Box 50, Elkton Road, Newark, DE. Sponsor: G. L. Kennedy, Jr.

Chlorsulfuron is the active ingredient in Glane® weed killer, a dry powder which is applied as a low concentration water spray. Ten male and ten female rats showed no apparent effects after a single, four-hour, head-only exposure to 5,900 mg/m³ chlorsulfuron dust. In a subchronic inhalation toxicity study, groups of 10 male rats were exposed, head only, to atmospheres containing either 0, 100, 500, or 2,500 mg/m³ of chlorsulfuron dust for 6 hours a day, 5 days a week for 2 weeks. Airborne particle mass median diameters ranged from 5.2 to 6.2 μm. Rates exposed to 2,500 mg/m³ gained less weight, had decreased blood lymphocytes, and increased lung and spleen weights compared to controls. Histological examination showed adverse effects only in the 2,500 mg/m³-exposed rats including: damage to the renal papilla and calyx, lymphoid hyperplasia in the spleen, decreased hemopoiesis in the bone marrow and a slight exacerbation of pneumonitis. None of these effects were present after a 12-day recovery period. Immediately after exposure urinary hematuria was seen in 3 of 9 and in 8 of 10 rats in the 500 and 2,500 mg/m³ groups, respectively. No significant clinical or pathological effects were seen in the 100 mg/m³ group.
The subacute toxicity of a new formulation of MATAILL (amincarb) was assessed by exposing male and female Sprague-Dawley rats via a nose-only technique to respirable (2.0-4.1 μm) aerosol at chamber concentrations of 22.5, 45 and 90 ug of insecticide/litre of air for 2 hr/day for 30 consecutive days, comparing the effects to those of rats exposed to vehicle aerosol or room air for the same duration. Randomly-selected rats of each group were blod at day 8, 15 and at sacrifice after 30 days treatment. Subgroups were killed 30 days post-treatment to study the regressive effects. Parameters measured included body wt., food intake, plasma, rbc and brain cho- linesterase and hepatic and renal carboxylesterases. Milk muscle tremors were observed occasionally in the intermediate and high dose groups. Among females, there was a dose-dependent inhibition of enzyme activity, though, within treatment groups, there were no differences associated with duration of exposure. Enzymes were normal by 30 days post-treatment. Lung weights were increased in vehicle and MATAILL-treated groups. Histological study revealed changes attributable to a nonspecific tissue response to a heavy burden of an oil-based irritant which was resolved by 30 days post-treatment.


It has been previously reported that skin sensory stimulation (SSS) produced by synthetic pyrethroids can be evaluated by use of a behavioral model, using guinea pigs. Present investigations were designed to further characterize fenvalerate mediated SSS. Studies were performed to determine association of SSS to overt skin irritation, alleviation/protection by Vitamin E and corn oil, and sensitivity of affected skin sites to further irritation. Guinea pigs were treated with fenvalerate solutions on one side of their shaved back and vehicle on the other side. Other treatments were applied to both sides of the back before or after fenvalerate. Fenvalerate-mediated SSS was not associated with classic skin irritation as determined by the Draize technique or by a method utilizing Evans blue dye. When animals were no longer responding to fenvalerate treatment (4 hrs after dosing), an irritant, oil of mustard, restimulated the behavioral response up to 72 hours after fenvalerate. Corn oil and to a greater extent Vitamin E were effective in alleviating and protecting against the sensation. Vitamin E also protected against irritant restimulation.

CHLORIDIMEFORM INDUCED DEPRESSION OF THE ISOLATED, PERFUSED RABBIT HEART. J.A. Krieger, and V.C. Ravikumar, Pharmacodynamics and Toxicology Laboratories, University of Oklahoma College of Pharmacy, Oklahoma City, OK.

Previous studies have shown that N'-[(4-chloro-o-toly)-N,N-dimethylformamidine] (CDM) exerts profound effects on the cardiovascular system of acutely intoxicated animals (Environ. Health Perspect. 14: 71, 1976; Toxicol. Appl. Pharmacol. 44: 357, 1976; Bull. Environ. Contam. Toxicol. 27: 707, 1981). The present study was undertaken to ascertain what effect CDM would have on isolated, perfused rabbit hearts. Dose dependent decreases in myocardial contractility, heart rate, and coronary flow rate were observed. Myocardial contractility was affected to a greater degree than the other two parameters. A concentration of 3.2 X 10^-4 M CDM in Chenoweth's solution produced near cardiac standstill. Heart rate was also depressed in a dose dependent manner, but not to the same extent as contractility. CDM appeared to exert a biphasic effect on coronary flow rate; relatively low concentrations of CDM decreased flow, whereas high concentrations appeared to transiently increase flow. The cardio-depressant effects of CDM were completely reversible. Cardiac contractility returned to near CDM levels when the heart was subsequently perfused with plain Chenoweth's solution, whereas heart rate and coronary flow rate were only partially reversed.


A 30-day feeding study combined with a one-generation reproduction study was conducted to make preliminary assessment of subchronic toxicity and reproductive effects of the plant growth regulator DS-29399. Six groups of rats were fed 0, 12.5, 25, 50,100 and 200 mg/kg/day DS-29399. In the 30-day feeding study, hypertrophy of the cortical tubular epithelial cells of the kidney was observed in all animals in all treatment groups. Hepato- cellular hypertrophy occurred in males at doses >25 mg/kg/day and in females at doses >100 mg/kg/day. Selected tissues from adult animals in the reproduction phase were examined microscopically. Hypertrophy of the tubular epithelial cells of the kidney was present in all animals at all dosage levels. The tubular-cell hypertrophy was not observed in the nuclei of the renal tubular epithelial cells. Microscopic changes in the liver occurred in animals at all dosage levels. Lesions included hepatocellular hypertrophy accompanied by vacuolation and necrosis. The time of appearance of microscopic changes in kidneys and liver of rats was unusually short. The toxic effects were cumulative. The lesions appeared at lower doses and were more severe with longer dosing periods.
A 30-day oral toxicity study was conducted in Beagle dogs with DS-29399 at doses of 0.1, 0.5, 1.5 and 10 mg/kg. Evaluation of daily physical observations, body weights, food consumption, ophthalmological and electrocardiographic examinations, clinical laboratory studies, and gross and microscopic findings revealed no differences between treated groups of dogs and the concurrent controls. Histopathological changes were observed in the prostate, testes or kidneys of male dogs. The prostatic lesions characterized by epithelial cell atrophy and/or glandular dilatation, were observed in all males at dose levels of 10, 5, and 0.5 mg/kg and in one of two males examined at the 0.1 mg/kg dose level. Testicular degeneration was observed in both high dose (10 mg/kg) males and in one male at both the 5 mg/kg and 0.1 mg/kg dose levels. In the inner cortex of the kidneys, hypertrophy of the tubular epithelial cells was observed in both high dose (10 mg/kg) male dogs and in one of two males at both the 5 and 0.5 mg/kg dose levels. No other histopathological changes attributable to the administration of DS-29399 were observed in the male or female dogs receiving DS-29399.

Pesticide formulations and their components are routinely tested for toxicity and irritation properties. These tests are performed to evaluate health hazards, establish handling precautions, and for registration purposes. One carrier for granular pesticide formulations is Pregelled Defatted Corn Grit. A part of the test battery performed on this carrier was a skin irritation test according to the Draize procedure. A 0.5 g sample of corn grit was placed on each of two dose sites (abraded and intact) located on shaved dorsal areas of 6 rabbits. All samples were moistened and the dose sites were occluded for the 24 hr exposure. This test resulted in severe irritation to rabbit skin that developed over a 72 hr period. Histologic examination provided no evidence of residual corn grit fines but indicated the presence of fungal hyphae. Fungal culturing of the corn grit yielded Aspergillus flavus and a Rhizopus sp. A corn grit sample was then sterilized with ethylene oxide and tested for skin irritation. This sample produced no irritation after a 24 hr exposure to rabbit skin. The study shows that skin irritation can be produced by the carrier of a granular formulation and demonstrates the systematic identification of this effect.

The comparative in vitro inhibitory activity of Acephate Technical on brain and RBC acetylcholinesterases (AChE) and plasma cholinesterase (ChE) was determined for the rat, monkey and human. Acephate Technical was shown to be a weak inhibitor of AChE and ChE for all the species. The 

100,000 times less effective than eserine as an inhibitor of AChE and 10,000 times less effective as an inhibitor of ChE. Acephate Technical was more effective against rat brain and RBC AChE (1.6 x 10^-3 M and 1.3 x 10^-3 M) than against mouse (3.4 x 10^-3 M and 2.7 x 10^-3 M) and human (5.4 x 10^-3 M and 2.7 x 10^-3 M) brain and RBC AChE (respectively). For plasma ChE, the IC50 value was lower for the monkey (2.3 x 10^-3 M) and human (1.8 x 10^-3 M) than for the rat (4.5 x 10^-3 M). In summary, Acephate Technical is a weak inhibitor of AChE and ChE in vitro. The species order of sensitivity (IC50) to Acephate Technical for AChE was rat < monkey < human, while the reverse order was observed for ChE, human < monkey < rat.
73 BEHAVIORAL EFFECTS INDUCED BY DIELDRIN IN RATS PREEXPOSED TO SITUATIONAL STRESS. J. Carlson. Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, NY. Sponsor: R. Abraham.

The interaction of 'situational stress' and various (0, 0.5, 1.5 and 4.5 mg/kg) levels of acute exposure to the chlorohydrocarbon pesticide Dieldrin on the production of behavioral effects was studied in male Sprague-Dawley rats weighing 300-350 gms. Situational stress in the form of a series of 40 0.8 mA randomly presented uncontrollable electric shocks preceded exposure to the compound at four dose levels for one group of animals. Another group received an identical series of controllable shocks and compound exposure, while a third group received the compound with no pre-shock exposure. All groups were subsequently tested on a sixty-trial fixed ratio 2 shock-escape task in a shuttle box. Deficits in the performance of the task occurred in an apparent dose-related fashion in animals pre-exposed to uncontrollable shock. No effect of the compound was noted in the other shock-pre-exposure groups. These effects are discussed with respect to the actions of situational stress on neurochemical systems and how these may interact with Dieldrin and other environmental chemicals. The model described here provides a good example for detection of behavioral effects at low levels of exposure. Supported by NIHES Grant No. ST32ES07058.


CTA has been proposed as a test for evaluating the toxic effects of chemicals on the central nervous system. This study examined the ability of different OP's in inducing CTA. Individually housed male rats were adapted to a restricted (10 min/day) water schedule. When intake had stabilized, rats were given a 0.15% (w/v) saccharin water solution. Dichlorvos (DDVP) (3.0, 4.5 or 6.0 mg/kg), parathion (PS) (2.0, 3.5, or 5.0 mg/kg) or dioisopropylfluorophosphate (DFP) (0.1, 0.2 or 0.8 mg/kg) were administered ip 15 minutes after this drinking session. Two days later, the rats were offered a choice of saccharin and water to drink. DDVP (4.5 and 6.0 mg/kg), PS (all doses) and DFP (0.2 and 0.8 mg/kg) significantly lowered saccharin consumption (as percent of total fluid consumed) indicating that they caused CTA. Only the highest dose of PS (5 mg/kg) gave slight signs of OP toxicity. At the dose of 4.5 mg/kg DDVP did not alter nociceptive threshold in the tail flick and hot plate tests, nor alter rat behavior and performance in the open field or the inclined plane tests. These results indicate that CTA can be induced by different OP's at doses which gave no other sign of toxicity, suggesting that CTA may be a useful test for evaluating the neurobehavioral toxicity of OP's. (Supported in part by grant HS 01831 from NIHES).

75 DFP INHIBITS RETICULOENDOTHELIAL CELL PHAGOCYTOSIS OF BACTERIA IN A MANNER DISTINGUISHABLE FROM CHOLINERGIC ACTIVITY. George A. W. Waterhouse, Stephen F. Dunn, David R. Steup, and Robert B. Forney. Department of Pharmacology and Toxicology and Department of Surgery, Indiana University School of Medicine, Indianapolis, IN 46223.

Male Sprague-Dawley rats (140-160 grams) were subcutaneously administered 0.1, 0.1, or 1.0 mg/kg diisopropyl fluorophosphate (DFP), a potent serine esterase inhibitor, 1 hour prior to the IV administration of 1 × 10⁹ 3H-N-labeled E. coli. All animals were sacrificed 10 minutes after bacterial challenge with sections of liver, spleen, lung, and kidney being removed for radioactivity determination. 1.0 mg/kg carbachol was administered similarly to the DFP in order to evaluate cholinergic influences on phagocytosis. Both carbachol and the high dose of DFP decreased liver uptake of bacteria. Splenic uptake was unaffected by DFP, yet markedly decreased bacterial sequestration. In lung, radioactivity was decreased by DFP while carbachol generated an increase in activity. Kidney uptake was inhibited by all three concentrations of DFP, but carbachol produced no measurable change from control (saline). These data suggest that the response to DFP and to carbachol are highly organ specific. Furthermore, it would appear that the mode of action for DFP can not necessarily be attributed solely to its cholinergic action. These findings support the hypothesis that serine esterases play an important role in normal immunologic function.

76 CHLORIDEMFORM (CDFM) EFFECTS ON MOTOR ACTIVITY IN RATS: INFLUENCE OF HOME-CAGE HOUSING CONDITIONS. O.D. Walker, R.C. MacPhail, and H.A. Tilton. Neurotoxicology Division, US EPA and Laboratory of Behavioral and Neurological Toxicology, NIHES, Research Triangle Park, NC 27711.

Exp. I determined the effects of CDFM-HCl (1.25-40 mg salt/kg h.wt., i.p., 10-min pre-sessions) in adult male LE rats. Rats were housed in groups of three and tested individually in a photocell device, according to a within-subjects design, during daily 25-min sessions. A small dosage of CDFM (5 mg/kg) produced substantial decreases in motor activity whereas a large dosage (40 mg/kg) had no effect, when compared to non-injection and saline-injection (1 ml/kg) control data. Similar effects of CDFM (2.5-40 mg/kg) were obtained (Exp. II) in male F-344 rats, housed in groups of four, and tested individually in a capacitance-sensing device according to a between-subjects design. Decreased motor activity was not produced by 5 mg/kg in rats individually housed and tested repeatedly in the photocell device (Exp. III). In rats housed individually and tested in the photocell device (between-subjects design) CDFM (1.25-40 mg/kg) decreased motor activity in a dose-related fashion (Exp. IV). CDFM-induced decreases in motor activity, therefore, depend to a large extent on the conditions of housing. Also, these data suggest that housing conditions may interact with dosing design.

Rats treated with the pesticide chlorideneform (CDM) exhibit pronounced but transient increases in both latency and amplitude of pattern reversal-evoked potentials (PREPs) (Dyer and Boyes, The Toxiconologist, 3:13, 1983). The present investigation explored the possibility that these functional changes were related to biochemical perturbations. The rate of rapid axonal transport (AT) in the retinal ganglion cells of saline-treated control and CDM-treated (40 mg/kg, i.p.) Long-Evans, hooded rats (n=5/treatment group) was measured using intraocular injection of a radiolabeled glycoprotein precursor (L-[6-3H]-fucose, 50 uci) 3 hrs before sacrifice. CDM decreased the amount of labeled glycoprotein transported to the axons (optic tract) and nerve endings (superior colliculus) to 67% and 55% of control, respectively, and produced hypothermia (35°C). When the body temperature of CDM-treated rats was raised to control levels (37.5°C), AT rate was normal. Therefore, retardation of rapid AT, like some PREP latency increase (Boyes and Dyer, The Toxiconologist, 1984), are related to CDM-induced hypothermia.

*Supported by a NRC postdoctoral fellowship.


Laying hens are routinely used to assess for OPCS toxicity. The present observations were made while studying the mechanism of action of S,S,S-Tri-n-butyl phosphorothiolate (DEP) and its metabolite n-butyl mercaptane (nBMP). Subchronic daily oral dosing of DEP (5,25,50,100 mg/kg/day) to laying hens, caused reduction in food and water intake, salivation, diarrhea, dehydration, hypothermia, loss of body weight and constriction of blood vessels and difficulty to breath. Following the first dose of DEP (>50 mg/kg/day) no eggs were layed. Few eggs were layed shell-less at 50 mg/kg/day of DEP. Similar observations were made after 14-21 days following daily and administration of tri-O-cresyl phosphate (TOCP - 20 mg/kg/day). Single high doses of DEP (1000 mg/kg) produced these effects acutely, while TOCP (750-1000 mg/kg) and leptophos (655 mg/kg) produced similar but delayed (14 days) effects in addition to paralysis. Severe reduction of spasm weight, immature decomposed egg yolk, and severe deposition of medullary bone were seen in all treated hens. Rooster, although, showed similar signs of toxicity. no effects were seen on the bone. OPCS may interfere with calcium mobilization and egg shell formation and egg laying mechanism possibly as a result of severe dehydration. Caution should be taken in explaining data obtained on the effects of OPCS on laying hens.

80 Acute Toxicity (oral & inhaled) of Methyl-DBCP


Acute studies were conducted on methyl-DBCP (1,2-dibromo-3-chloro-1,1-trichloropropane) because of structural and physical similarities to DBCP (1,2-dibromo-3-chloropropane) which has known carcinogenic and sterilant effects. Oral LD50 and LC50 of methyl-DBCP were 780 mg/kg and 950 ppm respectively for Sprague-Dawley rats. Whole-body exposures to a mixed vapor and condensation aerosol of methyl-DBCP were conducted at nominal concentrations between 547 and 1537 ppm. Inhaled analysis at 365 ppm gave concentrations between 845 and 1235 ppm. Toxicity in the oral study was characterized by ptosis, piloerrection, nervousness, hunched back, increased muscle tone and decreased reflexes and activity, ataxia and coma. Three rats showed hind limb paralysis. Death occurred within 3 days. In the inhalation study, thoracic swelling, respiratory distress, ataxia and coma were the main observations. Most deaths occurred within 7 days. Paresseal, paralitulosis and paralisis and or hemorrhage of the GI tract and thickening of the gastric fundus. Swelling and other pulmonary changes were observed grossly in the inhalation study and were supported by increases in lung weights. Histologically, acute or subacute bronchiitis was observed. Hepatocellular vacuolization and exsanguination occurred in a small proportion of treated animals. These studies suggest methyl-DBCP acutely is less toxic than DBCP.
81 THE CHRONIC EFFECTS OF DIETARY ADMINISTRATION OF CARBOFURAN ON BEAGLE DOGS. G.M. RAND, J.R. De PROSPERO AND M.J. FLETCHER. FMC CORPORATION, PRINCETON, NJ.

Beagle dogs (N = 12/concentration/sex/group) were administered dietary concentrations of 0, 10, 20 or 500 ppm of carbofuran (technical) for one year. Body weight loss and pharmacotoxic symptoms such as emesis and loose stools were evident in the 500 ppm group. Throughout the study, plasma cholinesterase activity was significantly depressed in males and females of the 500 ppm group and males of the 20 ppm group. A marked compound-related depression of hematocrit, hemoglobin and erythrocyte count was observed in the 500 ppm males. Changes in electrolyte values were concurrently observed (depression of calcium and sodium and elevation of potassium). Brain and heart weights were also significantly depressed in the 500 ppm males.

Based on the results of this study, it may be concluded that 10 ppm is the no-effect level and 20 ppm is the minimal effect level for carbofuran (technical) when administered to beagle dogs for one year.

83 A BEHAVIOURAL GUINEA PIG TEST FOR ASSESSING PYRETHROID INDUCED CUTANEOUS SENSORY EFFECTS. C.M. McKillop, J.A.C. Brock & G.J.A. Oliver, ICI PLC Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire SK10 4TJ, UK. Sponsor: I.P.H.Purchase.

Pyrethroids are known to induce cutaneous effects in man which are distinct from classical irritation/vascular responses. These effects are characterised by transient facial burning/tingling sensations. A guinea pig flank test (Cagen et al. 1982) has been developed to study this phenomenon and provides a quantitative means to evaluate non-erythematous irritation to the skin. Guinea pigs were treated with pyrethroid solutions on one side of their clipped flanks and the vehicle on the other side. The number of times the animals responded by licking, rubbing or biting the test sites in a 5 minute observation period was recorded. The behavioural response of the guinea pigs to application of a range of concentrations of permethrin, cypermethrin and deltamethrin was assessed. The relative potency of the pyrethroid in eliciting this behaviour was permethrin < cypermethrin < deltamethrin. Peak activity occurred 1 hour following application. The animals were still responding to the pyrethroid test site 6 hours post application but by 24 hours the effect had disappeared. Although this behavioural screen utilises an indirect measurement of skin sensation, it may prove useful in the prediction of cutaneous sensory effects induced by pyrethroid preparations.

82 PARAOQUAT (PQ)-INDUCED CONDITIONED TASTE AVERSIONS (CTA). THE DOSE-RESPONSE RELATIONSHIP. M.S. Dey, R.I. Krieger, B.E. Renzi, and R.C. Ritter. Veterinary Medicine, University of Idaho, Moscow, ID.

CTAs reduce the likelihood of repeated ingestion of food associated with toxic effects. PQ-induced CTAs (and weight loss) are mediated by the area postrema which responds to blood borne toxic substances (Dey 1983, Toxicologist No. 381). We examined the dose-response relationship between peak blood PQ concentrations and CTA formation. Male Sprague-Dawley rats (335 ± 65 g) were trained to drink an instant breakfast solution from calibrated drinking tubes. Rats were offered a novel flavored instant breakfast solution and consumption was measured over 30 min. Rats that consumed > 3.0 ml of the novel flavored solution were given PQ SC within 5 min. Blood (0.15 ml) was serially sampled between 10 and 35 min. after injection via an indwelling jugular cannula. Two days later these rats were again offered the same novel flavored solution and consumption was measured over 30 min. Dosages of PQ ranged from 0.48 to 48.0 µmoles/kg and responses ranged from no change in consumption to a consistent 100% decrease in the volume consumed, respectively. A dosage of 7.2 µmoles/kg resulted in peak blood PQ concentrations of 8.0 µmoles/ml and reduced consumption of the novel flavored solution an average of 52%. CTAs are dose-dependently produced by low blood PQ concentrations which do not produce lung or renal damage. (Supported in part by NIH Biomedical Research Development Grants 1 S08 RR 09073-01A2 and 2 S07 RR 07170).

84 A SENSORY NERVE PREPARATION FOR ASSESSING PYRETHROID INDUCED REPETITIVE ACTIVITY. C.M. McKillop, W.M. Reilly, J.A.C. Brock & C.J.A. Oliver, ICI PLC Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, SK10 4TJ. UK Sponsor: I.P.H.Purchase.

The principal effect of pyrethroids in the vertebrate nervous system is to induce repetitive activity, particularly in the sensory nervous system. This may be responsible for the transient facial symptoms reported after occupational exposure. The effects of a range of concentrations of permethrin, cypermethrin and deltamethrin on the dorsal cutaneous sensory nerves of the American bullfrog, Rana catesbeiana, have been investigated. Pyrethroid was applied to the left hand side of the dorsal skin surface, vehicle to the right hand side. The dorsal cutaneous nerves were exposed and the spontaneous activity of the IVth pair of sensory nerves was recorded at various time intervals. Permethrin induced short trains of nerve impulses (2-10 in total) lasting for tenths of a second. In contrast, cypermethrin and deltamethrin induced long trains of nerve impulses which could last for seconds and contain up to a hundred impulses. Peak activity with all three compounds occurred 1-2 hours post application. This direct measurement of sensory nerve activity may be useful in the future screening of pyrethroid preparations.
83 THE ACUTE TOXICITY OF AN INHALABLE FLUOROPHOSPHONATE (DIFLUORO) AND CHLOROPHOSPHONATE (DICHLORO) IN THREE SPECIES OF RODENTS. A.R. Dahl, T.C. Marshall, and C.H. Hobbs. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM

Methylphosphonic dichloride (DC) is a precursor in the preparation of methylphosphonic difluoride (DF) which is used in the binary weapon. Both chemicals rapidly hydrolyze under physiological conditions to hydrogen chloride and methylphosphonic acid (MPA), and hydrogen fluoride and methylphosphonofluoride acid (MF), respectively. The toxicity of inhaled DF and DC vapors and the oral toxicity of their hydrolysis products was determined in rats, mice, and guinea pigs. The toxicity of DF hydrolysis products was similar to that for fluoride ion alone (LD_{50}^f = 100 mg/kg in rats); but MF alone was also toxic (LD_{50}^f = 300 mg/kg) with symptoms quite different from those of acute fluorosis. DC hydrolysis products were relatively non-toxic (oral LD_{50} > 1000 mg/kg). The relative inhalation toxicities of DF and DC paralleled the oral toxicities of the hydrolysis products. (DC LD_{50} > 7000 μg/L, 1 hr exposure; DF < 2000 μg/L). Death due to acute fluorosis appeared likely in animals exposed to DF. Guinea pigs were more sensitive (DF LD_{50} < 1600 μg/L) with death possibly due to reflex-mediated bronchial constriction. (Research performed for the Chemical Research and Development Center under a Memorandum of Understanding with the U.S. Department of Energy under U.S. DOE Contract No. DE-AC04-76EV01013.)


Acute ACR administration caused: 1) an increase in dopamine and serotonin (5-HT) receptor binding 2) an increase in 5-HT metabolism and 3) the inhibition of retrograde transport. We studied the acute effects of ACR on cat spinal cord reflexes in an attempt to correlate functional changes with the biochemical alterations observed. The monosynaptic reflex (MSR), dorsal root reflex (DRR), and dorsal root potential (DRP) were recorded in spinal cord transected cats. Dorsal root L_s was placed on a stimulating electrode and L_s ventral and L_s dorsal roots were placed on recording electrodes. The evoked potentials were averaged and integrated by a computer. ACR 50 mg/kg was administered every 15 min. to a total dose of 500 mg/kg. The MSR, DRR and DRP were averaged and integrated just prior to the succeeding dose. The MSR exhibited a biphatic response. Initially, the MSR was depressed about 30%. This was followed by a 40% increase which occurred at about 400 mg/kg ACR. The DRR progressively increased to about 600% of control. This depolarization was accompanied by a depression in the DRP. These effects are very similar to the actions of quipazine, a 5-HT agonist, on spinal reflexes and may correlate with the acute biochemical changes in 5-HT metabolism mentioned above. Supported by NS18664.

86 STUDIES ON THE MECHANISM OF NEUROMUSCULAR EFFECTS OF N'-[(2,4-DXYLYL)-N-METHYL FORMAMIDINE HYDROCHLORIDE (U-40481A) IN RATS. V.C. Ravikumar and J.A. Rieger, Dept. of Pharmacol. & Toxicol., Univ. of Oklahoma Coll. of Pharm., Oklahoma City, OK 73190.

We have recently reported that the motor incoordination produced in rodents by U-40481A, at least partly, due to neuromuscular transmission blockade (NTB) and direct depression of skeletal muscle contractility (DSMC) (TOXICOLOGY, 27:71-80, 1981). Using the isolated hemidiaphragm preparation, we have now obtained evidence that the effects of U-40481A are due to interference with the role of calcium ions in the two processes. U-40481 produced significantly greater NTB and DSMC in Tyrode's solution containing 0.4 mM CaCl_2 than when 1.6 mM CaCl_2 (normal) was present. In normal Tyrode's solution, the NTB and DSMC by U-40481 were not reversed by adding CaCl_2 (3.6 mM) to the bath, but were significantly reversed by subsequently adding caffeine (1.9 mM). However, caffeine (1.9 mM) by itself significantly reversed both effects with no further reversal by subsequently adding CaCl_2 (3.6 mM). In Tyrode's solution containing 0.4 mM CaCl_2, both NTB and DSMC were reversed by CaCl_2 (1.8 mM) itself, with the NTB-reversal being more significant. Subsequent addition of caffeine (1.9 mM) further reversed the U-40481-induced effects, with the reversal of DSMC being more significant. Caffeine (1.9 mM) by itself did not reverse U-40481-induced NTB, but significantly reversed the DSMC. Subsequent addition of CaCl_2 (1.8 mM) reversed only the NTB significantly.


Sulfolane is a commercially important potentially neurotoxic solvent. This research examined the consequences of acute exposure to sulfolane upon the visual system, as measured using flash-evoked potentials (FEPs) and pattern reversal evoked potentials (PREPs). Based upon studies of 109 chronically implanted hooded rats, a single i.p. injection of either 1/2 or 1/4, but not 1/8 the reported i.p. LD_{50} of 1600 mg/kg produced significant increases in peak latencies of both FEPS and PREPs, but did not produce significant amplitude alterations. A time course study with 39 additional rats indicated that these effects were apparent within 1 hr and lasted longer than 6 hr. Extrapolation suggested return to control by about 12 hrs. In addition, sulfolane produced an ambient temperature- and dose-dependent hypothermia. Hypothermia itself produces increased FEP latencies and amplitudes. Therefore the effects of sulfolane upon FEP and PREP latencies may be secondary to hypothermia, but since no amplitude effects were seen here, prevention of hypothermia by warming the ambient environment may unmask a depression in amplitude and other effects of the compound. Nevertheless, the acute visual system toxicity of sulfolane does not appear to be high.

*Support by NRC research associateship.
Pesticides were compared to widely used drugs for behavioral effects using Long-Evans rats trained to nose-poke on a multiple schedule for food reinforcers. Daily sessions were divided into 12, 3 min periods. During odd periods all 16 rats were on a random ratio 40 schedule (RR40). During even periods eight rats were on a differential reinforcement of low rate 10 a schedule (DL10) while the others were on extinction (EXT). Once performances stabilised, d-amphetamine (dA), phenylpropanolamine (PPA), chloridormine (CDM), propranol (PXR) or saline was injected i.p. 15 min prior to session. All rats received all compounds in varying dosage sequences. During control sessions response rates were high and uniform on the RR40 but low and uniform on either the DL10 or EXT. On the RR40, all compounds produced rate decreases. EDSO were approximately 1.5 mg/kg dA, 15 mg/kg CDM and PPA, and 6 mg/kg PXR. On the DL10, all compounds produced rate increases. EDSO were approximately 0.75 mg/kg dA, 7.5 mg/kg CDM and PPA, and 8 mg/kg PXR. However, on EXT rates were increased only by dA (EDS0 3 mg/kg) and CDM (EDS0 10 mg/kg), while they were decreased by PPA (EDS0 20 mg/kg) and PXR (EDS0 8 mg/kg). These results indicate a rate dependent effect of dA and CDM but a more specific effect of PPA and PXR, increasing low rates only when they are maintained by reinforcement.

The organotin compound, triethyltin (TET), produces toxic effects in a variety of physiological systems. Thermoregulatory control appears to be especially susceptible to TET toxicity, as TET administration has been shown to cause a pronounced hypothermia in rats. To further elucidate effects of TET on thermoregulation we measured metabolic rate, evaporative water loss (EWL), body temperature, and preferred ambient temperature (Ta) of mice treated intraperitoneally with TET (bromide salt). At a Ta of 23 to 24°C, TET (6 and 8 mg/kg) inhibited metabolic rate by 23 and 66%, respectively. TET resulted in hypothermia at Ta's of 20 and 30°C but not 35°C. TET had little effect on EWL. Mice given TET at doses of 4, 6, and 8 mg/kg selected a cooler Ta (ca. 25°C) compared to controls (ca. 29°C). Thus, the mice selected a Ta associated with a hypo- thermic body temperature. At a relatively cool Ta, mice treated with TET had a reduced rate of heat production and, consequently, became hypo- thermic. At a relatively warm Ta, TET had no effect on heat production and did not increase active heat dissipation (i.e., EWL), thus the mice remained normothermic. The behavioral data indicate that TET evokes a type of regulated hypothermia in mice.
Aminoglycoside antibiotics are both nephrotoxic and ototoxic. This correlation, and the anatomical similarity between the renal tubule and the stria vascularis, suggest that some nephrotoxicants may also be ototoxic. We dosed male Sprague-Dawley rats by gavage for 21 days with CHCl₃ (0.2 ml/kg/day), 1,2-dichloroethane (0.2 ml/kg/day), or K₂Cr₂O₇ (10 mg/kg/day). At 1, 10 and 31 days after dosing auditory function of 2 rats from each group was assessed by measuring brainstem auditory evoked potentials. Auditory thresholds were recorded at 0.5, 1, 2, 4, 8, 16 and 32 kHz. No treatment related effects were detected at any frequency. Histological evaluation of the basal membrane and organ of Corti and cytocochleagram preparation showed no hair cell loss. The data suggest that CHCl₃, 1,2-dichloroethane and K₂Cr₂O₇ are not ototoxic agents. (Supported by NIH Training Grant 5 T32 ES07062.)


Oral administration of 2.2 mM 2,5-hexanediode (2,5-HD)/kg/day plus 2.2 mM methyl ethyl ketone (MEK) /kg/day, 5 days/wk, to male F-344 rats, resulted in a more rapid onset of distal neuropathy than rats receiving 2.2 mM 2,5-HD/kg/day alone. Motor-sensory deficit was evaluated biweekly using a balance beam and an accelerating rotorod. Rats receiving 2,5-HD+MEK demonstrated a rapid decline in balance beam performance after 33 days as compared to 58 days in rats receiving 2,5-HD alone. Performances on the accelerating rotorod gradually declined beginning at 10 and 26 days with rats only on the rotorod at all by 44 as compared to 82 days for the 2,5-HD+MEK and the 2,5-HD groups respectively. The time to onset of hindlimb drag was 48 vs. 85 days for the 2,5-HD+MEK and the 2,5-HD rats respectively. Blood 2,5-HD was measured from serial samples drawn from naive and chronically (7 days) treated rats following oral administration of a single dose of 2,5-HD or 2,5-HD+MEK. The disappearance of 2,5-HD from the blood in the 2,5-HD+MEK group was delayed in both naive and chronic treatments, causing a shift to the right of the elimination curve during the 3-9 hour period post-dose. Enhanced neurotoxicity of 2,5-HD by MEK probably results from higher and prolonged blood levels of the neurotoxicant and may, in part, contribute to the effect observed with other neurotoxic hexa carbons in combination with MEK.


A battery of microcomputer administered tasks has been developed for field testing impairment of performance in populations of individuals exposed to a putative toxic agent. The present data were collected to help determine the sensitivity of these measures in detecting such effects. Two exposure conditions were arranged: In the alcohol study, subjects were tested on two occasions - when sober and when blood alcohol was approximately 80mg/dl. In the Carbon Monoxide study, subjects were either exposed to normal air or to 200 ppm CO (blood levels approximately 7% COHb). Subjects were 18-30 year old males. Tasks in the battery included simple reaction time, visual choice reaction time, color/name classification (Stroop task), memory scanning (Sternberg task), keeping track of objects (modeled after Atkinson-Shifrin continuous memory task). In the alcohol study, these measures were related to standard neurocognitive measures. The battery was sensitive to these exposures and thus appears useful for field testing of toxic impairment.

COMPARATIVE EFFECTS OF THREE ORGANOCHLORINES ON HYPERREACTIVITY AND TREMOR IN RATS. H.A. Tilson, N.J. Peterson and C.F. Maclay. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

The purpose of this research was to compare the dose- and time-related effects of 3 representative organochlorine insecticides on hyperreactibility and tremor in rats. Male Fischer-344 rats, approximately 8-10 wks old, were tested i.p. with either corn oil, 20 or 40 mg/kg of chlordecone in oil. Rats were treated 1,3,5, or 24 hrs later for whole body tremor and responsiveness to 20 presentations of an 8 KHz, 110 dB tone. Similar experiments were done with 2p'-DDT (100 and 200 mg/kg) and lindane (25 and 50 mg/kg). The average startle response over 20 trials was augmented 24 hrs after the higher dose of each compound. Analysis of the data in five blocks of 4 trials each indicated that all compounds interfered with habituation to the tone. Spectral analysis of movement showed that 40 mg/kg chlordecone increased power in the 2.5-5 Hz regions 3 hrs post-dose, which was associated with visible tremor; 200 mg/kg DDT decreased power in the 2.5-5 Hz regions 1 hr postdose. Lindane produced a nonsystematic decrease in power 1,3 and 5 hrs postdosing. All three agents increased startle responsiveness, but at the doses used, only chlordecone produced tremor indicating that the neurotoxicological profile of chlordecone may differ from that of lindane and DDT.
Chlorine dioxide (ClO₂), an alternate water disinfectant, has been implicated as a potential anti-thyroid agent (Reucz et al., 1982). Because anti-thyroid compounds are known to alter neurobehavioral development, the present study was designed to determine if perinatal exposure to ClO₂ affects behavioral activity of rat pups. The exploratory cage system used to monitor pup activity between ages 14 and 21 days (Crofton et al. 1980) has previously detected delayed neurobehavioral development as a result of exposure to lead and propylthiouracil. Pups were exposed to ClO₂ either directly, by gavaging 14 mg/kg/day from age 5 to 20 days, or indirectly via their dams drinking water in concentrations of 2, 20, or 100 mg/L from gestation to weaning. Although the activity of the indirectly exposed group was not different from controls, the group gavaged showed significantly depressed activity for ages 18 and 19 postpartum compared to control animals. T₄ levels of 21 day old pups were significantly depressed in the 100 mg/L group versus control. The gavaged pups showed even greater depressed T₄ levels which strongly correlates with their activity. These data support the hypothesis that ClO₂ affects thyroid function and suggest that a slight depression in T₄ can result in developmental delays. (This abstract does not necessarily reflect EPA policy.)

Chlorine dioxide (ClO₂), a drinking water disinfectant, was evaluated for behavioral effects because it has been implicated as an anti-thyroid agent. Rat pups were intubated daily with ClO₂ (14 mg/kg), perchlorate (ClO₄⁻ 14 mg/kg—positive control), or distilled water from 5 to 20 days of age. At 21 days of age thyroid hormones T₃ and T₄ were measured. At the doses administered, ClO₂ disrupted thyroid function more than ClO₄⁻ in that T₄ was significantly depressed in both ClO₂ and ClO₄⁻ exposed animals while T₃ was significantly elevated only in the ClO₂ exposed animals. A significant depression of exploratory behavior was evident in both ClO₂ and ClO₄ treated rats at 28 days of age, but not at 60 days. In a different paradigm (residential cages) running-wheel drinking and feeding behaviors were quantified from 50 to 60 days of age. Rats exposed to ClO₂ exhibited decreased running-wheel and increased drinking activity in residential cages. ClO₄⁻ treated rats did not differ from controls in running-wheel activity levels but did show increased drinking activity. These data indicate that ClO₂ is a more potent antithyroid chemical than ClO₄⁻ and the effects are long lasting. (This abstract does not necessarily reflect EPA policy.)

Neurotoxic esterase, (NTE), has been proposed as the site for the initiation of organophosphorus-induced delayed neurotoxicity (OPIDN). Among the problems associated with the NTE hypothesis are the observations that NTE activity in brain returns to nearly normal levels before the onset of the neuropathy, and that NTE is present, and is inhibited by organophosphorus compounds in young animals which are insensitive to the neurotoxic effects of these compounds. We present data that indicate that differences in the recovery rates of NTE activity may account for these observations. First, it is demonstrated that the time of onset of the recovery of NTE activity in the most distal portion of the hen sciatic nerve occurs several days later than in the most proximal part of the nerve. This fact could explain greater sensitivity of longer axons to OPIDN, as well as suggest that NTE activity is depressed for a longer period at the site of the neuropathy than it would appear from measurements of NTE activity in brain. Secondly, it is shown that the rate of recovery of NTE activity is faster in both chick nerve and brain than in hen. A greater rate of recovery of NTE activity by either dephosphorylation or resynthesis could account, in part, for the resistance of younger animals to OPIDN. (Supported in part by NIHES Grant ES02717.)

The adult female chicken (hen) is the routine test species for organophosphorus-induced delayed neuropathy while some closely related species are resistant to this condition. After challenge with a dose of diisopropylfluorophosphate which induces neuropathy in hens, brain neurotoxic esterase (NTE) was inhibited 80% or more in hens and females of two resistant avian species, Japanese quail and Bobwhite quail. Only hens developed signs of neuropathy, however. Inhibition of brain NTE in vitro showed that enzyme from all species exhibited similar sensitivities to inhibitor. While brain NTE showed similar sensitivity to in vivo and in vitro inhibition in all species brain acetylcholinesterase showed wide species differences in sensitivity which correlated with species differences in susceptibility to acute toxicity. Further comparative studies between closely related species which show extensive inhibition of NTE yet differ in susceptibility to delayed neuropathy may provide insight into relevant events in the pathogenesis of this syndrome. Research supported by Training Grant ES07090 and Research Grant ES01831 from the National Institute of Environmental Health Sciences. 25
101 NEUROHISTOLOGICAL AND CLINICAL RECOVERY IN HENS AFTER ADMINISTRATION OF TRI-O-TOLYL PHOSPHATE.
G. L. Sprague, A. A. Bickford and T. R. Castles.
Stauffer Chemical Company, Richmond, CA and University of Missouri-Columbia, Columbia, MO.

This study examined recovery of hens from tri-o-tolyl phosphate (TOCP)-induced delayed neurotoxicity in order to correlate histologic changes with clinical improvement. Twelve adult, White Leghorn hens were treated twice with 500 mg/kg TOCP (p.o.) with 3 weeks between the treatments. All but 5 hens were terminated 3 weeks after the second treatment. The 5 remaining hens were terminated after a 120-day observation period. Equal numbers of control hens were treated with 10 g/kg corn oil. All hens treated with TOCP showed progressive leg weakness. Significant improvement first evident 9 weeks into the recovery period remained constant thereafter. Moderate to severe axonal degeneration within specific myelinated tracts of the brain stem and cervical, thoracic and sacro-lumbar spinal cord and in bilateral sciatic nerves was evident in all hens terminated 3 weeks after the second TOCP treatment. Regeneration within the CNS and sciatic nerves was clearly evident in hens held for 120 days and the severity of axonal degeneration in the spinal cord was reduced. Neuronal swelling was unique to TOCP-treated hens held to evaluate recovery. This study demonstrated clinical recovery during a 4-month period from the delayed neurotoxic effects of TOCP accompanied by histologic recovery within the CNS as well as in peripheral nerves.

102 THE EFFECT OF HEPATIC MIXED-FUNCTION OXIDASE ENZYME INDUCERS ON THE DEVELOPMENT OF TRI-ORTHO-CRESYL PHOSPHATE-INDUCED DELAYED NEUROTOXICITY.
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Sponsor: D. Polin

Adult female White Leghorn chickens were divided into 15 groups of ten birds each. Five groups received the Class I mixed-function oxidase (MFO) inducer phenobarbital (Pb), five groups received 8-naphthoflavone (BNF), a Class II MFO inducer, while the remaining five groups were administered corn oil. After 24 hours birds received a single oral dose of the delayed neurotoxin tri-ortho-cresyl phosphate (TOCP) at concentrations of 62.5, 125, 250, or 500 mg/kg body weight. Corn oil served as the vehicle control. Forty-eight hours after the administration of TOCP half the birds were killed for determination of whole-brain neurotoxic esterase (NTE) activity. The remaining birds were observed for onset of clinical signs characteristic of delayed neurotoxicity. Birds receiving BNF prior to TOCP were protected by the inducer when compared to birds receiving Pb and TOCP or TOCP alone. This was indicated by slower development of less severe clinical signs. The extent of NTE inhibition was significantly less in the BNF group at 62.5 gm TOCP/kg body weight and numerically less at the other dosage levels when compared to the Pb and TOCP or TOCP only groups. The protective effect offered by BNF may be due to the increased inactivating of the neurotoxic metabolite.

103 FORMATION OF SUBSTITUTED DIMETHYL PYRROLE ADDUCTS OF 2,5-Hexanedione with POLYAMINES IN VITRO AND IN VIVO.
T.B. Moore and R.J. Richardson, Toxicology Program, School of Public Health, The University of Michigan, Ann Arbor, MI 48109.

Rates of formation of substituted dimethyl pyrrole adducts resulting from the reaction of 2,5-hexanedione (HD) with primary amino moieties of a variety of compounds in vitro increased as follows: \( \epsilon \)-amino capric acid > poly-L-lysine > bovine serum albumin > ethanolamine > putrescine > spermidine > spermine. The overall second-order rate constants ranged from 0.005 to 3.45 \( \text{mol} \cdot \text{hr}^{-1} \cdot \text{cm}^{-1} \) after treatment with Ehrlich's reagent. The pyrrothiated adduct of spermidine was isolated by ion exchange column chromatography from acetic nerve, spinal cord, brain (medulla), and testes of rats dosed subacutely with HD, ip. Tissue concentrations of the modified polyamine were dose-related in spinal cord and sciatic nerve. No adduct could be isolated from the liver. Further study will be required to determine whether pyrrothiated polyamines in neural tissue have a causal role in HD neuropathy. (Supported in part by NIEHS Training Grant 5T32 ES 07062).

104 TOXICOLOGICAL CHARACTERIZATION OF MONOMERIC AND OLIGOMERIC CONTAMINANTS IN A CMATION POLYLYCONCEN TRATE COAGULANT AID.
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More than 50 million pounds of flocculants are used annually in drinking water purification. These polymers may contain low molecular weight contaminants or incomplete reaction products that may leach into water. Our purpose is to examine the extent of water contamination following treatment with an acrylamide-methacrylate based copolymer, and the toxicological significance of such contamination. Leachates were extracted from the flocculant with polar solvents. GPC/HPLC examination showed to distribution of products ranging from monomers to pentamers with free acrylamide as a possible contaminant. Ames test of the extract showed negative results in both frame shift and base substituted strains. The extract was tested for possible acute toxicity in ICR Swiss strain mice. High doses of more than 4g/kg body weight proved lethal, while moderate doses of 1-4g/kg body weight produced neurological complications with some loss of motor control. Thus, more extensive analytical and toxicological studies are needed regarding polymeric flocculant aids used to treat drinking water. Supported by EPA Contractual Agreement CR810196-01-0 (This abstract does not necessarily reflect EPA policy).

Urban aerosols contain heavy metals such as Ni, a known human carcinogen. We measured the deposition of NiCl₂ aerosols in the respiratory tract in order to determine the kinetics of Ni uptake from the lung. Rats were exposed to NiCl₂ aerosol (600-600 µg/m³; 0.7% MMAD and a g 2.13) for 2 hours. After exposure, a cannula was inserted in the trachea toward the head, and 1 ml 1% trypsin was injected through the cannula into the nasopharyngeal zone (NP). After 5 min, the NP was lavaged with 3 x 5mls of water. The trachea and main bronchi (TB) were trimmed from the rest of the lung (P). The 3 samples were acid digested and analyzed by atomic absorption spectrometry. The fractions of Ni deposited were 1.5, 0.5 and 1.5% in the NP, TB and P zones, respectively. The deposition in the NP zone agreeing with the traditional model previously described, Ni is eliminated from the lung with a half-life of 60 hr, and the elimination may be concentration dependent. (Supported by an EPA Cooperative Agreement and NIH Grant ES01859.)


HCl gas is an upper respiratory tract irritant that has recently been compared to formaldehyde (Albert et al, JNCl 68:597, 1982; Perera and Wetto, Science 216:285, 1982). Since concentration response data on HCl-induced nasal passage toxicity has not been reported, we undertook a series of studies in Sprague Dawley and F-344 rats. Animals were exposed to 0, 10, 20 or 50 ppm HCl by inhalation for 6 hrs/day, 5 days/week for up to 90 days. Male and female rats of both strains exposed to 50 ppm HCl had discolored hair coats and female F-344 rats had a significant decrease in body weight gain. Concentration related changes were not detected in organ weights, clinical chemistries, or hematology. To date, detailed histopathological analyses have been completed on the nasal passages of rats exposed to HCl for 4 days and killed 18 hours later. This corresponds to the time of maximal irritation/destruction of the nasal passages in rats exposed to formaldehyde. Significant HCl-induced lesions were confined to rats exposed to 50 ppm and consisted of local degeneration, epithelial hyperplasia and early squamous metaplasia on the dorsal tip of the maxilloturbinate of the most anterior section. Based on this preliminary data we conclude that HCl is approximately one-tenth as potent as formaldehyde in the production of nasal toxicity.


Male guinea pigs (330-450g) were used. HCl inhalation resulted in both sensory and pulmonary irritation with the onset of sensory irritation taking 6 min during HCl exposures at 320 ppm but less than 1 min at 680, 1040 and 1380 ppm, while the onset of pulmonary irritation showed a concentration-response relationship in that the higher the concentration the earlier the onset. Following HCl exposures, animals were evaluated using a pulmonary function test based on the hyperventilatory response to CO₂. The response to a mixture of 10% CO₂, 20% O₂ and 70% N₂ measured by tidal volume increases was reduced by 1040 and 1380 ppm HCl at 0.5 hr and from 1 to 5 days post exposure, but by 320 ppm at 0.5 hr and on day 3 only. The tidal volume increase induced by CO₂ remained lower than the controls on day 10 in the 1040 ppm HCl group. By day 15, all the HCl groups had recovered. In contrast, HCl exposures did not lead to any significant reduction of the CO₂-induced increase in respiratory rate. The baseline respiratory rate, however, showed a HCl concentration-dependent reduction following exposures. In conclusion, the CO₂-challenge method was useful in detecting the respiratory effects of HCl and the recovery from over time. (Supported by N.I.E.H.S. Research Grant 5-K01-ES02747.)

We have previously shown that vitamin C (VC) deficiency in guinea pig (GP) enhances the pulmonary toxicity of NO₂. To further define this effect, lung concentrations of VC, vitamin E (VE), and non-protein sulphydryl (NPSH) were determined simultaneously in the lungs of GP fed rabbit chow (RC) for up to 17 days. Our assay showed VC levels in RC to be similar to normal GP chow. Lung VC dropped quickly to 50% of normal after 4 days on RC then declined more slowly. Body weights were similar in both diet groups. Exposure to NO₂ (4.8 ppm, 3 hr) caused increased lavage fluid protein (edema) which became significant after 7 days of RC feeding. After 14 days on RC, lung VC was reduced to 20% of normal, lung NPSH was increased by 25%, and lung VE was decreased by 10%. Exposure to NO₂ (4.5 ppm, 16 hr) increased wet lung weight by 25% only in RC-fed GP. VE, VC and NPSH were not affected by NO₂ exposure in both RC-fed and normal GP. Lavage fluid contained detectable amounts only of VC, and levels were not altered by NO₂ (4.8 ppm, 4 hr). Thus, NO₂-induced pulmonary edema occurs when little oxidation of VE, VC, and NPSH is detectable by our methods. The protective effect of these interdependent antioxidants appears to be only partially effective, or else their oxidation may be compartmentalized or rapidly reversible. (This abstract does not necessarily reflect EPA policy.)

111 CHLORINE: CHRONIC INHALATION TOXICITY STUDY IN RHEUS MONKEYS. D.R. Klomme, C.E. Ulrich, M.G. Riley, C.S. Barlow, T.E. Ham, Jr., and K.T. Morgan, International Research and Development Corporation, Mattawan, MI and Chemical Industry Institute of Toxicology, Res. Tri. Park, NC

Exposure of rats to chlorine (Cl₂) by inhalation for 30 days (1.3, or 9 ppm) elicited pathologic changes in the respiratory tract, liver and kidney, as well as alterations in urinary and clinical chemistry parameters (Barrow et al., Tox. Appl. Pharm., 49:77, 1979). The purpose of this study was to investigate the chronic effects of Cl₂ inhalation in monkeys. Rhesus monkeys (4 males and 4 females/group) were exposed to concentrations of 0.1, 0.5 or 2.3 ppm, 6 hr/day, 5 days/week for one year. Pulmonary physiology, body weights, urinalysis, electrocardiograms, hematology and clinical chemistries were evaluated monthly; blood gases and ophthalmology were evaluated less frequently. Monkeys exposed to 2.3 ppm exhibited signs of ocular irritation during exposures. No effects were found during evaluation of the other parameters studied. After one year all monkeys were subjected to a thorough necropsy and histopathologic evaluation. Histopathologic changes were limited to focal, concentration-related non-keratinizing squamous metaplasia of the respiratory epithelium of the nasal passages. The results indicate that, at concentrations up to 2.3 ppm, Cl₂ acts only as an upper respiratory irritant in monkeys and that this species appears to be less sensitive to Cl₂ toxicity than the rat.

112 RESPIRATORY RESPONSE AND NASAL DEPOSITION IN NOSEPIECE EXPOSURES OF SPRAGUE-DAWLEY RATS FOLLOWING SUBCHRONIC INHALATION OF FORMALDEHYDE. C.E. Dallas, J.C. Theiss, and E.J. Fairchild, Environmental Sciences, University of Texas School of Public Health, Houston, TX

Since formaldehyde has been shown to induce respiratory depression and altered deposition after acute exposure in rodents, this study endeavored to determine which patterns of changes in these parameters, if any, occurred in rats after long-term exposure to the compound. Male Sprague-Dawley rats were exposed to 0.5, 3, or 15 ppm of formaldehyde for 6 hours/day, 5 days/week. After intervals of 8, 16, and 24 weeks of exposure, animals were removed and individually treated by nosepiece exposure sequentially to: 1) the same concentration which they received in the long-term exposure, and 2) after a post-exposure equilibration period, a 30 ppm "challenge" dose of formaldehyde. Respiratory rate, minute volume, and tidal volume were measured at one minute intervals and exhaled breath collected over ten minute intervals. No significant changes in respiratory response were detected after exposure to 0.5 ppm while animals exposed for 16 weeks to 15 ppm showed less depression in respiratory parameters than the corresponding controls or animals exposed for 8 weeks to 15 ppm. Very high deposition was demonstrated in all animals. Changes in respiratory response to formaldehyde were thus shown to occur after long-term exposure and to be dependent on both concentration and length of exposure.
Bis-chlorethyl-nitrosourea (BCNU) is widely employed for therapy of a variety of tumors in man. Use of the drug in patients has been associated with the development of pulmonary fibrosis. A better understanding of the mechanisms leading to such BCNU-induced adverse effects in the lungs should be achievable by experimentation in animals. Therefore, we studied the pulmonary toxicity of BCNU in F344 rats using a multiple dose regimen comparable to the dose schedule commonly given patients. Morphological aspects were investigated by light and electron microscopy. The lungs were also assayed for hydroxyproline as an indicator of changes in lung collagen and hence, possible biochemical marker of lung fibrosis. All animals developed pulmonary fibrosis accompanied by formation of nodular granulomas. Moreover, a significant number of animals demonstrated hyperplasia and early atheromatous metaplasia of airway epithelia and peripheral submucous glands. Early morphological alterations, suggestive of changes in surfactant production, were found in alveolar type II cells after 1 and 2 doses of BCNU. No measurable increase in hydroxyproline levels were found in any of the BCNU-treated animals.

The partitioning of respiratory mucosal DNA into interfacial (IF) and aqueous (AQ) fractions by extraction of tissue homogenates has been described (TAP 20, 121 (1983)). CH₂O exposure increased the percentage of IF DNA at concentrations > 6 ppm, indicating the formation of DNA-protein cross-links. To test this hypothesis, rats were exposed to atmospheres containing [¹⁴C]- and [³⁴S]CH₂O at concentrations of 0.3, 2, 6, 10, or 15 ppm for 6 hr. The major route of macromolecular labeling at all concentrations was metabolic incorporation. However, significant differences in the labeling of AQ and IF DNA were found at 6 ppm but not at 0.3 or 2 ppm: (1) the incorporation of [¹⁴C] into IF DNA was 65% less than into AQ DNA; (2) the [³⁴S]/[¹⁴C] ratio of IF DNA was 25% greater than that of AQ DNA; (3) the [³⁴S]/[¹⁴C] ratios of IF DNA and proteins were highly correlated (r = 0.94), but those of AQ DNA and proteins were not correlated. The difference between AQ and IF DNA in extractability and specific activity implies a difference in structure induced by CH₂O at 6 ppm. This difference is probably due to DNA-protein cross-linking, since cross-links would be expected to inhibit DNA synthesis, and methylene bridges to proteins should increase the [³⁴S]/[¹⁴C] ratio of IF relative to AQ DNA. These results provide additional evidence that CH₂O forms DNA-protein cross-links in the respiratory mucosa at 6 ppm.

GSH is a cofactor for the oxidation of formaldehyde (CH₂O) catalyzed by formaldehyde dehydrogenase (FDH), which is present in the rat respiratory mucosa. We have postulated that inhaled CH₂O at concentrations > 6 ppm forms DNA-protein cross-links in the respiratory mucosa, as measured by an increase in the percent of interfacial (IF) DNA (TAP 70, 121 (1983)). Therefore, depletion of GSH coupled with exposure to CH₂O should increase the percent of IF DNA relative to that found in nondepleted animals exposed to CH₂O. Concentrations of nonprotein sulhydryls in the respiratory mucosa (2.4 ± 0.4 molal/g) of male F-344 rats were depleted by 90% within 1.25 hr and remained at that level for at least 3 hr after i.p. injection of phenone (250 mg/kg) in corn oil. Absolute increases in % IF DNA over controls as a result of exposure to CH₂O (6 ppm, 3 hr; exposures begun 1 hr post-injection) were as follows: (A) phenone-injected rats: 15.1 ± 1.7; (B) corn-oil injected rats: 3.0 ± 2.7; (C) un.injected rats: 4.0 ± 2.0. Thus, GSH depletion increased the percent of IF DNA resulting from exposure to CH₂O by approximately 3-fold. However, phenone alone had no significant effect on the percent of IF DNA from unexposed animals. These results show that FDH protects against CH₂O-induced toxicity and indicate that exogenous CH₂O is the primary source of DNA-protein cross-links.

In rat hepatocytes depleted of glutathione (GSBH) by diethyl maleate (DEM), the oxidation of formaldehyde (CH₂O) was decreased while lipid peroxidation and cytotoxicity were both increased (Ku and Billings, Proceedings of the Third International Congress on Toxicology, in press). The enhanced lipid peroxidation caused by CH₂O (5 mM to 10 mM) in DEM pretreated cells suggests that CH₂O is exerting toxicity via a free radical-mediated mechanism. The addition of antioxidants, α-tocopherol, butylated hydroxytoluene, or ascorbate, produced a dose-dependent decrease in the extent of lipid peroxidation which was mirrored by a decrease in cytotoxicity. Lipid peroxidation was determined by measuring absorbance of thiobarbituric acid-reactive compounds at 535 nm. Cytotoxicity was assessed by NAAD stimulation of lactate dehydrogenase activity. CH₂O alone caused cytotoxicity at concentrations greater than 10 mM but no lipid peroxidation was observed. The addition of antioxidants was ineffective in decreasing the cytotoxic effects caused by CH₂O alone. These results suggest that in GSH-depleted cells, CH₂O cytotoxicity is due to enhanced lipid peroxidation and CH₂O toxicity is mediated via free radicals. However, cytotoxicity occurs by a different mechanism in cells not previously depleted of GSH. (Supported by NIEHS Grants E052868 and ES07090).
RESPIRATORY RESPONSE AND DEPOSITION IN THE LOWER RESPIRATORY TRACT OF SPRAGUE-DAWLEY RATS FOLLOWING SUBCHRONIC INHALATION OF FORMALDEHYDE.

C.E. Dallas, J.C. Theing, and E.J. Fairchild, Environmental Sciences, University of Texas School of Public Health, Houston, TX.

This study examined the effects of long-term formaldehyde inhalation on the respiratory response from subsequent test exposures to the lower respiratory system. Male Sprague-Dawley rats were exposed to 0.5, 3, or 15 ppm of formaldehyde for 6 hours/day, 5 days/week, for intervals of 8, 16, or 24 weeks. Sample anesthetized animals were then administered formaldehyde by inhalation through a miniaturized one-way breathing valve inserted into the trachea. Exposures were conducted sequentially: 1) the same concentration received in the long-term exposure, and 2) after a post-exposure equilibration period, a 30 ppm "challenge" dose of formaldehyde. Respiratory rate, minute volume, and tidal volume were measured at one minute intervals and exhaled breath collected over ten minute intervals. Animals subchronically exposed to 3 ppm formaldehyde exhibited slight respiratory depression after both 3 and 30 ppm tracheal exposure, while the 0.5 ppm subchronically exposed animals did not demonstrate significant lower respiratory irritancy. Following 8 weeks exposure to 15 ppm, tracheal exposure to 15 and 30 ppm also demonstrated additional depression relative to controls. It is suggested that at sufficient exposure levels, formaldehyde may cause irritancy to the lower respiratory tract after long-term inhalation.


The scientific validity of quantitative risk estimates obtained with administered dose as the independent variable hinges on the validity of this dose measure as a linear proxy for the dose of ultimate carcinogen reaching target tissue. Any disproportionate difference between the high-and low-dose structure or function of delivery, metabolism, and lesion repair systems conflicts with the linear proportionality assumption and mandates use of delivered dose in quantitative risk assessment models. For formaldehyde, which causes nasal cancer in rats, four alternative measures of the dose delivered to rat nasal cavity respiratory epithelium (RE) were examined for possible departures from linear dependence on ambient air concentration. Deposition rate per unit surface area was non-linear, but only near 15 ppm, a concentration that induced the respiratory depression reflex. Neither percent interfacial (IF) DNA extracted from homogenates of RE nor the $\frac{3H}{14C}$ ratio in IF DNA following exposure to doubly labeled formaldehyde were significantly non-linear. At 2 ppm, the percent $^3$H-labeled RE cells following injection of tritiated thymidine was significantly lower than that predicted by a linear response from 0 to 6 ppm. It follows that if this measure is a valid proxy for delivered dose, then low-dose risk estimates based on ambient air concentration may be an order of magnitude too high.

A STUDY OF THE EFFECT OF DISULFIRAM ON ACUTE ETHYLENE GLYCOL POISONING IN RATS.

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Sponsor: W.D. Grey

Disulfiram was concurrently administered at varying dose levels with ethylene glycol by oral gavage to male and female TAC: N (SD) FBR rats to determine its effect on ethylene glycol-induced toxicity and lethality. Body weight, clinical sign, ethylene glycol and acetaldehyde concentrations in plasma, blood gas (pH, $pCO_2$ and $pO_2$), body temperature, lethality and gross necropsy data were obtained.

Disulfiram administration delayed the time of death for males and females. The frequency of lethality and metabolic acidosis, the severity of hypothermia and the plasma ethylene glycol concentration were reduced in females.
121 CYTOTOXICITY OF CITRININ EVALUATED IN CULTURED BOVINE KIDNEY CELLS. M. Yoneyama, R.P. Sharma and S.I. Kleinshcuster. Toxicology Program, Utah State University, Logan UT 84322.

Citrinln produced by various species of Aspergillus and Penicillium has been found to be nephrotoxic to several animal models in vivo; however, only a few studies have been conducted to investigate its cellular effects in cultured cell systems in vitro. Therefore, in this study bovine kidney cell line (MDBK) was cultured with citrinin to determine its morphological and biochemical effects in vitro. After 24 hrs of culture with citrinin (10-100 μM), a concentration-dependent decrease in cell survival was found with distinctive morphological changes at 30 and 100 μM illustrated by scanning electron microscopy. Cells after 1 hr treatment followed by 24 hrs culture treatment followed by 24 hrs culture with fresh medium required 100-1000 μM of citrinin to decrease the survival. Trypan blue exclusion test after 24 hr-treatment showed 0% of viability at 100 μM and 7% of viability at 300 μM. When cells were cultured for 3 days with citrinin, no multiplication of cells was found at 50 μM and all cells were clumped at 100 μM. Specific activity of K+-dependent phosphatase and acld phosphatase and acid phosphatase in 24 hr-treated cells decreased significantly at 100 and 500 μM whereas succinic dehydrogenase was resistant to these concentrations. Incorporation of [3H]-leucine to cellular protein decreased at 30 μM.


The effects of ochratoxin A (OA) were investigated in partially nephrectomized (PN) rats. PN rats compensated for an initial loss of renal function except for inulin clearance, which remained significantly impaired in the model. Sham-operated (SO) rats cleared inulin and p-aninohipurate (PAH) at rates of 3.94 and 7.49 ml/min, respectively, while PN rats cleared inulin and PAH at 2.51 and 8.84 ml/min. Daily administration of low levels of OA produced an enhanced decrease in urine osmolality, urine flow and body weight with a modest increase in urinary protein of PN versus SO rats. OA treated rats cleared inulin and PAH at rates significantly lower than non-treated controls. SO and PN rats cleared OA at 0.109 and 0.078 ml/min, respectively. The clearance rates were reduced to 0.031 and 0.039 ml/min for SO and PN rats, respectively, by pre-treatment with probenecid. Probenecid had no effect on liver and kidney concentrations of OA, yet histopathology revealed less extensive focal necrosis in kidneys from the combination OA-probenecid group than the OA controls. These results verify that the nephrotoxic action of OA is elicited mainly in renal proximal tubules and is enhanced in the PN rat. The mechanism of nephrotoxicity may be related to both reabsorption and secretory processes. (Supported by CCM 16820 and DOD Project DAMG29-83-G-0088.)

123 EFFECT OF CEPHALORIDINE ON CELLULAR ATP CONTENT & MITOCONDRIAL SUCINATE DEHYDROGENASE ACTIVITY IN PRIMARY CULTURES OF RAT KIDNEY CORTICAL EPITHELIAL CELLS. M.A. Smith and D. Acosta. The University of Texas, Division of Pharmacology/Toxicology, Austin, TX.

Primary cultures of rat kidney cortical epithelial cells were used as a tool to evaluate the renal toxic effects of cephaloridine (Cph). Cells were exposed to Cph concentrations of 10-3, 2X10-4 and 2X10-5 M. Cell cultures were prepared from 10-12 day old Sprague-Dawley rats and cultures were grown in hormone & D-valine supplemented media. After a complete cell monolayer had formed, cells were exposed to Cph for periods of 2, 4, 8 & 12 hrs. Previous studies in our laboratory have shown that Cph did not cause significant changes in in vitro indices of toxicity until 8 to 12 hours after exposure to Cph. The purpose of this study was to determine where mitochondrial alterations fit into the sequence of Cph induced renal toxicity. Cellular ATP content and mitochondrial succinate dehydrogenase (SDH) activity were used as indices of mitochondrial function. No significant changes in cellular ATP content were evident until 12 hours after exposure to Cph. The two highest Cph concentrations caused ATP content to decrease by approximately 30%, while the lowest Cph concentration caused a 20% decline in ATP content. Changes in SDH activity occurred as early as 4 hours after exposure to Cph. SDH activity decreased by as much as 50% after a 4 hour exposure to Cph. These data suggest that early mitochondrial changes may be involved in Cph induced nephrotoxicity.


Renal and hepatic toxicities of CHC13 appear to require metabolism of CHC13 by cytochrome P-450 to a reactive intermediate. Phenobarbital (PB) induction of P-450 potentiated hepatic toxicity and metabolism of CHC13, but does not alter renal P-450 nor nephrotoxicity of CHC13 in rats or mice. However, PB does induce renal P-450 in rabbits. Thus, the effect of PB (60 mg/kg i.p. for 4 days) on renal CHC13 metabolism was evaluated in this species. CHC13 toxicity was enhanced in renal cortical slices prepared from rabbits pretreated with PB, as indicated by a decreased ability of renal cortical slices to accumulate radioison after preincubation of slices with CHC13. Likewise, PB potentiated the metabolism of [14C]CHC13 to covalently bound radioactivity and 14CD2 in rabbit renal cortical slices and microsomes. Addition of cysteine reduced the in vitro covalent binding of [14C]CHC13 to rabbit renal cortical microsomal protein and concomitantly trapped a reactive metabolite, presumably phosphene, as 2-oxythialosedine-4-carboxylic acid. Thus, these data support the hypothesis that CHC13 is metabolized to the reactive metabolite, phosphene, by a PB-inducible form of cytochrome P-450 in the rabbit kidney.
Comparisons were made in male, Fischer 344 rats of the renal effects of 1,2-dibromo-3-chloropropane (DBCP) and a hydrochloric acid (HCl), to indicate whether the latter could be the cause of injury. Subcutaneous administrations of 0-80 mg/kg DBCP or 0-125 mg/kg CLA caused dose-dependent renal proximal tubular necrosis. The damage with DBCP was restricted to the pars recta, but was more widely distributed with CLA. Both compounds increased fluid intake, urine volume, proteinuria and glucosuria by an ensuing 3 day period, and reduced creatinine clearance when administered at 80 mg/kg (DBCP) or 100 mg/kg (CLA); these doses also increased kidney weights and serum urea nitrogen and creatinine concentrations. Accumulation of organic ions in vitro and glycolysis (metabolism to CO₂) by renal slices were reduced whether DBCP or CLA were administered in vivo or were added in vitro. The oxidations of pyruvate and lactate were similarly impaired in vivo and in vitro. Thus, the toxic effects of these agents may occur secondary to an inhibition of energy metabolism. These results document synchronous effects of DBCP and CLA on renal function and biochemistry, suggesting that CLA may be the cause of DBCP nephrotoxicity. The differences in localization of structural damage may be due to site-specific metabolism of DBCP to CLA.

**AT-125 (ACICIN) DOES NOT PREVENT HEXACHLOROBUTADIENE (HCB) NEPHROTOXICITY.** M. E. Davis, West Virginia Univ. Med. Ctr., Morgantown, WV 26506

γ-Glutamyltranspeptidase (γ-GTP) catalyzed degradation of a glutathione: HCB conjugate has been proposed to yield reactive intermediates which are the actual nephrotoxic agents. AT-125 pretreatment was used to inhibit γ-GTP and HCB toxicity assessed. Male rats were injected with AT-125 (10 mg/kg), or saline, followed 1 hr later by HCB (200 mg/kg) or oil. Renal γ-GTP activity was 3% of control at 1 and 2 hrs after AT-125 and was still greatly decreased (17% of control) at 24 hrs. AT-125 had minimal effects on renal function. HCB caused significant impairment of renal function but AT-125 pretreatment did not alter these effects. During the first 24 hrs after HCB urine volume was increased similarly in AT-125 and saline groups (24.7±6 ml and 25.5±5.2 ml, vs oil controls 7.6±1.3 ml) while urine osmolality was decreased. AT-125 pretreatment did not affect HCB proteinuria (AT-125 104% of saline) or glucosuria (AT-125 101% of saline). BUN and plasma creatinine were similarly elevated (BUN: 147±75 mg/dl, AT-125; 101±7, saline) (creatinine: 3.9±1.7 mg/dl, AT-125; 3.8±0.35, saline) from oil treated controls (BUN: 19±1 mg/dl creatinine 0.59±0.2 mg/dl). Thus pretreatment with AT-125 did not decrease HCB-induced impairment of renal function. The present studies do not support the hypothesis that renal γ-glutamyltranspeptidase activity is a critical step in the activation of HCB to a nephrotoxic intermediate. (NVU Medical Corp. and NIH Grant 5 S01-R054-18).

**THE ROLE OF METABOLISM IN STRAIN DIFFERENCES IN ACETAMINOPHEN (APAP) NEPHROTOXICITY.** J.F. Newtown, D.A. Pasino and J.B. Bock, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI.

APAP produces renal necrosis restricted to the straight segment of the proximal tubule in Fischer 344 (P344) but not in Sprague-Dawley (SD) rats. These strain differences in APAP-induced nephrotoxicity apparently cannot be attributed to differences in in vitro renal P-450 activation or deacetylated-dependent metabolic activation (DDMA). Furthermore, in vitro renal toxicity of APAP and its nephrotoxic metabolite, p-aminophenol (PAP) was identical in both strains. In contrast, arylation of renal macromolecules in vivo in P344 rats was 4x greater than in SD rats. This difference in covalent binding (CB) resulted primarily from DDMA. At the time of maximal CB, APAP renal cortical concentrations were 2x greater in P344 than in SD rats. These studies suggest that strain differences in APAP nephrotoxicity are due to differences in DDMA. Strain differences in the renal concentrations of APAP may be a contributing factor in strain differences in DDMA in vivo.
We have previously shown that 3-alkylfurans damage metabolically active organs of the body. 3-Pentylfuran is a particularly potent nephrotoxin, and it was important to establish that this effect resulted from metabolic activation of the furan. Uptake of p-aminophenol and tetra-ethylammonium by kidney slices was used to assess the functional integrity of the kidney, and measurement of serum urea nitrogen was carried out as an in vivo measurement of kidney function. Time course studies indicated measurements were best made 24 hours after dosing. Phenobarbital (60 mg/Kg i.p.) protected mice against pentylfuraz nephrotoxicity (295 mg/Kg i.p.), probably as a result of a liver effect. beta-Naphthoflavone (80 mg/Kg i.p.) had no effect. Although other inhibitors had little effect, N-0ctylimidazole (20 mg/Kg i.p.) significantly reduced pentylfuran nephrotoxicity, as well as that due to the corresponding dihydrofuran (4-pentyl-2,3-dihydrofuran). These results point toward metabolic activation as the basis for the nephrotoxicity of both compounds. Support: NIH GM-26,366.

The purpose of this work was to determine the effects of mercury and chromium on whole animal renal function when given simultaneously in doses that were not toxic acutely. Early work in this laboratory demonstrated that the metals mercury and chromium when given in combination can be shown to alter organic ion transport in rat kidneys. Chromium administered in a dose of 10 mg/kg subcutaneously has no apparent effect on renal function as measured by urine output at 25, 60, and 24 hours after drug administration. This lack of an effect by chromium at a 10 mg/kg concentration on organic ion transport has been reported earlier (Baggett and Berndt, 1982). Mercury administered in a dose of 4 mg/kg had effects on renal function. Urine flow tended to increase over several days and urine osmolality was reduced. Food consumption was reduced as was body weight. However, when the two metals were administered coincidentally, there was a significant increase in urine output after two hours, but by day 2 posttreatment urine volume was only 10% of control. Surviving animals regained normal urine output by 96 hours posttreatment. Food consumption fell to 20% of control at 24 hrs, and by 96 hrs posttreatment there was a 20% decrease in body weight compared to beginning weights.

Studies with anesthetized animals have shown no relation between the effects of the two metals on renal function and renal blood flow. (Supported by USPHS BS 0123-01).

Nephrotoxicity of Trimethyltin Chloride (TMT) in Rats. D.G. Robertson, R.H. Gray, S.H. Kim, and P.A. de la Iglesia. Environmental & Ind. Health, Univ. of Michigan; Pathology & Exper. Toxicology, Warner-Lambert / Parke-Davis Pharm. Res., Ann Arbor, MI.

TMT is primarily known as a neurotoxicant and its nephrotoxicity is not well described. This study reports the effects of TMT on rat kidney. Male Long-Evans rats (150-175 grams) were dosed with 12.25 mg/kg TMT. Animals manifested overt toxicity signs, lost weight and mortality ensued. Moribund animals were killed on day 8 and showed marked proximal tubular damage with dilatation, loss of brush borders and cytoplasmic vacuolization. Survivors were killed after 35 days and showed no nephropathology. A group of male Wistar rats was dosed with 10 mg/kg TMT and killed 6 days later. Kidney revealed pathological changes. BUN levels were significantly elevated; no change was seen in serum alanine transferase, gamma-glutamyl transpeptidase or alkaline phosphatase. This brief study indicates that TMT produces kidney pathology in addition to the well established neurotoxic effects.

Subchronic Ethanol Administration Enhances the Hepatocellular Genotoxicity of Several Pyrolysate Mutagens. D.J. Loury and J.L. Byard, Dept. of Env. Tox., Univ. of Calif., Davis, CA 95616.

Alcoholism has been associated epidemiologically with an increased incidence of liver cancer. The present study evaluated the effect of subchronic ethanol ingestion on the genotoxicity and metabolism of the pyrolysate mutagens Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, IQ and MeIQ in primary cultures of rat hepatocytes. Male Sprague-Dawley rats were pair-fed for 8 days liquid diets containing either 8% v/v ethanol or an equi-caloric sucrose solution. Genotoxicity was determined by the induction of unscheduled DNA synthesis (UDS). HPLC techniques were used to measure the concentrations of pyrolysates in the culture medium after 0, 2, 5, and 16 h exposures. Ethanol pretreatment significantly enhanced (p<0.05) the UDS response to Glu-P-1, Glu-P-2, IQ and MeIQ but not to Trp-P-1 or Trp-P-2. Statistically significant increases in UDS responses ranged from 1.9 fold for IQ to 3.4 fold for Glu-P-2. Following 16 h exposures, the concentration of parent mutagens in the culture medium decreased by 75-96%. No appreciable changes in the culture of pyrolysate metabolism could be attributed to ethanol pretreatment. A selective increase in the metabolic activation of Glu-P-1, Glu-P-2, IQ and MeIQ following subchronic ethanol exposure may account for the present findings. (Supported by NIEHS PHS ES07059-03 and IBM grant S3B2403).
THE MUTAGENICITY OF GAS-PHASE ARC WELDING FUMES.

The effects of chronic exposure to complex ambient gas-phase mixtures have typically been investigated by assaying the extracts of particulates collected on filters for mutagenicity in the Salmonella/microsome system. We have used the Salmonella strains 1535 and 1538 to determine the mutagenicity of the particulate fraction and the filtered flow-through fraction from manual arc welding on mild steel. We report a small increase in revertants in both strains associated with the particulate fraction. The flow-through fraction, when tested in the aqueous phase by bubbling the fumes through a suspension of cells, was also slightly mutagenic for both strains. However, when strain 1535 cells were preincubated and exposed to the gaseous phase, a linear dose-dependent increase resulted in which a 100-fold increase in revertants and a 200-fold increase in mutation frequency could be measured. Only a two-fold increase was obtained in strain 1538 in the gaseous system. Thus we conclude that, while the particulates from arc welding fumes are somewhat mutagenic as reported by others, there is a large mutagenic activity associated with the filtered gaseous phase.

CONTRIBUTION OF PRIMARY AROMATIC AMINES TO THE MUTAGENICITY OF GASIFIER TARS AND COAL OILS.
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Primary aromatic amines (PAA) are believed to contribute significantly to observed bacterial mutagenicity and potential health hazards associated with coal liquefaction products. Two reactions which chemically alter PAA were developed to determine the contribution of these compounds to the indirect bacterial mutagenicity of a low Btu gasifier tar trap tar. Nitrous acid was shown to generate substantial direct mutagenicity from the tar when used with 0.3 N HCl (pH 1.2). This direct mutagenicity limited interpretation of results. At a pH of 4.0, nitrous acid only partially eliminated the indirect mutagenicity of a PAA standard. At the optimal pH of 2.5, no direct activity was generated for the tar, and virtually all of the indirect activity for PAA standards was eliminated. For low Btu gasifier tar, 61% of the indirect activity was eliminated from a coal oil. The second reaction, acetylation, reduced the indirect activity of most PAA standards by 79% or more. Acetylation of the tar likewise reduced part, but not all, of the mutagenicity, whereas most of the activity of coal oil was eliminated. These results indicated that a much lower percent of mutagenic activity of low Btu coal tar samples was due to PAA than was the case for coal oil. (Research was supported by U.S. Department of Energy Contract No. DE-AC04-76EEO1013.)

COMPARISON OF IN VIVO AND IN VITRO TESTS FOR CARCINOGENICITY OF COMPLEX MIXTURES.

Our laboratory has been studying the toxicity of the complex organic by-products of coal gasification using two in vitro mammalian tests (SECE and alveolar macrophage function), the Ames test, and an in vivo test for dermal carcinogenicity. To further characterize the toxicity of these complex mixtures, which contain hundreds of compounds, a coal gasification tar was fractionated into components with neutral, basic, or acidic characteristics. These fractions were then studied for mutagenicity and cytotoxicity in the CHO/HGPRT assay and for carcinogenicity after chronic dermal exposure in mice. The carcinogenicity results showed that the neutral fraction produced the earliest time-of-onset and a greater percentage of malignant tumor induction than the unfractonated tar. The acid fraction was the least active with the base fraction intermediate. The CHO/HGPRT assay produced similar results (neutral fraction > base fraction > acid fraction) for mutagenicity. The presence of hepatic S9 in the assay protected against the cytotoxicity of the base and neutral fractions, but not the acid fraction. This close correlation between the CHO/HGPRT system and the in vivo dermal carcinogenicity assay is probably due to the known sensitivity of the CHO/HGPRT assay to mutagenic polycyclic aromatic hydrocarbons. (Work supported by the U.S. Department of Energy under contract No. W-31-109-ENG-38.)

EVALUATION OF BUTHIDAZOLE FOR MUTAGENICITY WITH SALMONELLA.

The mutagenic potential of buthidazole was evaluated in a battery of in vitro bacterial assays designed to detect frameshift or base-pair mutations. Buthidazole was added directly to the agar plates with or without liver microsomal preparations from uninduced mice or Aroclor 1254-induced mice and rats. In addition, male and female mice and rats were fed buthidazole to determine if mutagenic metabolites could be detected in urine collected from the treated animals. The results of these studies indicated that buthidazole did not cause a significant increase in the number of revertants/plate in any of the tester strains with or without metabolic activation nor was there apparent mutagenic activity in the urine collected from these rodents.

N-Substituted polycyclic aromatic hydrocarbons, including arylamines, have been observed as environmental contaminants. We have irradiated 2-aminofluorene (2AF) with either a sun lamp, cool white, black, blue, or yellow fluorescent light or incubated the compound in the dark prior to assaying for direct mutagenicity using Salmonella typhimurium strain TA98. The effectiveness of these exposures in potentiating the mutagenicity of 2AF was sun > black > cool white > blue > yellow > dark. By using optical filters, the wavelengths of light responsible for the poten-
tiation were found to be between approximately 300 nm and 450 nm. Studies using radical scavenger,

139 THE MUTAGENIC ACTIVITY OF TOXAPHENE AND TOXAPHENE FRACTIONS IN THE AMES TEST. J.F. Rogauskas and G.A. Pollock. WO1 Regional Program in Veterinary Medicine, University of Idaho, Moscow, ID; SGS Biotech Inc., Palmit-

sville, OH.

The mutagenic activities of toxaphene insecticide and two fractions of toxaphene (polar and non-polar) were determined by the Salmonella/microsome assay for mutagenicity. The mutagenicities were determined using TA100 with and without metabolic activation. S-9 fractions from animals induced with the different test mixtures or Aroclor 1254 were used for the assays in order to compare differences in induction caused by the various exposures.

Toxaphene and the fractions were all directly mutagenic but incubation with S-9 caused a decrease in revertants. Toxaphene (6000 mg/plate) caused a 2-3 fold increase in revertants per plate in the direct assay and was more potent than the two fractions. The number of revertants approached control levels for all S-9 fractions tested, but this decrease was apparently due to a physical effect (sequestration) rather than metabolism.


Acrylamide (ACM), a vinyl monomer widely used in industry, has been reported to induce tumors in mice (Laurie et al., 1983). Presently, there are inconsistent results concerning the genotoxicity of ACM in several in vitro tests. This study examined the effect of ACM on unscheduled DNA synthesis using the HPC/DNA repair test and C562 gradients. Hepatocytes were isolated from male Fisher 344 rats and exposed to ACM (0-10^-4 M) and [H]-thymidine (Tdr) for 18 hours. Incorporation of H-Tdr into DNA was determined by autoradiography. No DNA repair was observed at ACM doses up to 10^-2 M. These findings were confirmed with C562 gradients. ACM doses greater than 10^-2 M were cytotoxic as determined by cell survival, uptake of trypan blue, and release of aspartate from cells. Doses of ACM which were found to be cytotoxic had previously been reported as positive in the HPC/DNA repair test (Mast et al., 1983). The possibility that ACM inhibits DNA repair processes, thereby obscuring possible genotoxic effects, was investigated. Preliminary results indicate that ACM does not inhibit UV-induced DNA repair. These results suggest that ACM is not genotoxic in the rat.


Six different ethyleneamines, including samples which varied in purity and composition, were tested for genotoxic potential using the Chinese Hamster Ovary (CHO) gene mutation assay, the Sister Chromatid Exchange (SCE) test and a primary rat hepatocyte assay to detect production of unscheduled DNA Synthesis (UDS). The CHO gene mutation and SCE tests were performed both with and without rat liver homogenate metabolic activation. Diethylenetriamine and aminoethylethanolamine did not produce significant, dose-related effects in any of the three tests. Slight increases in SCEs produced by one diethylenetriamine sample were not observed with two purified samples with reduced aminooethylpyperazine content. Amino-

ethylpyperazine itself produced significant effects only in the SCE test with comparatively greater activity observed in the test without metabolic activation. Tetraethylenepentamine and a complex mixture of higher polyamines produced significant effects in both the SCE and UDS tests. Triethylentetramine produced positive effects in all three tests but genotoxicity was reduced or eliminated following purification or sodium borohydride reduction. Variable composi-
tion and purity of the higher alkylamines apparently can affect significantly the genotoxic potential of the test material.
Lack of mutagenicity of hydroquinone (Hq), tetramethylthiuram monosulfide (TMTM), and tetramethylthiuram disulfide (TMTD) in Drosophila.

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Interest in the development of a battery of quick, complementary mutagenicity assays which will accurately predict the mutagenic potential of different classes of chemicals is increasing. Proper evaluation of the strengths and weaknesses of different short-term assays requires comparison of both negative and positive data. Negative data obtained for three industrial chemicals with the sex-linked recessive lethal test are reported here. Recessive lethal mutations (rims) were found in 0.16% (7/4377) and 0.25% (11/4435) of the F2 cultures from males fed 1000 ug Hq/ml and a 1% sucrose solution, respectively. Rims were also found in 0.14% (7/5160) and 0.11% (5/4612) of the cultures from males fed 750 ug TMTM/ml and a 0.5% Tween-80/4% sucrose solution, respectively. Finally, rims were found in 0.12% (6/5023) and 0.19% (8/4278) of cultures from males fed 600 ug TMTD/ml and a 0.4% Tween-80/1% sucrose solution, respectively. No statistical differences were detected when these data were evaluated with the Kastenbaum-Bowman test (P < 0.05).

Measurement of unscheduled DNA synthesis (UDS) in mouse hepatocytes following in vivo exposure.


The in vivo-in vitro hepatocyte DNA repair assay has been shown to be a useful indicator of the carcinoenic potential of chemicals in rat liver (Environ. Mutagen. 4:553). We have modified this assay for measurement of UDS in mouse hepatocytes following in vivo exposure to genotoxic agents. B6C3F1 mice were treated by gavage and hepatocytes were isolated by liver perfusion and incubated with $^3H$-thymidine. UDS was measured by quantitative autoradiography as net grains/nucleus (NG). The percent of cells in repair (R IR) was calculated as the percentage of cells with >5 NG. Controls yielded 0% with <2% IR. Mice sacrificed 2 hr after treatment with the hepatocarcinogen dimethylaminobenzylideneamine yielded 9.9 and 24.7 NG (59 and 85% IR) at 2 and 10 mg/kg, respectively. 4-Aminobiphenyl is a mammary carcinogen in rats, but produces liver and bladder tumors in mice. Treatment of mice with 200 mg/kg yielded 6.4 NG and 55% IR. Hepatotoxicity can also be measured as increased DNA replication; 48 hr after treatment with the hepatotoxin carbon tetrachloride, 6.0% of cells were undergoing S-phase synthesis compared with <0.2% in controls. These results indicate that this assay may be useful for the study of genotoxic and hepatotoxic chemicals in the mouse and should permit better comparisons of the effects of hepatocarcinogens between species.

Trichloroethylene: Tumor promoting effects.

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In certain geographic locations the population is exposed to low levels of trichloroethylene (TCE) and other halogenated hydrocarbons. Since these agents are often carcinogenic in short-term bioassays, we examined the ability of chronic TCE or CCl4 administration to promote the development of phenotypically altered foci and hyperplastic nodules in the livers of CFW Swiss Webster mice initiated with dimethylaminobenzylideneamine (25 ug/mouse) within 30 hr of birth. After weaning, TCE or CCl4 was administered in drinking water at two concentrations (1.0 mg or 5.0 mg TCE/ml) and 0.1 mg or 1.0 mg CCl4/ml) for 12 or 20 weeks. Serum enzymes (GOT, GPT, and alkaline phosphatase) were performed as indicators of hepatocellular injury. With the 1.0 mg TCE/ml and 0.1 mg CCl4/ml treatments enzyme levels did not vary significantly from controls. With the 1.0 mg CCl4/ml and 5.0 mg TCE/ml treatments serum enzyme levels were 2-5 times greater than control values. The preneoplastic response was evaluated by assessing the development of foci and nodules resistant to iron accumulation. The 5.0 mg TCE/ml and 0.1 mg CCl4/ml treatments produced slight increases (1.5-3 times DNA-alone treated animals) in the number of focal and nodular intersections per sq. cm of liver tissue. The 1.0 mg CCl4/ml treatment enhanced the number of focal and nodular intersections to 3-6 times DNA-alone treated animals. (Supported by BRSG 2507 RR05605.)

Two-year feeding studies with 2-acetylaminofluorene (2-AAF): Comparative histopathology in Sprague-Dawley rats and CD-1 mice.

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Chronic feeding/oncogenicity studies with 2-AAF were conducted to evaluate interspecies differences in histopathology relative to dose and sex. Rats received 200 ppm (25/sex) or 60 ppm (65/sex); mice, 500 ppm (25/sex) or 30 ppm (100/sex); negative controls included 170 rats/sex and 250 mice/sex. No signs of toxicosis were observed, but after two years survival was 7% for treated rats vs. 76% for controls; 2% for high-dose mice and 40% for low-dose mice vs. 57% for controls. The incidence of non-neoplastic lesions in rats and mice, treated and control, were similar to that previously reported. Both species had treatment-related increases in liver neoplasia; in rats, the incidence was greater in males and dose-related in females; in mice, the incidence was dose-related in females. Metastasis involved numerous organs in rats, but none in mice. Rats had a dose-related increase in mammary gland neoplasms. Other subcutaneous neoplasms occurred in 11% of male and 36% of female treated rats and in only 1% of the controls. Transitional cell, urinary bladder neoplasms occurred in 40% of high-dose male and 32% of high-dose female mice, but in no controls. Bladder neoplasms did not occur in rats; however, transitional cell hyperplasia occurred in 20% (5/25) of the high-dose females, in 1.6% (1/64) of the low-dose males, and in no controls.

Kathon CG (1.5% active ingredient) was applied to the dorsal skin of 40 male CD-1 mice at a concentration of 400 ppm (active ingredient) and a dose of 25 uL 3 times a week for 30 months. A positive control group of 40 male mice was similarly treated with 3-methylcholanthrene (3-MC 1000 ppm) while a negative control group was similarly treated with distilled water. Necropsies were performed on all mice. Histopathologic evaluation was performed on selected organs and tissues from each group. Kathon CG had no effect on 30-month survival; 10 of 40 control mice died during the study. Kathon CG-treated mice survived 30 months. All 3-MC-treated mice died within 16 months. Brown staining, eschar and/or desiccation, and flaking of the skin were seen at the application site of Kathon CG-treated mice. All 3-MC-treated mice developed lesions at the application site within 6 months. Two Kathon CG-treated mice developed lesions after the application site (1 hemangioendothelioma, 1 hemangiosarcoma); however, neither was treatment related since similar vascular tumors were seen in the spleen, liver, and tail of 3 control mice. There were no indications of a treatment-related increased incidence of neoplasms systemically or at the application site in the Kathon CG-treated mice. Kathon CG-treated skin showed epidermal necrosis, eschar, hyperplasia, hyperkeratosis, dermal inflammation, and increased dermal collagen. All 3-MC-treated mice developed squamous cell carcinomas at the application site. Thirty months of continued application of Kathon CG to male mice showed no local or systemic tumorogenic potential.

146 STUDIES ON THE MOUSE LUNG TUMOR ASSAY AS A SCREEN FOR CARCINOGENS. L.H. Smith and H.P. Witschi, Biology Division, ORNL, Oak Ridge, TN and R.N. Maronpot, Natl. Toxicology Program, Research Triangle Park, NC.

The objective of our study was to validate the mouse lung tumor assay. We evaluated 5 mixtures from sunflower processes, 8 nitrated toluenes and 30 compounds that had been tested in a 2-year NCI type carcinogenesis bioassay. Male A/J mice were injected with the substances for 3 weeks a week for 8 consecutive weeks. Four months after the last injection, the mice were killed and carcinogenicity of the substance was evaluated by determining the number of tumors on the lung surface (multiplicity) and the number of mice with tumors (incidence). These end points were compared to those of appropriate controls, and Chi-square and t-tests were applied to determine significant differences at the 5% level. Few of the substances were found to give a unequivocally positive response. Shale oil and 2 of its derivatives and 2 tar mixtures from a coal gasifier were clearly positive. The 8 nitrate toluenes were negative. Out of 18 compounds known to be human carcinogens or to be positive in a conventional 2-year bioassay in rodents, the lung tumor assay was unequivocally positive for 5. Thus, it might be appropriate to reconsider the usefulness of the lung tumor assay as a short-term in vivo screen for carcinogenesis. Research sponsored by the Office of Health & Environ Res, US Dept of Energy, under contract W-7405-eng-26 with Union Caribide.


Sprague-Dawley rats (70 males and 70 females/group) were exposed to 0, 10, 50, and 150 ppm morpholine, a secondary cyclic amine, 6 hr/day, 5 days/week for 104 weeks; 10 males and 10 females from each group were sacrificed after 52 weeks.

Survival for mid- and high-concentration males was slightly poorer than controls; survival for females and mean body weights of both sexes were not significantly affected. Corneal irritation, uveitis, and diffuse posterior lens capsule cataracts were observed in rats from all groups; anterior eye segment effects were more pronounced at the high dose level. High-level exposures caused focal erosion and focal squamous metaplasia of the epithelium of the anterior nasal cavity in most high-concentration males and females, which occasionally involved the nasal turbinates and maxillary turbinates. The incidence of metaplasia normally occurring in aging Sprague-Dawley rats was not significantly increased by morpholine.

148 EFFECTS OF 17a-ETHYNYLESTRADIOL (17a-EE,) ON HEPATIC ESTROGEN ACTION IN RATS TREATED PREVIOUSLY WITH DIETHYLNITROSAMINE (DEN). D.B. Campen, A.E.M. Vickers and G.W. Lucier. NIH/NIH, Research Triangle Park, NC 27709

Estrogen increases the incidence of hepatocellular carcinoma by acting as a tumor promoter. We are using the rat two-stage model to evaluate the role of hepatic estrogen receptors (ER) in the promotion process. Adult ovariectomized Sprague-Dawley rats were administered DEN (200 mg/kg i.p.). Silastic capsules containing 17a-EE, were then implanted subcutaneously every 28 days to produce dose groups of 0, 0.09 (low dose) and 0.43 (high dose) mg 17a-EE,/kg/day. Cytosolic ER, microsomal epoxide hydrolase (EH) activity, and GGT-positive foci were quantified in hepatic tissue after 10, 19 and 40 weeks of estrogen treatment. At 10 weeks, microsomal EH activity showed no difference among treatment groups. Significant decreases in concentration of unoccupied ER were detected in estrogen-treated animals. At 19 weeks, the area of GGT-positive foci were different among treatment groups. Values in mg/mg CM: controls, 0.1; DEN-cholesterol, 4.8; DEN-low dose 17a-EE,, 20.6; DEN-high dose 17a-EE,, 19.3; and saline-high dose 17a-EE,, 5.0. Our study demonstrates increases in prae-neoplastic lesions using DEN as an initiator followed by continuous administration of 17a-EE, as a promoter. It appears that interaction of 17a-EE, with the hepatic ER may be playing a role in the carcinogenic process.
To evaluate the carcinogenic potential of methylene chloride in B6C3F1 mice, levels of 0, 60, 125, 185 and 250 mg/kg/day were administered in deionized water to 650 male and 350 female mice (unbalanced study design) for two years. Minimal effects were noted in mean leukocyte count following one year of treatment. Treatment-related histomorphologic alterations of the liver were noted in the high-dose males and females consisting of a marginal increase in the amount of Oil Red O positive material. No treatment-related increases in neoplastic histomorphologic findings were observed. Statistical analyses of results failed also to yield positive significance for trend. Based upon the conditions of this study, methylene chloride did not induce a carcinogenic response. A "no-effect" level of toxicologic and non-neoplastic histopathologic effects was observed at a dose level of 185 mg/kg/day.

Firemaster BP-6 (FM), a mixture of polybrominated biphenyls, has been shown to cause hepatic tumors in rats and mice. FM has also been shown to act as a tumor promoter in hepatocarcinogenesis bioassays in rats. Due to the extremely long retention times of FM in animal tissues, we hypothesized that tumor promotion could occur after short-term exposure to FM. Female Sprague-Dawley rats weighing 185-215 g were 2/3 partially hepatectomized and 24 hours later given 10 mg/kg diethylnitrosamine ip. On days 30-36, rats were gavaged with FM in corn oil, at total doses of 0, 3, 30, 150, or 300 mg/kg per rat. These doses approximate the total amount of FM a rat consumes if fed 0, 1, 10, 50, or 150 ppm FM, respectively, in the diet for 180 days. Rats were killed on day 150. The average numbers of gamma-glutamyl transferase positive foci/cross-section of liver for groups of six rats were: control, 65; 2 mg FM, 98; 30 mg FM, 347; 150 mg FM, 395; and 300 mg FM, 127. Numbers of foci were significantly increased in rats given 30 or 150 mg FM. Decreased weight gains were seen in rats given 30 mg or more FM. Two of six rats died after receiving 300 mg FM. These results indicate that hepatic tumor promotion can occur after short-term exposure to FM. (Supported by NIEHS Grant ES-02781 and by the Mich. Agr. Exp. Station).

Assessment of damage at restriction endonuclease (RE) cleavage sites in hepatic DNA following exposure to the hepatocarcinogen 2-acetylaminofluorene (AAF). R.L. Vorce and J.L. Goodman, Department of Pharmacology and Toxicology, Center for Environmental Toxicology, Michigan State University, East Lansing, MI 48824.

Our objective was to test the hypothesis that following exposure to chemical carcinogens the damage which occurs to the DNA of target tissue is distributed in a nonrandom fashion within the genome. AAF was administered to male rats (200 g) via gastric intubation at doses ranging from 1 exposure of 5 mg/100 g up to 10 mg/100 g 3 times/day for 2 days. Rats were killed at 18 h after the last dose. Hepatic DNA was isolated, digested with a RE (Kpn I, EcoR I, Hind III, or Hae III), electrophoresed in a 0.9% agarose gel and transferred to nitrocellulose paper. The mobility of specific DNA fragments was assessed by hybridization with 32P-labeled rat serum albumin gene cDNA, and visualized as bands by autoradiography. Following a single dose of AAF, 5 mg/100 g, the bands observed under control conditions following digestion with Kpn I were not seen. In these experiments a single high molecular weight band was seen, indicating that some recognition sites for Kpn I had not been cleaved. AAF at the highest dose regimen failed to inhibit either EcoRI, Hind III, or Hae III. These results indicate a high degree of selectivity with which AAF interacts with portions of the genome. Those DNA adducts which inhibit the action of RE might cause aberrant gene expression as a result of modifying interaction of regulatory proteins and/or RNA polymerases with DNA, in addition to the possibility of being promutagenic. (Supp. by USPHS Grant CA-30,639)


Omadine® MDS, a pyridinethiol-N-oxide derivative, is intended for use in cosmetics and shampoos. Previous studies have established its safety for a wide variety of applications. In this study, the carcinogenic potential of Omadine® MDS was evaluated in an 18-month skin painting study in mice, where it was incorporated in a shampoo vehicle and applied dermally 3 times/week at dosage levels of 2 and 20 mg/kg. Two control groups (receiving deionized water as the shampoo vehicle) were evaluated concurrently. Moderate skin lesions occurred in the vehicle control and Omadine® MDS groups. A preliminary evaluation of the macroscopic and microscopic data indicated that there was no carcinogenic effect. 2-Methylsulfonyl-pyridine (2MSP) was recovered from plasma samples obtained from mice sacrificed in extremis during the study, giving evidence that the persistent metabolite occurs in mice and confirming the systemic absorption of Omadine® MDS in this study.
male rats were given three i.p. injections of either phenobarbital (PB), piperonyl butoxide (PBO), β-naphthoflavone (BNF) or α-naphthoflavone (ANF) prior to a single dose of diethyl-nitrosamine (DEN) or saline. The rats were fed a basal diet for 7 days followed by a choline deficient diet for 6 weeks and were iron loaded before necropsy. Sections of liver (alcohol-fixed) were stained for γ-glutamyltransferase (GGT) and iron.

Altered foci were more easily identified by the GGT than the iron staining technique. Quantitative stereology of GGT+ foci showed that PB and BNF, inducers of mixed-function oxidases, increased the numbers, sizes and numerical density of DEN-induced foci; only slight increases in density of foci were seen after PBO or ANF pretreatment.

These results suggest that the induction of DEN metabolism enhances the development of altered hepatic foci in rats.

The purpose of the present work was to adapt and further develop an in vitro mouse skin system for studying the effect(s) of the tumor promoter phorbol myristate acetate (PMA) on antioxidant defense enzymes, specifically catalase. In previous studies we found that PMA stimulates H2O2 production in vivo. Studies by others in vivo and confirmed by us, showed that mouse skin catalase decreases in a dose-dependent manner upon PMA application. Dorsal skin explants from CD-1 mice were cultured for 24 hours at 37°C in Dulbecco's MEM in the presence of fetal bovine serum and gentamycin. PMA in acetone (0.9 μg/ml final conc.) or acetone (1% final conc.) was added. After 24 hours the explants showed structural integrity, exclusion of Trypan Blue and reduced tetrazolium chloride. PMA was not toxic as seen by the lack of increase of lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase in the culture medium. In PMA-treated explants catalase activity increased by 90% compared to control explants. Catalase activity in the latter was equivalent to that observed in vivo. In vitro dose response studies are in progress to elucidate the difference observed with respect to the effect of PMA on catalase in vivo.

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157 EVALUATION OF THE GENOTOXIC POTENTIAL OF PICLORAM HERBICIDE IN F344 RATS. T. R. Fox, R. H. Reitz, and P. G. Watanabe. Toxicology Research Laboratory, Dow Chemical U.S.A., Midland, MI 48640.

The genotoxic potential of Picloram (4-amino-3,5,6-chloro-picolinic acid) was evaluated in two ways: (1) by determination of unscheduled DNA synthesis (UDS) in primary rat hepatocytes, and (2) by measuring the radioactivity associated with liver DNA following gavage with 14C-Picloram. Picloram did not induce UDS at concentrations from 5 x 10-6 to 5 x 10-4 M (5 x 10-4 was cytotoxic). Z-AAF was active in this system at 1 x 10-7 M. DNA isolated from the livers of rats dosed with 1000 mg/kg Picloram (8 x 10+8 dpm) contained less than 30 dpm per 5 mg DNA. Further experiments to characterize these very low levels of radioactivity will be reported. Additional studies revealed that Picloram failed to stimulate cellular regeneration in liver after gavage with either a single dose of 1000 mg/kg or repeated (9) doses of 800 mg/kg. These data suggest that the liver lesions observed after Picloram treatment have arisen from nongenotoxic mechanisms.


In previous studies we showed that the toxic α,β-unsaturated aldehydes acrolein, crotonaldehyde, 4-hydroxynonenal, and trans,trans-muconaldehyde inhibit superoxide anion radical (O2-) production in vitro on phorbol myristate acetate (PMA)-stimulated human granulocytes. The concentration at which 50% inhibition (IC50) was observed varied from 2.1 x 10^-3- 3.3 x 10^-2 M depending on the aldehyde. Using the cytochrome C assay to measure O2- production, the present studies indicate that a similar inhibition occurs in digitonin and concanavalin A-stimulated human granulocytes pre-treated with the aldehydes at or near the IC50 found for PMA-stimulated granulocytes. When the calcium ionophore A-23187 was used as an oxygen burst initiator, human granulocytes preincubated with aldehydes at or near the IC50's exhibited enhanced O2- production up to almost twice the amount observed in control cells. These data confirm previous studies in our laboratory which suggest that the membrane oxidase which produces O2- can exhibit a biphasic behavior which may be of significance with respect to oxygen radical toxicity by environmental agents. Supported by NIH Grants ES02510 and CA33270.


In the first 7 to 10 days after topical application of a single dose of benzo[a]pyrene (BaP) to the dorsal skin of C3H mice, the half-lives of BaPDE/DNA adducts and of DNA were determined to be approximately 5 days. These data indicate that, in proliferating mouse skin, BaPDE/DNA lesions are not repaired, but are diluted from the genome at a rate equivalent to DNA turnover (i.e., replication vs degradation). Subsequent to this initial period, BaPDE/DNA adduct removal continues, but at a much reduced rate. At 30 days post treatment with BaP, approximately 15% of the adducts are still detectable, however, their half-life has increased to 30 days. Similar experiments with a hairless strain of mouse showed that, although the amount of BaPDE/DNA adduct formation was lower initially, the kinetics of adduct disappearance and persistence were essentially the same as found with the C3H mouse. This work, and that of others suggest that either: (1) BaPDE/DNA adducts continue to exist in a small subpopulation of essentially dormant epidermal cells long after their predicted disappearance by DNA turnover; or (2) transfer of this type of lesion to newly synthesized DNA occurs. Research sponsored by the Office of Health & Environ. Research, U.S. Dept. of Energy under contract W-7405-eng-26 with Union Carbide.


High boiling coal liquids contain substantial concentrations of benzo(a)pyrene (BaP) and other biologically active polynuclear hydrocarbons. The concentration of BaP has been used as an indicator of carcinogenic potency for complex mixtures. However, carcinogenesis studies suggest that the expression of BaP activity is markedly influenced by the composition of the mixture. For example, two coal liquids (CL-1 and -2) contain equal concentrations of BaP but CL-1 is about 5 times as tumorigenic as CL-2. Differential reductions in metabolic activation and DNA binding may be responsible for these differences in biological activity. To evaluate this hypothesis, radiolabeled BaP was incubated with rat liver S9 in the presence or absence of CL-1 or CL-2. In the absence of CL, 50% of the BaP was metabolized within 2 minutes; however, this time increased to 5 and 15 minutes, respectively, with CL-1 and CL-2 at a ratio of 12:1 CL:BaP. When calf thymus DNA was added to the incubation mixtures, the highest DNA binding was found with BaP alone. CL-1 and CL-2 reduced DNA binding to 10 and 1%, respectively, of that found with BaP alone. These data indicate that the rate of BaP metabolism and amount of DNA binding was greatest for the most carcinogenic CL. (Supported by U.S. Dept. of Energy Contract No. DE-AC06-76RLO-1830)

To determine whether the toxic effect of methylmercury chloride (MeHg) on reproduction and neoplasia was dose dependent, 4 levels of MeHg (0, 5, 10 and 15 ppm) were fed from weaning age until parturition to female Wistar rats in combination with 50 mg sodium nitrite and 100 mg ethyurea (EU) per kg body weight by gavage on day 17, 18 and 19 of gestation. The number of pups weaned per female was 8.1, 9.8, 6.1 and 0.9 and the tumor incidence in the progeny after one year was 58, 50, 80 and 71% for 0, 5, 10 and 15 ppm MeHg respectively. Ten ppm MeHg had no effect on the labeling of fetus carcass or liver and brain DNA one hr after IV injection of H2S2C-methylthiourea (ENU) at day 20 of gestation. The 90-day-old progeny of females treated with 10 ppm MeHg and nitrite-EU had more extensive hyperplasia and neoplasia with 4 brain glioblastomas and 2 trigeminal nerve tumors in MeHg progeny compared to 1 and 0 in the nitrite-EU control progeny. These data suggest that MeHg does not affect initiation of ENU induced neoplasia but probably promotes the development of neoplasia, although the dose-response for MeHg was not linear for all parameters. (Supported in part by USPHS Grants ES-00210 and ES-00040).

162 AN INVESTIGATION OF FORMALIN AND PARA-FORMALDEHYDE AS AGENTS OF SYSTEMIC TUMORGENESIS IN STRAIN A MICE. M.E. Broodeu, J. C., Thieis, R.K, C.E. Dallas, R.E. Billings. University of Texas Health Science Center at Houston, Houston, TX

The effect of formaldehyde as an agent capable of producing a carcinogenic effect at a site in the body distant from the site of administration was assessed utilizing the mouse pulmonary adenoma assay. In this study, formalin and paraformaldehyde, two types of formaldehyde, were administered intraperitoneally (i.p.) and orally. In a 3-week range finding study, the MTD for formalin was 150 mg/kg/day orally and 25 mg/kg/day i.p. while the MTD for paraformaldehyde was 225 mg/kg/day orally and 40 mg/kg/day i.p. to ensure that the test compounds reach the target tissue the rate of expiration of radioactivity in mice exposed to C14-labelled formalin or paraformaldehyde by oral and i.p. routes was assessed. While formalin and paraformaldehyde were expired at similar rates, expiration of radioactivity in mice given paraformaldehyde orally was sustained over a more extended duration. To test the complete carcinogenicity and the initiator (+BHT) and promoter (+urethan) capabilities of formalin and paraformaldehyde strain A mice were treated daily, i.p. and orally, over a period of 8 weeks. Sixteen to 24 weeks following the start of treatment the mice will be sacrificed. The lung and forestomach adenomas, endpoints of the study, will be quantified.


NDELA, a contaminant present in cutting oils, various cosmetic formulations and tobacco smoke, causes a high incidence of tumors in the liver, nasal cavity and respiratory tract of rodents. However, NDELA is negative or only weakly positive in short-term tests such as the Ames assay. We have examined the ability of NDELA to initiate hepatic GPT+ foci in male F-344 rats using the initiation/promotion protocol developed by Cayama/Farber. NDELA (4.0 g/kg) induces GPT+ foci when administered (p.o.) at various times following 2/3 partial hepatectomy (PH). Maximal response was obtained when NDELA was administered 18h post-PH with decreasing responses observed 15h and 12h post-PH. Decreasing doses of NDELA resulted in decreased foci induction when administered 18h post-PH: 4.0 g/kg, 25 + 7 (foci/cm2 + SEM); 2.0 g/kg, 18 + 8; 0.4 g/kg, 6 + 2; 0.2 g/kg, 4 ± 2; no foci were detectable in the control group. These results indicate that NDELA induces GPT+ foci in a time-and dose-dependent manner. Therefore, the Cayama/Farber hepatic initiation/promotion protocol is a better indicator of the initiation potential of NDELA than other short-term tests such as the Ames assay.


Several studies have shown that the administration of zinc (Zn) will prevent cadmium (Cd)-induced tumor formation, both at the injection site and in distant tissues (i.e. testes). Interactions of these metals with DNA could possibly account for this antagonism. Therefore, the binding of Cd to DNA in vitro and the effect of Zn on such binding was investigated. Various concentrations of CdCl2 (0.1 to 250 μM) containing radiolabeled Cd (109Cd) were incubated for 1 hr with 100 μg purified double-stranded calf thymus DNA (0.1 mg/ml in 2 mM Tris, pH 7.4) both with and without ZnCl2 (40 μM). Bound and free Cd were separated by gel filtration (Sephadex G-25) and quantitated by gamma spectrometry. Scatchard analysis revealed two Cd-binding sites and a positive slope at Cd levels ≤ 2 μM, indicative of cooperative binding. Cooperativity was not seen when heat-denatured DNA was used. Analysis of high affinity (HA) sites showed 0.93 μmoles of HA sites/mg DNA. Double reciprocal plots indicated a dissociation constant (Kd) for the Cd-DNA complex of 23 μM and showed Zn to be a competitive antagonist of Cd binding to HA sites. The Kd of Zn was determined to be 31 μM. The results indicate the cooperativity of Cd binding to double stranded DNA and competition by Zn for Cd binding sites in DNA.
ABSENCE OF PROMOTING ACTIVITY OF ARSENITE AND ARSENATE FOR THE MOUSE SKIN AND LUNG. R.J. Bull, M. Robinson, and R.D. Laurie, Toxicology and Microbiology Division, HERL, USEPA, Cincinnati, OH 45268

Arsenic (As) compounds have been linked with the development of skin and lung tumors in humans. An experiment was devised to test whether arsenic could promote the development of skin and/or lung tumors in the Sencar mouse. Sodium arsenite or arsenate was administered in the drinking water at concentrations of 80 or 160 mg/L to 8 groups of animals (40 animals/group). In 4 groups animals were first treated with a single dose of ethyl carbamate (EC) at 500 mg/kg p.o. The remaining 4 groups received vehicle. Two additional groups were initiated with EC as above and were supplied with distilled water. One of these groups received tri-weekly applications of 1.0 μg TPA to the shaved back. Skin tumors were recorded on a weekly basis. Animals were sacrificed at 32 weeks and examined for the development of lung adenosmas. Animals receiving TPA promotion developed an average of 1.6 tumors/animal. No As-treated animals developed skin tumors. Animals that had received EC alone developed 0.80 lung adenosmas/animal. Groups of animals receiving EC and As developed between 0.45 and 0.89 lung adenosomas/animal. Those not receiving EC averaged between 0.06 and 0.13 adenosomas/animal. Therefore, these data failed to support the hypothesis that As acts as a tumor promoter in the lung and skin. (This abstract does not necessarily reflect EPA policy.)

ABILITY OF MOUSE SKIN INITIATION PROMOTION STUDIES TO DETECT CARCINOGENS OF DIFFERENT CHEMICAL CLASSES. R.D. Laurie, M. Robinson, and R.J. Bull, Toxicology and Microbiology Division, HERL, USEPA, Cincinnati, OH 45268

A study has been undertaken to determine whether mouse skin initiation/promotion experiments can identify carcinogens of different chemical classes. Twenty chemicals have been tested in female Sencar mice as tumor initiators. These known carcinogens were administered p.o., i.p., s.c. or topically. Two weeks later, 1.0 μg TPA was applied to the shaved back for 20 weeks. Seven of twenty carcinogens gave positive results. Carcinogenic PAHs (B[a]P, DMBA, 3-MC) were consistently detected. Some nitrosamines gave positive tumor yields (MNW) while others were negative (DENA, DBNA). Ethyl carbamate and nitrosamines (FANFT) gave positive results but the latter responses were weak. Hydrazines, aromatic amines, sulfonates, azo compounds, lactones, chlorinated phenols, halogenated hydrocarbons, dioxane, benzodioxane, and heteroaromatic compounds were negative. Cumulative papilloma yields at 24 weeks were fairly reliable predictors of the number of animals that would develop carcinomas by 1 year as long as the cumulative papilloma yield was between 1.0 and 10.0. Mouse skin initiation/promotion assays cannot be depended upon alone to detect carcinogenic chemicals. However, the use of papillomas as predictors of the incidence of malignant tumors does seem reliable within defined limits. (This abstract does not necessarily reflect EPA policy.)

THE SENCAR MOUSE: ITS USE IN BOTH THE LUNG ADENOMA AND SKIN INITIATION/PROMOTION ASSAYS. M. Robinson, R.J. Bull, G. Knutson, and R.P. Shields, Toxicology & Microbiology Division, USEPA, Cincinnati, OH 45268, Pathology Associates Inc., Ijamsville, MD 21754, and Univ. Florida, Gainesville, Fl. 32610

Four strains of mice: Sencar, BALB/c, A/J and ICR-Swiss, were administered 4 carcinogens by either the oral or i.p. routes. Two weeks later of 1-5 μg of TPA in 0.2 μl acetone/mouse was applied 3 X weekly to the shaved back for 20 weeks. All strains displayed increases in the yield of lung adenosmas in response to ethyl carbamate at 32 weeks. Benzo(a)pyrene increased lung adenosmas in only the Sencar and A/J strain whereas FANFT increased lung adenosmas only in the A/J strain. Only the Sencar and ICR-Swiss mice gave positive responses in the skin. In the Sencar this was seen with all 3 chemicals, however, FANFT gave a marginal response. The ICR-Swiss responded only to ethyl carbamate. Sencar mice were treated with a single oral dose of diethylnitrosamine (50 mg/Kg) followed by tri-weekly application of 1 μg TPA. 51/57 animals developed lung adenosomas vs. 5/57 control animals. No treatment related skin tumors resulted. Histopathologically confirmed lesions indicate that the spectrum of chemicals detected in the Sencar mouse can be broadened in a combined bioassay. (This abstract does not necessarily reflect EPA policy.)

MODULATION OF CHEMICAL HEPATOTOXICITY BY CHOLESTASIS IN RATS. W.C. Kerrish, T.B. Leonard, C.L. Lagg, and J.G. Dent. SKF Labs., and Phila. College of Pharmacy and Science, Phila., PA.

In cholestasis, hepatic bilirubin and bile salt concentrations are elevated. Because of their deterrent properties bile salts can disrupt both structure and function of cellular membranes. These studies were designed to assess whether cholestasis predisposes liver cells to the hepatotoxic action of xenobiotics. Dietary administration of 1-naphthylisothiocyanate (ANT) to rats for 2 and 4 weeks was employed to induce cholestasis. ANIT was fed in the diet at 0.022% and 0.047% by weight. Animals were administered (ip) minimally toxic doses of either allyl alcohol (AA) (25 mg/kg), carbon tetrachloride (CCL4) (200 mg/kg), or galactosamine (GTA) (200 mg/kg) 1 day following cessation of ANIT feeding and sacrificed 24 hr later. Serum alanine transaminase activity (ALT), total serum bile acid concentration, liver/body wt. and histology were evaluated. ANIT feeding decreased the AA (80-90%) and GTA (97%) mediated elevation of serum ALT. Conversely, the CCL4 mediated increases in serum ALT were enhanced by ~390% (2 wk) and ~52X (4 wk). These observations are consistent with the hypothesis that cholestasis modifies the hepatotoxic response to xenobiotics and may be explained by decreases in metabolism (AA) and alterations in antioxidant status and/or membrane stability (CCL4).
169 EARLY EVENTS IN CCl₄ TOXICITY IN PERFUSED RAT LIVER. R.C. Thurman, Dept. of Pharmacology, U. of North Carolina, Chapel Hill, NC; U. of Maryland, Baltimore, MD.

Addition of CCl₄ or BrCl₃Cl to hemoglobin-free perfused rat livers produced a rapid (t½ = 4-6 min) increase in O₂ uptake. This increase in O₂ uptake was about 30 µmol/g/h with low (160 µM) concentrations of CCl₄; however, higher (800 µM) doses of CCl₄ or a range (80 to 800 µM) of BrCl₃Cl concentrations produced a smaller increase (8-10 µmol/g/h) followed by a large decline. The changes in O₂ uptake were observed in phenobarbital-treated but not in normal or 3-methylcholanthrene-treated rats and were blocked by metyrapone. The effect of CCl₄ on O₂ uptake was unaffected by depletion of GSH with phorone, by infusion of KCN or by perfusion with Ca²⁺-free buffer. The changes in O₂ uptake were, however, blocked largely by infusion of the antioxidants DPPD and vitamin E into the preparation. With BrCl₃Cl, an increase in bilirubin GSSC release indicative of oxidative stress preceded a decrease in bile flow, release of LDH into the perfusate and swelling of the liver. Perfusion for 90 to 120 min with haloalkanes was followed by uptake of trypan blue in pericentral regions of the liver lobule. These data are consistent with the hypothesis that increases in O₂ uptake are due to peroxyl and/or lipid radical formation within minutes after administration of CCl₄. Moreover, the perfused liver is an ideal model for identification of the time course of events leading to zonal toxicity due to haloalkanes (ES-02759).

171 ROLE OF INDUCTION AND NORMOTHERMIA IN TWO MODELS OF VOLATILE ANESTHETIC HEPATOTOXICITY. R.C. Lind, A.L. Gandolfi and T.C. Sipes, Dept. of Anesthesiology, University of Arizona, Tucson, AZ.

The enflurane-hypoxia heat [EHH: phenobarbital (PB)-induced male rats exposed for 2 hr to 1.5% enflurane (E) at 10% O₂, external heat to maintain normothermia] anesthetic hepatotoxicity model is based on an anoxia/ischemia injury. This model has been extrapolated to explain the hepatic necrosis arising from the chemogetic halothane-hypoxia (HH) model [PB-induced male rats exposed for 2 hr to 1% halothane (H) at 14% O₂]. Therefore, the conditions necessary to produce necrosis in these differing anesthetic models was investigated. Anoxic/ischemic hepatic injury causes an immediate rise in SGPT and a watery vacuolization (WV) in the centrilobular areas. These conditions were found in the EHH model (SGPT = 170±167, WV highly evident, immediately postexposure, with or without PB induction), while 1.5% E at 10% O₂ without heating caused no hepatic damage. Exposures to severe hypoxia only (5% O₂) produced similar results. The HH model required PB induction and followed a typical chemogetic toxic time course for development of hepatic necrosis. SGPT levels were normal until 6 hr postexposure then rose to 1076±61 by 24 hr. Warming to maintain normothermia in the HH model did cause slight WV immediately postexposure but no elevation in SGPT; 24 hr SGPT were identical to HH (no heat) values. The differences in requirements for hepatic injury in these two models implicates separate mechanisms of necrosis. (NIH AM 16715).

170 INCREASE IN CATALASE-H₂O₂ AND INHIBITION OF KETONE GENESIS BY VALPROATE IN PERFUSED RAT LIVER. M.J. Olson and R.C. Thurman, Dept. of Pharmacology, U. of North Carolina, Chapel Hill, NC.

Valproate (Val), a widely used anticonvulsant, has been shown to produce hepatic steatosis predominantly in perihilar regions of the liver lobule in man and rats. Catalase-H₂O₂, NADH fluorescence and oxygen uptake were monitored continuously during the infusion of Val bound to defatted albumin into hemoglobin-free, perfused livers isolated from fasted rats (n=8). Val caused a rapid (t₀.₅ = 5-6 min), irreversible increase in the steady-state of the catalase-H₂O₂ complex which was maximal at 500 µM Val (2.2 ± 0.32%). Ketone body (acetacetate + β-hydroxybutyrate) production and NADH fluorescence decreased by Val in a concentration-dependent manner; maximum inhibition of ketogenesis (79%) was observed with 500 µM Val. Addition of Val decreased O₂ uptake maximally by 12 ± 3%. Val had no effect on the rate of bile flow but decreased the rate of GSSG release into bile by 45%. Under these conditions, Val did not increase LDH release into the perfusate during 2 hrs of perfusion. Treatment of rats with phenobarbital did not alter the effect of Val on any of the parameters of hepatic function which were monitored. These data are consistent with the hypothesis that inhibition of β-oxidation plays a role in the mechanism of hepatic steatosis caused by Val. In addition, the increase in catalase-H₂O₂ compound may result from an increase in the generation of toxic H₂O₂ via peroxisomal β-oxidation. (ES-07126, ES-02759)

172 CHLOROQUINE-INDUCED PHOSPHOLIPIDOSIS IN RAT LIVER: EFFECTS ON ACID PHOSPHOLIPASE A ACTIVITY. M.J. Reasor and K.Y. Hostetler, Dept. of Medicine, VA Medical Center and Univ. of California, San Diego, CA 92161.

Previous studies from our laboratory have indicated that the treatment of rats with the chloroquine (CQ) results in the induction of a phospholipid (PL) storage disorder in liver. PL accumulation could result from a cellular depletion of phospholipase activity or through an inhibition of PL catabolism. Experiments were performed to test these theories. Rats were treated with CQ for 7 days (100 mg/kg/day, p.o.). Liver was homogenized and subjected to differential centrifugation. CQ treatment resulted in a 35% increase in PL content of the homogenate with the increase occurring almost exclusively in the heavy mitochondrial (M) fraction. This was accompanied by a shift of N-acetyl-β-D-glucosaminidase (NAG) activity (a lysosomal marker enzyme) from the light mitochondrial fraction to the M-fraction. Acid phospholipase A activity in the homogenate doubled following CQ-treatment and had a subcellular localization identical to that of NAG. Therefore, the CQ-induced accumulation of PL appeared to be lysosomal in nature and paradoxically, occurred concomitantly with an increase in lysosomal acid phospholipase A activity. The results indicate that CQ induces a hepatic phospholipidosis not by a loss of lysosomal phospholipase activity, but principally through an inhibition of PL catabolism.
173 SUBCELLULAR FRACTIONATION OF MOUSE LIVER DURING ACETAMINOPHEN (APAP) HEPATOTOXICITY. G.L. Ginsberg and S.D. Cohen. Toxicology Program, School of Pharmacy, Univ. of Conn., Storrs, CT

The mechanism whereby intracellular covalent binding of APAP-derived electrophiles produces hepatotoxicity is unknown. We studied the effect of APAP on marker enzyme distribution among liver organelle fractions after differential ultracentrifugation. Marker enzymes were NADPH cytochrome c reductase (NCR), acid phosphatase (AP), succinate cytochrome c reductase (SCR), and monoamine oxidase (MAO) for microsomes, lysosomes, inner and outer mitochondrial membranes, respectively. Eighteen hours after APAP (600 mg/kg, po, in fasted mice) total liver NCR activity in the nuclear fraction was increased 77%, and decreased 51% in the microsomal fraction. NCR and O-demethylase specific activities in the microsomal fraction decreased by 31% and 16%, respectively. This is consistent with APAP activation and electrophilic interaction with the endoplasmic reticulum. SCR and MAO total liver activities were unchanged in the mitochondrial fraction, but were 45% and 68% elevated in the nuclear fraction, respectively. AP total activity was elevated 63% in the nuclear fraction. The altered distribution of marker enzymes among the fractions suggests increased lysosomal autophagy in response to damage. (GLG was supported by a Stauffer Chemical Co. Fellowship in Toxicology.)

174 THE PROTECTIVE EFFECTS OF α2-ADRENERGIC BLOCKERS ON THE HEPATOTOXICITY OF COCAINE. M. Schieffer, R.D. Harbison, J.G. Pounds, and R.C. James. Division of Interdisciplinary Toxicology, Univ. of Arkansas for Medical Sciences, Little Rock, AR and the National Center for Toxicological Research, Jefferson, AR.

Previous studies in this laboratory indicated that anesthetic given subcutaneously, lowers the hepatic glutathione (GSH) content, i.e., reduced sulfhydryls, to a 50% of control levels, and doubles the activity of serum glutamate-oxaloacetate transaminase (SGOT) within 2-4 hours of administration. These effects on GSH levels were regulated by the α2-adrenergic system. The hepatotoxicity of cocaine, a drug of abuse with sympathomimetic activity, is modified by liver GSH status. These studies were initiated to evaluate the role of adrenergic function in the hepatotoxicity of cocaine. Male B6C3F1 mice (20-25 g) were administered 0-60 mg cocaine/kg with or without prior injection of phentolamine (5 mg/kg) or yohimbine (5 mg/kg). Adrenergic blockers decreased the toxicity of cocaine at all toxic doses tested. SGOT levels were 50% of control values. Time course studies revealed that adrenergic blockers delayed both the onset, and decreased the magnitude of toxicity. These observations were confirmed by histopathological evaluation. These results correlated well with the prevention of cocaine-induced GSH depletion by the adrenergic blockers. These studies suggest that the α2-adrenergic activity of cocaine enhances the inherent hepatotoxicity of the compound.

175 A NEW METHOD TO STUDY GLUTATHIONE CONJUGATION IN DIFFERENT REGIONS OF THE LIVER LOBULE. C. Harris and R.G. Thurman, Dept. of Pharmacology, U. of N.C., Chapel Hill, NC 27514.

Many hepatotoxins exert their effects preferentially in distinct regions of the liver lobule. To study mechanisms involved in such toxicities, a new non-invasive method was developed using tissue reflectance at 366 nm to monitor glutathione (GSH) conjugation in distinct regions of the lobule in the perfused rat liver. The model compound 1-chloro-2,4-dinitrobenzene (CDNB), a substrate for all of the major hepatic glutathione-S-transferases, undergoes a red spectral shift upon formation of the glutathione adduct.

A large tipped, multi-fiber light guide was used to correlate rates of production of S-(2,4-dinitrobenzene)-glutathione by the entire liver with initial rates of change in reflectance at 366 nm from many lobules. A good (r=0.98) correlation was observed. Micro-light guides, constructed to include a single (80 µm) glass fiber which conducts light to the surface of the liver and 6-emission fibers which collect reflected light, allowed simultaneous monitoring (366 nm light) from periportal and pericentral regions of the liver lobule. Initial rates of change of 366 nm reflectance showed that the CDNB-GSH adduct was formed 2.3 times faster in periportal (10.6 µmol/g/h) than in pericentral (4.5 µmol/g/h) regions of the liver lobule. Regional differences in GSH adduct formation followed the reported sublobular distribution of glutathione in the liver (BS-02759).

176 HISTOLOGICAL AND METABOLIC INJURIES OF RAT LIVER FOLLOWING ACETAMINOPHEN TREATMENT IN VIVO AND IN VITRO. S. Ji1, L. Cheng1, A. Gosh2, F. Mitschinsky2, C. Malinick3 and R. Trelech2,1 College of Pharmacy, Rutgers University, 2School of Medicine University of Pennsylvania and 3Rutgers Medical School, Piscataway, N.J. Sponsor: R. Snyder.

Livers isolated from 18 hour-starved rats pretreated with acetaminophen (AA) overnight shows extensive hepatocellular necrosis confined to the periportal region of the liver lobules. To define the nature of the early events preceding necrosis, we studied the metabolic and histological changes caused by infusion of AA into the perfused liver. Livers from Sprague-Dawley female rats were perfused with Krebs-Renseleit bicarbonate buffer at 37°C (via the portal vein; constant flow condition). Treatment with 25 nM AA led to the following results: 1) glycogen depletion from both periportal and pericentral regions, 2) disappearance of Kupfer cells from sinusoids and appearance of inflammatory cells in the extrasinusoidal spaces in the pericentral region, 3) increased uptake of trypan blue by Kupfer cells and hepato- cytes, 4) inhibition of hepatic O2 uptake (max. inhibit. 60%; half-max. dose 5-10 mM AA), 5) increased rate of glycolysis (50-100%), and 6) changes in the tissue levels of ATP, ADP and AMP by 15%, +102% and +48%, respectively, in 20 s after infusion of AA. Therefore we conclude that AA can cause rapid metabolic and immunological injuries to the liver prior to localized necrosis.
177 EFFECT OF 2-HEXANONE (Hx) UPON CCI₄ NEPHROTOXICITY AND HEPATOTOXICITY. M. Raisbeck, E. Brown, and W. Hewlett. UNIV. OF MIO., COLUMBIA, MIO., AND SK&F LABS., PHIL., PA.

Ketonic solvents potentiate the hepatotoxic effects of several haloalkanes. Additionally, Hx potentiates the nephrotoxic effects of CCl₄. This experiment was undertaken to see if the potentiating effects of Hx extend to CCI₄. F-344 rats were pretreated with Hx (0, 0.1, 0.25, 0.5 ml/kg ip). Renal and hepatic effects were evaluated 24 and 48 hr after challenge by changes in plasma creatinine (Cr), urea nitrogen (BUN), and alanine aminotransferase (ALT) and histopathologically. Hx pretreatment increased CCl₄ lethality at 48 hr and markedly potentiated CCl₄ hepatotoxicity as seen by increases in ALT and by increased centrifugal necrosis. BUN, Cr, and kidney weight/body weight ratio were significantly (p < .05) increased above corn oil treated controls in rats treated with either Hx or CCl₄. There was a trend (not significant - p < .05) toward a further increase in these parameters in rats treated with both Hx and CCl₄. Histologically, CCl₄ did not cause marked changes in the kidney, but there were slightly more tubular casts and/or degenerating epithelium in rats treated with Hx and CCl₄ than in rats treated with either agent alone. Although Hx markedly potentiates CCl₄ hepatotoxicity, it did not appreciably alter CCl₄ nephrotoxicity.


Experiments were undertaken to examine the ability of selenium to protect against acetaminophen-induced toxicity in male rats. Pretreatment with sodium selenite (1 mg Se/kg ip) produced a significant protection against the hepatotoxic effects of acetaminophen (1 g/kg) as assessed by plasma AST and ALT activities. Pretreatment with Se also reduced the in vitro covalent binding of acetaminophen metabolites to hepatic protein. Se did not significantly affect microsomal cytochrome P-450 content suggesting that selenium did not significantly alter the metabolism of acetaminophen to reactive metabolites. Se significantly increased glutathione (GSH) levels in the liver and inhibited the decrease in hepatic GSH content following hepatotoxic doses of acetaminophen. There was an increase in the activity of γ-glutamylcyclisteine synthetase, the rate-limiting enzyme in GSH synthesis, which may account for the increased glutathione availability in selenium treated animals. Se also increased the activity of glutathione-S-transferase, leading to the speculation that this enzyme may facilitate the catalytic interaction between GSH and reactive acetaminophen metabolites and, thus, prevent the hepatotoxicity. (This work was supported in part by NIH grant ES-02425 and a Burroughs-Wellcome Toxicology Scholar Award).

178 o-DICHLOROBENZENE TOXICITY AND METABOLISM IN ISOLATED HEPATOCYTES. C. Babik and J. Brodeur. Dép. méd. trav. et hyg. mil. Fac. de méd., Univ. de Montréal, Montréal, Canada. H3C 3J7

The in vitro metabolism of o-dichlorobenzene (DCB) was investigated to evaluate its cytotoxicity, with the parameters being cellular glutathione (GSH) content and release of alanine aminotransferase (ALT) from hepatocytes in suspension. Isolated hepatocytes from untreated, phenobarbital (80 mg/kg/day i.p., 3 days) or SKF 525-A (50 mg/kg i.p., 1 hr prior to sacrifice) pretreated male rats were employed. DCB solubilized in dimethylformamide (DMF) was added to the hepatocyte suspension at 0.5, 1, 2.5, 4 or 5 mM, and aliquots were sampled every 15 min over a 2 hr incubation period. Control cells showed a spontaneous release of ALT over the 2 hr of about 3% and a 20% decrease in GSH. DMF produced no significant effect on cellular integrity. Consequent to the addition of DCB, ALT liberation increased and GSH content decreased in a time- and concentration-dependent manner. GSH depletion was highest in cells from phenobarbital-treated rats while unexpectedly ALT release was lowest, denoting an incompatibility between these two parameters in the evaluation of treatment effect on toxicity. Determination of ALT release appears to be more indicative of parent compound toxicity, whereas GSH depletion tends to relate more directly to metabolite production. (Supported by IRSSS, Québec).


Experiments were conducted to examine the effect of zinc on bromobenzene (BB) hepatotoxicity in male rats. Pretreatment with zinc (6 mg Zn/kg, ip x 2 days) 24 hours prior to administration of BB (7.5 mmole/kg, ip) prevented the BB-elevation of plasma AST by 25% and plasma ALT by 50%. Zinc administered simultaneously with BB did not alter the hepatotoxicity by BB. Mechanism experiments showed that zinc treatment did not alter the pattern of the hepatic subcellular distribution radioactivity following the administration of 14C-BB. However, the covalent binding of 14C-BB metabolites in hepatic tissue was decreased significantly in zinc treated animals. Further experiments showed that 14C-BB metabolites did not bind to hepatic metallothionein induced by zinc. Zinc treatment depressed cytochrome P-450 content, inhibited the metabolism of aniline, and lowered the activity of NADPH cytochrome c reductase. The hepato-protective effect of zinc did not involve changes in hepatic glutathione content. Therefore, it is concluded by these studies that zinc protection of bromobenzene hepatotoxicity is most likely mediated through changes in the metabolic conversion of BB to a toxic metabolite. (Supported by Burroughs-Wellcome Toxicology Scholar Award).

Cycling of microsomal mixed function oxidases during substrate biotransformation generates O2- and H2O2 thereby imposing an acute oxidative stress. Hydrogen peroxide formation accompanies the metabolism of ethoxycoumarin (EC) and ethylmorphine (EM) in microsomal preparations and in intact hepatocytes. The significance of this oxidative stress was considered in an analysis of the toxicity of EC and EM using primary hepatocyte cultures. Concentration-dependent killing was observed with both compounds in cells prepared from phenobarbital pretreated rats (EC LC50 = 96 μM, EM LC50 = 280 μM). 70% of total cell killing occurred within 4 hrs. SKF 525A (10 μM) protected against EC and EM toxicity. The SH reagents L-cysteine, N-acetylcysteine β-mercaptoethanol, and α-mercaptoacetylonylglycine also protected. The antioxidants N,N’-diphenyl-p-phenylenediamine (DPDP) and promethazine prevented cell killing for 4-6 hrs. MDA accumulation accompanied cell killing by both EC and EM, and DPPD prevented this manifestation of lipid peroxidation. The data indicate that EC and EM are toxic to hepatocytes and that the toxicity is dependent upon metabolism. Since neither compound is at present known to form an electrophilic product, the toxicity would seem to be a consequence of oxidative stress. (Supported by NIH Grant AM 31114)

182 THE EFFECTS OF MIXED-FUNCTION OXIDASE MODULATION ON PREOCCEIN II INDUCED HEPATOTOXICITY. S.K. Duddy and M.T.S. Hsia, Env. Tox. Ctr. and Dept. of Entomology, Univ. of Wisconsin, Madison, WI

SKF525-A, cimetidine (CD), phenobarbital (PB) and 3-methylcholanthrene (MC) are all well known modifiers of xenobiotic metabolism. The effect of these compounds on precoecine II (P-II) induced hepatotoxicity was studied in male Sprague-Dawley rats. Three separate groups of animals were pretreated with PB, MC or CD for 3 days, then received P-II (175mg/kg for PB & MC; 200mg/kg for CD) on day 4. A 4th group was given SKF525-A 1 hr before and after receiving P-II (200mg/kg). Necropsies were performed 24 hr after P-II treatment, with changes in SGOT and SGPT levels serving as primary indices of response. Given without P-II, SKF525-A, PB, MC and CD increased SGOT and SGPT to no more than 3X control levels. P-II at 175mg/kg increased SGOT and SGPT to 47X and 35X controls, respectively, and 200mg/kg raised SGOT and SGPT to 70X and 28X controls, respectively. When P-II was given together with CD or SKF525-A, only slight increases in SGOT and SGPT resulted (4-5X controls). When P-II was given after PB and MC induction, SGOT rose not more than 3.5X control levels and SGPT increased not more than 6.0X controls. These results indicate that the profile and levels of xenobiotic metabolizing enzymes are crucial in determining the extent of hepatic damage induced by P-II. (Supported by USDA Hatch Grant #2730 and the College of Ag. and Life Sci., Univ. of Wisconsin, Madison)

183 HEPATOTOXICITY OF 4,4-METHYLENE-2,6-DIISOPROPYL ANILINE AND 4,4-METHYLENE DIISANILINE IN THE RAT AND HAMSTER. C.R. Short, K.M. Kerr, T. Pullin, and M. Hardy. Louisiana State University School of Veterinary Medicine, Baton Rouge, LA

Both MDPA and MDA are known to be hepatotoxic, and MDA is hepatocarcinogenic. This study was undertaken to compare the hepatic lesion of MDPA between species and between agents. MDPA was administered in corn oil to rats by gavage at doses ranging from 10.5 mg/kg to 87.5 mg/kg, daily for 5,10 or 28 days. Controls were corn oil gavaged. A separate group of rats was dosed for 28 days and sacrificed on the 56th day. Outbred Syrian Golden hamsters were given MDPA by the same route at 87.5 mg/kg and 875 mg/kg for up to 28 days. MDA was administered daily to hamsters for 5,10 or 28 days at 87.5 mg/kg. MDPA produced a toxic hepatopathy in rats at the 3 highest dose levels at 5 days. Lesions consisted of hepatocytic vacuolar change and/or periacinar vascular degeneration. Incidence and severity of the lesions decreased at 10 and 28 days of continual treatment, and was essentially absent at 56 days. Hamsters appeared to be more resistant, showing no definite toxic effects at 87.5 mg/kg/day for 5,10, or 28 days. Hepatic lesions at 875 mg/kg were similar to those seen in rats at 87.5 mg/kg, but were more severe. MDA produced hepatic lesions that were different from those of MDPA treated hamsters, in that biliary hyperplasia, biliary fibrosis, and granulomatous cholangiohepatitis were predominant features.


Liver and lung injury induced by the plasticizer dl-(2-ethylhexyl)phthalate (DEHP), has been reported. However, the mechanism of this toxic action is not known. Peroxidation of membrane lipids is believed to be the mechanism of toxicity for many chemicals. This study was undertaken to examine if lipid peroxidation is involved in the toxicity of DEHP. Homogenates of the rat liver and lung were incubated at 37°C for 60 minutes with varying concentrations (0.0 to 2.0 nM) of DEHP. A dose dependent increase in the production of the thioribarbituric acid-reacting lipid hydroperoxides was observed indicating increased lipid peroxidation. This observation suggests that lipid peroxidation is involved in the DEHP-induced liver and lung toxicity. (Opinions expressed here are those of the authors and they do not necessarily represent the official views of the U.S. Consumer Product Safety Commission.)

A Hamilton Micro Lab®M diluter/dispenser was adapted for dispensing measured doses to laboratory animals. The device is made up of a microprocessor programming unit which controls a high-resolution stepper-drive motor which drives a precision gas-tight syringe. The accuracy of the system is better than 1% when dispensing at least 5% of the syringe volume. The syringe volume range is 0.1ml to 25ml.

The system was designed for long term gavage studies where the animals receive a daily dose based on a weekly body weight. The daily dosages are programmed into the microprocessor once each week. The unit is then used to dispense each animals individual dose on command. The advantages of the system are in the accuracy and in the time saved. The system has been used to dispense solutions, aqueous suspensions and corn oil suspensions without any problems. The entire liquid handling system is chemically inert.


We propose the use of a dynamic integrated mathematical model to estimate regional lung burdens for man from urban aerosols. Previously, we determined the kinetics of NH₄NO₃ uptake and distribution in rats. This model was validated by measurement of blood NO₃ following head-only exposure of intratracheally cannulated rats to an aerosol of 0.7 MMAD (g. 1.3) for 2 hrs. The model predicts 15% of the aerosol deposited in the respiratory tract (tracheobronchial (TB) zone 3%; pulmonary (P) zone 12%). When the growth of the aerosol particle within the lung due to humidity is considered, the fraction deposited is 17% (TB, 8%; P, 9%). We have also found that NH₄NO₃ is absorbed from the lung by diffusion with a half-life of 14 minutes, and is distributed according to a 2 compartment model. It is eliminated from the blood with a half-life of 100 minutes. As an illustration of the use of dynamic models in estimating human exposure from air pollution, a human exposure was simulated by applying rat kinetic data, human lung morphology and an aerosol concentration of 100 ug/m³. A steady state is reached at 2 hrs with a lung burden of 1.2 ug. (Supported by EPA Cooperative Agreement and NIH Grant ES01897.)

SINGLE DAY, QUANTITATIVE DETERMINATION OF THE MUTAGENIC POTENTIAL OF CHEMICALS USING THE S. TYPHIMURIUM ASSAY. B.A. Brooks, and J.G. Bahish. Department of Preventive Medicine, New York State College of Veterinary Medicine, Cornell University Ithaca, New York.

The microbial reversion assay using Salmonella typhimurium (Ames Test) is widely accepted as a screening test for chemical mutagens. An alternate methodology for the in vitro determination of chemicals as mutagens was examined. The microbial reversion assay using S. typhimurium was modified so that viable revertant organisms were quantitated in 24 hr.

S. typhimurium (his⁻) TA 100, was incubated overnight at 37°C in brain heart infusion medium with the mutagen N-methyl-N'-nitro-N-nitrosoguanidine. The cultures were then centrifuged at 9000 xg for 10 min, and the pellet suspended in Vogel-Bonner broth containing no histidine.

Viable revertant organisms were detected at 4 and 8 hr; after exposure to the mutagen by the determination of AFE using the firefly luciferin-luciferase bioluminescence reaction. The results were positively correlated with the plate incorporation microbial reversion assay.

The variables of incubation media, incubation time, size of inoculum, and growth characteristics of TA 100, have been optimized using the mutagen N-methyl-N'-nitro-N-nitrosoguanidine.

DEVELOPMENT OF A NON-INVASIVE METHOD TO MEASURE AIRFLOW RESISTIVE WORK IN GUINEA PIGS DURING AIR BREATHING AND DURING CHALLENGE WITH 10% CO₂. K.L. Wong and Y. Alarie, Dept. of Ind, Env Hth Sci., Univ. of Pittsburgh, Pittsburgh, PA 15261.

A method was developed to non-invasively measure pulmonary flow resistive work in guinea pigs during air breathing as well as during challenge with 10% CO₂. To measure resistive work, a guinea pig was placed in a whole body plethysmograph into which a head chamber was fixed. The head of each animal was positioned within this chamber through a latex dam making a seal at the neck. Tidal volume (VT) was measured by integrating airflow in and out of the head chamber measured via a pneumotachograph. The pressure in the whole body plethysmograph (∆P) was monitored by a pressure transducer. Both VT and ∆P were continuously monitored on a Gould oscillograph and the signals simultaneously digitized and stored on a MINC-23 microcomputer. Plotting VT vs. ∆P was accomplished from the digitized signals and these loops for each breath were displayed on the video terminal. The area of each loop, which is proportional to flow resistive work, was calculated. Measurements were made with the animals breathing air, followed by a challenge with a mixture containing 10% CO₂, 20% O₂ and 70% N₂. These measurements were conducted repeatedly with a group of guinea pigs and the method appears promising for repeated measurements in toxicological studies. Supported by NIEHS grant 5-R01-ES02747.
INTRAGASTRIC AND INTRADUODENAL DELIVERY SYSTEM IN INTACT DOGS
A. Davdovich, Department of Toxicology and Pathology, Hoffmann-La Roche Inc., Nutley, NJ 07110. (Sponsor R. M. McClain)

A non-invasive and non-traumatic procedure to deliver experimental compounds intragastrically and/or intraduodenally in intact dogs by means of a flexible gastrointestinal fibroscope will be described. Dogs are anesthetized after 18 hours fasting from solid food and positioned in left lateral recumbency.

The procedure allows visualization of all the esophageal, gastric and duodenal mucosal surface, as well as evaluation of the cardia and pylorus, in intact dogs. Photographs may be obtained to document local effects, monitor pH, biopsies may be taken for histological examination, gastric or intestinal secretions may be collected. The procedure is relatively simple, fast and reliable, and it can be repeated to follow the course of the local effects.


We (Ind. Uiv.) developed an improved method for detecting and quantitating low levels of enrichment of 18O in complex biological samples. Tracing studies using 18O labeled ozone (18O3) were performed by 1) formation from 18O2 and cryogenic purification of the 18O3, 2) exposure of mice to 1 ppm 18O3, 3) removal and lyophilization of tissues, 4) quantitative conversion of tissue oxygen to CO2 using a Schütze-Unterzaucher procedure, and 5) determination of the fractional abundance of 18O in the CO2 (18F) by isotope ratio mass spectrometry. Mouse lung 18O was linearly increased in proportion to the time of in vivo exposure to 18O3, with significant effects observable after 17 min. A 30 min exposure resulted in about 10 nmole of O3-derived oxygen in the total respiratory tract, of which 56% was present in the nasopharynx, 5% in the trachea, and 39% in the lungs. Increased blood 18O was only rarely detectable, and no changes were observed in the liver. These results suggest that direct O3 binding to tissue molecules occurs, and they demonstrate the feasibility and sensitivity available for tracing 18O in a complex biological system. (This abstract does not necessarily reflect EPA policy.)

BHT TOXICITY IN MURINE LUNG AS STUDIED BY HISTOLOGICAL SECTIONS MORPHOMETRY. P. Coulombe and M.G. Côté, dept. of pharmacology, faculty of medicine, University of Montreal, Montreal, Quebec, Canada.

After butylated hydroxytoluene (BHT) stimulation, the development of alveolar lung lesions in the mouse follows a reproducible, cell-specific time course. This model of lesion-repair was thus used as a tool to assess the potential of an evaluation technique of alveolar damage following a toxic aggression.

Swiss-Webster mice (18-22 g) were treated with BHT (400 mg/ Kg i.p. vehicled in corn oil) and the left lung taken at 0, 1, 3, 5, 7 and 15 days after treatment. The technique of morphometric evaluation on histological sections embedded in glycol methacrylate (GMA) was used to quantify possible modifications of alveolar cells. Quantitative evaluation includes determination of Aa and Na ratios (MOP III, Zeiss). These measurements were done on cells recognized to be involved in the alveoli primary reaction: alveolar macrophages, polymorphonuclear cells, type II and intermediate pneumocytes.

This method of evaluation could be used to readily evaluate the pulmonary toxic potential of any substance. This work was supported by the Medical Research Council of Canada.

AUTOMATED EXPOSURE SYSTEM FOR METERED-DOSE AEROSOL PHARMACEUTICALS. C.E. Ulrich, D.R. Klonne, S.V. Church, International Research and Development Corporation, Mattawan, MI 49071.

Metered-dose aerosol pharmaceuticals present some unique problems and constraints when toxicity testing is required in large numbers of rodents. The most significant problem is one of maximizing the exposure concentration in the face of limited output rate from the metered-dose vial. Maintaining the contents of the metered-dose vial in a mixed state and ensuring homogeneous chamber distribution are also important considerations. The system to be described provides flexibility to permit the optimization of the various inter-related variables for a given experimental design. The system provides control over: chamber volume, number vials actuated simultaneously, rate at which vials are actuated and the mixing of vials contents between actuation periods. All variables related to the timing sequence are controlled by a mini-computer which maintains a consistent exposure regimen from day to day, while simultaneously permitting ease and flexibility in altering the timing variables to meet widely divergent exposure protocols. The system is completely automated such that once an exposure is started, operator involvement is not required.
The NTP is exploring the feasibility of using microencapsulation as a means of incorporating volatile, reactive and/or unpalatable chemicals into rodent feed for toxicologic studies. For this purpose, the encapsulation process must not alter any chemical properties of the test compound, the capsule shell materials must not have any adverse toxicologic effects, the encapsulated chemical must be stabilized when blended in rodent feed as well as when held under simulated dosing conditions, and the chemical must be readily released from the microcapsules after ingestion. A chemical currently under consideration is trichloroethylene (TCE) which was encapsulated with a gelatin:sorbitol (70:30) mixture. The concentration of TCE in the microcapsules was 43% (w/w). Particle size ranged from 100 to 425 μm with a majority of particles having a diameter of 300 μm. Less than 0.01% of TCE was removed by washing the microcapsules with toluene. The loss of TCE from microcapsules held in an open vessel at room temperature for 14 days was less than 2%. Microencapsulated TCE is being evaluated for its stability and homogeneity in rodent feed, and its bioavailability in rats and mice.

Good laboratory practices specify the need to take statistically randomized samples to establish homogeneity of test substance in diet. A literal translation of this into actual practice is an expensive, time-consuming, and nearly self-defeating procedure. We have made an evaluation of methods to establish the homogeneity of test substance in diet. Methods of obtaining representative samples from animal diet jars of the correct weight for analysis were evaluated. A diet blend was systematically sampled by several procedures which include a moderate number of stratified random samples and a large number of statistically randomized samples. The statistically randomized samples were taken by assignment of sequential numbers to animal food jars of a given dose as they were filled for placement into the cages. An a set of statistically randomized numbers were generated using a 10% sampling frequency for the total number of jars filled from that blend. As a random number jar was filled, it was set aside for this study. Results will be presented that show a small number of stratified samples can be used to establish homogeneity of diet blends with the advantages of faster sampling, ability to analyze diets before they are presented to animals and at reduced cost.

The need for a method to express the tendency of different compounds to cause cumulative effects when administered repeatedly is so obvious that several independent attempts have been made to improve on the old method of stating what fraction of a dose LD50 could be tolerated daily. Sufficient detail to permit use of the method introduced by Kagan in the Soviet Union has been unavailable to English-speaking toxicologists. This paper (a) summarizes his method including the constraints that form part of it, (b) applies it to 2 compounds that have different cumulative effects, and (c) compares it with other methods. According to Kagan's method, the cumulative percent mortality on each day is plotted on log probit paper against the log of the total dose that the animals dying on that day had consumed. The quotient of the chronic LD50 (read from the resulting curve) divided by the acute LD50 is called the cumulation coefficient. Coefficients less than 1.0 signify slight cumulation; those greater than 5.0 signify slight cumulation. Application of this method confirmed earlier evidence that warfarin is highly cumulative and parathion is only slightly cumulative. However, there are practical difficulties in using the method, and the exact coefficient obtained varies with the dosage chosen. It is concluded that Kagan's method is not as precise as either of the basically similar methods proposed by Boyd or Hayes.

Furosemide (FS, Lasix), a potent diuretic which causes rapid fluid loss, diminished concentration of prohibited drugs in urine and improved appearance, is a common drug of abuse in livestock shows. In order to evaluate its misuse and to determine the feasibility of post-show analytical screening, 5 pigs (crossbred barrows, 200 lb) were dosed with FS I.M. or orally (0.5-2.0 mg/kg) and urine (5 animals) and blood (1 animal) samples were collected. FS was partitioned into ethyl acetate following acidification and quantitated by reverse phase HPLC using UV (254 and 260nm) and fluorescent (254nm excitation, 375nm emission) detection (solvent system, 10% acetic acid: CH3CN, 80:20). Recovery of FS from spiked blood and urine samples was 80-90%. FS was rapidly cleared from plasma (t1/2α = 2.16 hr, t1/2β = 9.51 hr), but could be detected in urine 4-5 days after administration at concentrations as low as 0.05 ppm. Incubation of urine samples with β-glucuronidase showed that 12% of FS was excreted as the glucuronide conjugate. Total FS excreted (corrected for recovery and glucuronidase formation) ranged from 23% to 89% of the administered dose. These data indicate that post-show testing for FS is expedient and urine is clearly the specimen of choice. Due to increased sensitivity and selectivity, fluorescent detection is recommended.
201 A COMPARISON OF THE MORPHOLOGICAL FEATURES ASSOCIATED WITH REPARATIVE AND PRENEOPLASTIC HYPERPLASIA IN THE RAT URINARY BLADDER
D.M. Creasy and W.H. Butler. British Industrial Biological Research Association, Woodmansterne Road, Surrey, SM5 4DS, U.K.

Interpretation of epithelial hyperplasia in the urinary bladder relies on the ability to distinguish reparative hyperplasia from the progressive preneoplastic hyperplasia which precedes overt tumour development. This study in female Fischer 344 rats compares the hyperplastic response induced by formalin, trypsin, freeze ulceration and cyclophosphamide with the response from the known bladder carcinogen N-Butyl-4-(hydroxybutyl)-nitrosamine (BBN) administered at a dose calculated to produce 100% tumour incidence. The hyperplastic response to the various cytotoxic agents varied both in the cellular growth pattern and the subcellular features exhibited. Nodular downgrowths, subepithelial cell nests and intraepithelial capillary growth were present after formalin whilst considerable cellular and nuclear atypia followed cyclophosphamide administration. BBN treated animals showed progression from a regular simple hyperplasia to an exophytic papillary growth pattern or nodular downgrowth. (Supported by Ministry of Agriculture Fisheries and Food, U.K.)

202 THE POSSIBLE ROLE OF PHYSIOLOGICAL CHANGES IN THE OCCURRENCE OF BLADDER TUMORS IN SACCHARIN TREATED RATS. C. P. Schoenig, Consultant to the Calorie Control Council, Atlanta, GA. Sponsor: E. I. Goldenthal

The ingestion of high dose levels of sodium saccharin (NaS) is somehow related to the occurrence of urinary bladder tumors in rats. Since NaS does not bind to DNA nor is it metabolized, some indirect mechanism may be involved. This mechanism may be related to one or more of the physiological changes which have also been observed in rats fed NaS at dose levels at which tumors occur. As part of a two-generation bioassay which employed 2500 second generation male rats and in several adjunct studies, many of these physiological changes have been examined in some detail. Findings from these studies suggest that physiological changes may play an important role in the occurrence of urinary bladder tumors in NaS-treated rats.

203 EFFECTS OF THE TUMOR PROMOTER PHORBOL-12-MYRISTATE-13-ACETATE ON OOCYTE DIFFERENTIATION AND DEVELOPMENT IN HABROBRACON JUGLANDIS.
J. G. Best, D. S. Grosch. North Carolina State University, Raleigh, NC.

The etiology of the wide spectrum of physiological, biochemical, and genetic effects of the tumor promoter phorbol-12-myristate-13-acetate (PMA), though extensively studied, has not as yet been precisely defined. Much progress has been made through the application of a variety of biological systems to narrow in on the necessary and sufficient events giving rise to tumor promotion and the many pleiotropic effects which accompany it in higher organisms. The wasp, Habrobracon juglandis, is the subject of this study. The female wasp has four synchronized ovarioles, each with a single series of differentiating oogonial cells. Unfertilized eggs develop parthenogenetically into haploid males which express both dominant and recessive mutations. PMA administration in the wasp results in a very rapid drop in egg production, decreased egg hatchability, aberrant differentiation of oocytes, and necrosis of the ovarioles. Gamma rays or benzoyrene pretreatment show little synergism with PMA, suggesting that DNA repair changes and maturation of pre-mutational lesions have little to do with PMA toxicity.

The similarity between the response of mammalian, avian, and insect differentiating cells to PMA suggests a target molecule which is common to a wide range of organisms.


Alteration in the pH of the culture medium upon the addition of text article in vitro mutagenicity studies has been shown to be a passive factor in altering the uptake and effectiveness of test material. This study describes the effect of low pH caused by the addition of glacial acetic acid (CH₃COON) and hydrochloric acid (HCl) to the culture medium on the induction of chromosome aberrations in CHO cells. Briefly, the pH of the culture medium with or without S9 activation mixture was reduced with either CH₃COON or HCl and the cells were treated with such media at a pH range of 3 to 7.2. In a parallel experiment, the pH of the culture medium was reduced but readjusted to pH 7.2 prior to treating the cells. The cells were exposed for 2 hours with S9 activation and for 12 hours without activation. None of the cultures treated without S9 mixture exhibited any increase in the chromosome aberrations frequency. However, cultures treated with S9 mixture without readjustment to pH 7.2 showed 64% aberrant cells with CH₃COON at pH 5.5 and 68% aberrant cells with HCl at pH 5.25. Even when the pH was readjusted to pH 7.2 prior to the treatment of cells, approximately 50% of the cells showed chromosome aberrations. The results indicate that reducing the pH of the medium containing S9 mixture by itself may cause genetic damage in in vitro mutagenicity studies.

The first metaphase stage following aberration induction is the ideal time to observe chromosomal structural changes in their entirety. On the other hand, sister chromatid exchanges are visible only at second mitoses following 5-bromo-2-deoxyuridine (BudR) substitution of chromosomal DNA. Thus, determination of cell generation time, i.e., the cell kinetics, has a direct influence upon the quality of cytogenetic scoring in genotoxicity assays. BudR was incorporated subcutaneously either in tablet form or in an osmotic pump into the dorsal-thoracic area of anesthetized male Sprague-Dawley rats. Animals received continuous BudR treatment until the time of harvest. Samples of bone marrow, spleen, lymph node, and spermatogonia were collected in the presence of colchicine. Differentially stained BudR-incorporated chromosomal preparations were scored for 1st, 2nd, 3rd, or subsequent divisions. Based on the number of metaphases per cell cycle and the length of BudR treatment, the average cell generation time for rat bone marrow, spleen, lymph node, and spermatogonia was estimated to be 12, 14, 6, and 45 hours, respectively.


The metabolically-activated, genotoxic carcinogen DMN has been shown to induce tumors in multiple tissues and by various routes of administration. Male P-344 rats were exposed to a nominal concentration of 1000 ppm DMN by inhalation for 4 hr. Primary cell cultures were prepared from the epithelium of the nasoturbinate (NT), ethmoid turbinate (ET), maxilloturbinate (MT) and trachea (TE), as well as hepatocytes (H) and pachytene spermatocytes (PS). Cells were incubated with 3H-thymidine, and the induction of DNA repair as UDS was determined by autoradiography. DMN induced UDS in: NT (2.1 nC grains/nucleus); control (C) = 0.5 nC; ET (3.7 nC); C = 0.5 nC; MT (2.6 nC); C = 0.7 nC; TE (8.7 nC); C = -2.1 nC; and H (50.8 nC); C = -5.1 nC, but not PS (0.02 nC); C = 0.0 nC. Thus, inhalation of DMN induces DNA damage in epithelial cells of the upper respiratory system. DMN also entered the systemic circulation as evidenced by the positive response in the hepatocytes. DMN or its active metabolites apparently did not reach the testes in sufficient concentrations to cause DNA damage in spermatocytes. These results illustrate the potential of this assay scheme for assessing the organ-specific genotoxicity of environmental chemicals.

207 EXPRESSION OF BENZO(A)PYRENE AND 6-AMINOCRYSTATE INITIATING ACTIVITY IN COMPLEX MIXTURES FROM COAL D.D. Mahlum. Pacific Northwest Laboratory, Richland, WA.

Reports in the literature indicate that expression of mutagenic and carcinogenic activity of BaP and 6-AC in complex mixtures is strongly influenced by other components of the mixtures. We therefore directly examined the influence of coal-derived mixtures on skin tumor initiating activity of BaP and 6-AC. In the first experiment, a single dose of 10 or 50 μg of BaP in either acetone or a high boiling coal liquid (CL) was applied to the backs of female Charles River CD-I mice. Controls received CL alone. Two weeks later, the mice received twice weekly doses of 5 μg of phenol myristate acetate. The incidence and total tumors per group were recorded biweekly. Although both CL and BaP alone resulted in tumor development, the response to BaP in CL was significantly less than the response to BaP in acetone. A similar experiment was performed in which an initiating dose of 25 μg of 6-AC was applied in acetone or in the nitrogen-containing (NPAC) fraction derived from a high boiling CL. Again, more tumors were produced and they appeared earlier when 6-AC was applied in acetone than when applied in the NPAC fraction. These data demonstrate that the skin tumor initiating activities of BaP and 6-AC were suppressed by components of coal-derived materials. These results suggest that concentrations of BaP and 6-AC in complex mixtures may not be indicative of their tumorigenic activity (USOEY Contract DE-AC06-76R01830).


These studies evaluated the chronic toxicity and carcinogenic potential of RDX in F344 rats and B6C3F1 mice after dietary feeding for 24 months. Rats received 0, 0.3, 1.5, 8 or 40 mg/kg/day of RDX were administered 0, 1.5, 7, 35 or 100 mg/kg/day. This last dose was reduced from 175 mg/kg/day in Test Week 11 due to high mortality. RDX at 40 mg/kg/day was lethal to most of the male rats. Surviving high dose male rats demonstrated anemia with splenic hemosiderosis being observed. Female rats at this dose showed only slight anemia without histologic changes. Additional toxic responses seen for high dose rats included convulsions, fasting, hypoglycemia, hypertriglyceridemia, hypocholesterolemia, decreased SGPT levels, hepato-megal.) without histologic lesions, urogenital lesions (males), and lenticular cataracts (females). For mice, clinicopathologic parameters were unaltered by RDX. Histologic changes seen for high dose males included chronic dermatitis and possibly urinary tract lesions. Hepatic tumors may have been treatment-related for females, however this was not seen for males nor for rats of either sex. (Supported by the U.S. Army Medical Bioengineering R & D Laboratory under Contract No. DAMD17-79-C-9161.)

Ethyleneediamine dihydrochloride (EDA-2HC1) was incorporated in the diet of Fischer 344 rats at dosage goals of 0.39, 0.10, or 0.02 g/kg/day for two years. Few treatment-related effects were seen in the first 6 months; most toxicologic responses were seen at the 12-month sacrifice and thereafter. EDA did not exhibit carcinogenic potential. However, chronic toxicity was evident, primarily at the highest dosage level, as reflected by the higher mortality rate (especially in males), changes in body weight and some organ weights (mainly liver, kidney and spleen), marginal alterations in certain clinical pathology parameters (depression of erythrocyte counts, hemoglobin and hematocrit values in high level males) and increased prevalence of hepatocellular pleomorphism, rhinitis and tracheitis. The principal histologic lesion, hepatocellular pleomorphism, strongly implicates the liver as a target organ. At the highest dosage level, EDA also suppressed markedly the incidences of testicular interstitial cell adenoma which is a common, age-related, naturally occurring lesion in Fischer 344 rats.

211 CARCINOGENICITY OF ACRYLATES, LONG-TERM INHALATION STUDIES ON METHYL ACRYLATE (MA) and 2-BUTYL ACRYLATE (BA) IN RATS. H.-J. Klinisch, Dept. of Tox, BASF Aktiengesellschaft, Ludwigshafen, FRG, and W. Neislinghaus, INESC, Koenig, FRG. Sponsors: Harry Kerl.

Sprague-Dawley rats, 86 per sex and group, were exposed to 0, 15, 45, 135 ppm MA or BA for 6 hours per day, 5 days per week for 2 years. BA exposed animals were reared for 6 months post-exposure. Interim tests were performed after 12 and 18 months for MA and BA, and after 24 months for BA exposed animals. A temporary retardation of the body weight gain was observed in animals exposed to 135 ppm MA. No tumorogenic effects were attributed to the acrylates. A dose-dependent increase could not be established for the overall incidence or for any of a variety of observed tumor types, which were judged to be of spontaneous nature. No signs of systemic toxicity were observed. Examination of the nasal turbinates revealed local effects by irritation at the nasal mucosa. A dose-related atrophy of the neurogenic portion of the olfactory epithelium with proliferation of the reserve cells to a multilayered epithelium was found. These changes were primarily observed at the anterior portion of the olfactory mucosa at the transition of the respiratory to the olfactory epithelium. A regeneration (replacement of the olfactory epithelium by respiratory epithelium) was observed after the post-exposure period in the BA exposed animals. No atypical cellular or any neoplastic changes were found in the nasal mucosa or elsewhere in the respiratory tract. The interpretation of these changes must take into account that rats are obligatory nasal breathers and according to the studies with other irritant vapors are very susceptible to changes at the nasal mucosa. Dose-related cellular atrophy and vascularization of the eyes were observed with MA. These changes were observed only at 135 ppm with BA. A partial reversibility was observed in the post-exposure period in the BA exposed animals.


The chronic toxicity and carcinogenicity of ethylene oxide (EO) and propylene oxide (PO) were evaluated in a 2-yr inhalation study. Male weaning F344 rats (50 per group) were exposed to 0 (control), 50 and 100 ppm EO and 100 and 300 ppm PO (7 hr/day, 5 days/week). Significant histopathologic findings included a statistically significant increase in the incidence of mononuclear cell leukemia in the 50 ppm EO rats compared to controls (interim deaths and the final sacrifice combined). When the leukemia incidence in the control and 100 ppm EO rats were compared (final sacrifice), a statistically significant exposure-related increase in leukemia incidence was observed. A significant association of exposure and increased occurrence of peritoneal mesotheliomas was found for the 100 ppm EO group compared to the controls. Trend analysis indicated a statistically significant increase of mixed-cell gliomas with increased EO exposure. Among rats exposed to PO, there was a treatment-related increase in the incidence of proliferative epithelial lesions of the nasal epithelium of the possible contribution of a concurrent Hemophilus pleumoniae infection to the observed nasal lesions is uncertain.

212 DNA BINDING AND CARDIAC SUPEROXIDE PRODUCTION STUDIES OF PYRIDO[4,3-C]QUINONES. J.P. Nachman, E.T. Roginski, J.W. Banning, H.N. Abramson, and H.C. Wormser, Department of Basic Pharmaceutical Science, College of Pharmacy and AHP, Wayne State University Detroit, MI 48202

Pyrido[4,3-c]quinones (AQ) are potent antibacterial agents especially against gram-positive organisms. We tested two major biologic actions of these compounds: DNA intercalation and superoxide (O2-) production in sarcomas. Using the bathochromic and hypochromic shifts induced by intercalation, followed by Scatchard analysis, we calculated dissociation constants and the number of binding sites per base pair for four analogues. We compared O2- production in sarcomas using acetylated cytchrome c reduction according to Dornshow and Reeves. Results indicate that the unsubstituted AQ does not bind to DNA or change the melting temperature (Tm). However, placing a morpholino- or piperidyl-group at C-5 enhances the binding to DNA (Kd = 1.06 x 10^-6 M). All AQ compounds were roughly equipotent at producing O2- and were about 20-fold more toxic than cycloheximide. The presence of nitrogen on the C-5 side chain appears to increase DNA intercalation.

In summary, the unsubstituted nucleus accounts for O2- production but the side chain determines whether DNA intercalation will occur. (Supported in part by NCI Grant CA 32036 and Wayne State University Grant-in-aid 167-1007)

We have prepared and tested in vitro a series of 1,3-disubstituted 2-oximinoethyl imidazolium halides as reactivators of AChE inhibited by ethyl p-nitrophenyl methylphosphonate (EPPM) and soman (GD). The imidazolium substituents are 1-N-methyl, 3-N-methoxy-R, where R is varied in size from methyl to 1-naphthylmethyl. These compounds are more lipophilic (log P's -3.00 to 40.88) than 2-PAM and HI-6, and thus may penetrate better to active sites. Their log P's are correlated (.92) with eel AChE competitive inhibition potency (IC50's 0.3 to 0.007 nM). All have oxime pKa's of about 8.0. Reactivation of eel AChE inhibited by EPPM and GD is best when R is n-alkyl or planar, with potencies vs. EPPM up to that of 2-PAM, and vs. GD up to half that of HI-6. Reactivation potency vs. EPPM is somewhat correlated with that vs. GD (.65), but not with affinity for AChE (.05). These compounds are of great interest as non-pyridinium reactivators of AChE, for the reactivation structure-activity information which they provide, and for their potential as antidotes to GD and other organophosphates. (Supported by the U.S. Army Medical Research and Development Command, Contract DAMD17-82-C-2194).

DOSE-RESPONSE TOXICITY STUDIES ON TRIBUTOKYETHYL PHOSPHATE (TBOP) ORALLY ADMINISTERED TO SPRAGUE-DAWLEY RATS. S. Laham, G. Long and K. Schrader, Environmental Health Directorate, Health and Welfare Canada, Ottawa KIA OL2; and J. Szabo, Department of Anatomy, University of Ottawa, Ottawa K1H 8M5.

The response of the peripheral nervous system to various dose levels of TBOP was studied in Sprague-Dawley rats. Groups of randomized female and male rats (ave. wt. 196 g for females, 284 g for males; 10 rats/sex/dose level) were given a single oral dose of TBOP (dose levels: 1.00 to 3.20 mL/kg for females; 1.00 to 9.00 mL/kg for males). Electrophysiological parameters were measured in surviving rats three weeks following TBOP administration. A significant reduction (p<0.05) in nerve conduction velocity (NCV) was observed. Both relative refractory period (RRP) and absolute refractory period (ARP) were increased in the two higher male dose levels, whereas ARP was increased in only one female group. NCV reduction was dose-related in rats of both sexes.

Light and electron microscopic examination of sciatic nerve showed degenerative changes in both myelinated and unmyelinated fibres in the higher dose levels of both female and male groups, whereas advanced degeneration was observed only in the highest dose level of both sexes. Although similar morphological changes were observed in both sexes, females were more susceptible than males to the toxic effects of this compound.

NITE IN EXTRANERVOUS TISSUES. COMPARISON BETWEEN MAN AND MICE. A. Moretto and N. Lotti. Istituto di Medicina del Lavoro, Università di Padova, Italy.

Neuropathy Target Esterase (NTE) is one of the several esterases which hydrolyse phenyl valerate (PV). NTE is believed to be the target protein in the nervous system for development of organophosphate-induced delayed neuropathy (OPIDN) in man and most non-human primates. The animal model for OPIDN is hen. We report a comparison of NTE activities and sensitivities to inhibitors among several human and extraneurous tissues. To define NTE we used the complete titration curves with non-neurotoxic Paraoxon 0.1-10000uM) and neurotoxic (Methylparaoxon 0.1-5000uM) inhibitors of PV esterases. (0.01-0.05% of total activity). Reported data were obtained with the standard method (NTE=PV esterases sensitive to Methylparaoxon among the Paraoxon-resistant ones). No significant difference in NTE activity between humans and mice was found in brain (W=2323229101, U=22621, C=23724); liver (W=532.88; H=33.54, spleen (W=1498.123; H=1176.60) and testis (W=151.968; H=718.109), respectively). Human liver and kidney contain much higher NTE activity (2714.397; 2105.282, respectively) than the hen ones (only just detectable). In both organs the percentage of NTE activity among Paraoxon-sensitive PV esterase is higher (65% liver, 73% kidney) in man than in the hen (about 20% both). Blood lymphocytes NTE is higher in man (1005.99) than in the hen (592.42). NTE sensitivity to several inhibitors is similar among different tissues in man and hen (log within 3 fold differences). NTE activity seems to be more distributed among human tissues than in the hen ones (NTE is present in man also in lung, thyroid, colon, adrenal, prostate, epididymis, ovary, ov. duct, red munci, submand. gl.)

QUANTIFICATION OF LEG MOTOR DEFICITS AND LEG NERVE AND MUSCLE CHANGES IN CHICKS WITH NEUROPATHY FOLLOWING IN DUO TcCP TREATMENT. L. Sheets and S. Norton, Dept. of Pharmacology, Toxicology and Therapeutics, Univ. of Kansas Medical Center, Kansas City, KS.

White Leghorn chicks injected with trilorthocresyl phosphate (TCP) dissolved in corn oil into the albumen on incubation day 14 had impaired leg motor function and histologic changes consistent with delayed neurotoxicity. Embryos which survived a 90% lethal 230 μl/kg egg weight dose of TcCP were completely ataxic from hatching through 3 weeks posthatching. Embryos treated with 62 μl TcCP/kg egg weight were not grossly ataxic and gained weight normally. Observation of functional motor damage from posthatching days 7 through 21 showed impaired perching and a consistently short stride. Chicks at the low dose were killed on posthatching days 5, 15 and 25 and leg nerve and muscle were analyzed morphometrically. Muscle fibers from 4 muscles were uniformly smaller on day 5 and were hypertrophic on days 15 and 25. Motor endplates were smaller than controls on day 15. Increased terminal axonal branching was present. The evidence is consistent with partial denervation compensated by muscle fiber hypertrophy and axonal sprouting. Further analyses of nerve changes are underway. The chick embryo is susceptible to TCP-neuropathy and may be a very useful model for analyses of other chemical neuropathies. (Supported in part by USPHS Grants ES07079 and NS16694 and a Staufer Chemical Company Fellowship).

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A rodent model of OPION has been developed using biweekly dosages of tri-ortho-creosyl phosphate (TOCP). Long-Evans hooded, male rats, (n=30), treated prophylactically with atropine sulfate (15 mg/kg), were exposed via gavage to an LD50 (1160 mg/kg) dose of TOCP and sampled for histopathology every 6-wk over an 18-wk exposure period. Progressive dysfunctional changes e.g., ambulation, were correlated with spinal cord (CNS) and peripheral (PNS) neuropathological damage. In both the PNS and CNS tissues, the largest and longest nerve fibers were preferentially damaged. In all treated animals, CNS damage preceded PNS degeneration. Teased nerve fibers and electron microscopy revealed the morphological lesions to be neurofibrillary axonal swellings. The distribution of spinal cord damage and the differential vulnerability of the various PNS nerves examined, support a "dying-back" classification for OPION in the rat.

Chlordecone (CD) is a polycyclic chlorinated compound with profound effect on the central nervous system. It is postulated that CD produces neurotoxic symptoms by altering synaptic function in the brain. Calmodulin, a calcium binding protein, has been shown to regulate various synaptic functions. The present studies were initiated to study the effect of CD on the regulatory activity of calmodulin on calcium ATPase in rat brain synaptosomes (RBS). RBS were prepared from male Sprague-Dawley rat brains by sucrose-ficol gradient centrifugation. Calcium ATPase was determined by inorganic phosphate method. The effect of CD on basal and calmodulin activated calcium ATPase was determined by incubating the RBS in the presence of different concentrations of CD. Calmodulin at 1-50μg activated RBS calcium ATPase in a concentration-dependent manner. CD inhibited calcium ATPase in the absence of calmodulin in a concentration dependent manner with IC50 of 10μM. The calmodulin activated calcium ATPase was also inhibited by CD with an IC50 of 5μM. However at 0.5 and 0.1μM concentration CD did not alter the basal enzyme activity but decreased the calmodulin activated enzyme activity significantly. These results suggest that CD induced neurotoxicity may be due to alteration of calmodulin regulated processes in the brain. (Supported by NIH grant #ES02443).

A rodent model of organophosphorous-induced delayed neuropathy (OPIDN), induced by TOCP has been developed with functional and neuropathological changes that parallel those seen in TOCP-treated chickens. We attempted to exacerbate the onset of this neuropathy by metabolic interference with the mixed-function oxidase inhibitor, piperonyl butoxide (PnPB). Groups of Long-Evans hooded, male rats (n=15) were pretreated with PnPB (50 mg/kg), 1-2 hr before administration of an LD50 (1160 mg/kg) dose of TOCP. Another group (n=15) received a similar dose of TOCP without PnPB. All animals were treated with 7.5 mg/kg atropine sulfate. Controls consisted of PnPB-treated and untreated rats. Animals were exposed once a week for 3 weeks after which time they were sacrificed for histopathology. No central (CNS) or peripheral (PNS) nerve damage was seen in rats treated with TOCP alone or in either control group. In the PnPB pretreatment group however, severe degeneration of the large and long, ascending and descending CNS tracts was recorded in all animals. These data indicate that metabolic interference with PnPB exacerbates TOCP-induced neuropathy in rats and suggest that the rodent's resistance to OPIDN may have a hepatic basis.

Plictran (P) is an organotin compound known to be a specific acaricide with low mammalian toxicity. The previous data showed that it was a potent inhibitor of fish and mite ATPases with an IC50 of about 10-11M. The present studies were initiated to study its effects on a mammalian brain ATPase system. Rat brain synaptosomes (RBS) were prepared and Na+,K+ and mitochondrial Mg2+ ATPases were determined in the presence and absence of different concentrations of P. Further studies were carried out to characterize the inhibition using SH reagents such as dithiothreitol (DTT), glutathione and cysteine. P inhibited both mitochondrial Mg2+ ATPase and Na+,K+ ATPase significantly with IC50 of 0.05 and 2μM respectively. Maximum inhibition occurred at physiological pH and at 37°C. Kinetic analysis showed the Km and Vmax were reduced significantly with respect to ATP. A significant increase in activation energy values were found at 17-22°C and at 32-37°C in the presence of P. DTT but not glutathione and cysteine protected the P inhibition of Na+,K+ ATPase in vitro. These results suggest that mammalian ATPase is less sensitive to P as compared to lower organisms correlating well with its toxic effect. The inhibition of Na+,K+ ATPase by P in RBS may be due to its effect on specific SH groups at substrate independent sites. (Supported by NIH grant #ES02443).

Beta-N-oxalyl-L-alpha, beta-diaminopropionic acid (BOAA) has been proposed as the neurotoxic principle responsible for the development of human neurotoxicity after chronic ingestion of the Labruscathus acutus legume. The excitotoxic properties of BOAA may result from blockade of high-affinity glutamate uptake and/or from action as a direct agonist of glutamate receptors. High-affinity glutamergic, GABAergic, glycineergic and cholinergic uptake systems were studied, in vitro, in rat spinal cord and brain after preincubation with BOAA (1mM) in order to define the tissue and uptake specificity of BOAA action in CNS tissue. A crude synaptosomal fraction (P2) was used for all uptake assays. High-affinity uptake of 3H-GABA, 3H-glutamate, 3H-glycine and 3H-choline was examined after preincubation of tissue with BOAA for 15 and 30 min. 3H-Glutamate uptake in spinal cord was reduced by 33% and 37% of control values after preincubation with BOAA (1mM) for 15 and 30 min, respectively. 3H-Glutamate uptake in brain and uptake of 3H-GABA, 3H-glycine, and 3H-choline in spinal cord and brain was not affected. These data suggest that BOAA interferes specifically with glutamate uptake in the spinal cord, in vivo, without altering the uptake capacity of other neurotransmitter systems. This may be related to the reported onset of irreversible spastic paraparesis in rats treated intrathecally with BOAA. (Supported by NIH-NS-13106, NIMH-MH-15788, NS19611, and in part by the Muscular Dystrophy Association and the Amyotrophic Lateral Sclerosis Society of America).


2,5-hexanedione produces a distinct axonopathy in the cat optic tract. The present study examined neuronal and functional changes in the visual system during such intoxication to determine if the toxicity was selective.

Cats received 0.5% 2,5-hexanedione in drinking water for a period of at least 40 days. During this time their visual acuity and fliker resolution decreased approximately 15-20% during dosing while visual acuity was relatively unaffected.

One day after dosing was terminated, large unilateral injections of horseradish peroxidase (HRP) were made into the lateral geniculate nucleus of each cat. The cats were euthanized 24-48 hours after these injections and HRP transport visualized by reacting retinal wholemounts with tetramethylbenzidine. Large (alpha) ganglion cells throughout the retina showed a reduced density of label compared to other cell types and to large cells in control animals. Since the affected fibers correspond to the physiologically identified Y cells, it is likely that 2,5-hexanedione exposure may cause selective alterations in visual system physiological responses. This may be the basis of the visual threshold changes described above. (Supported by Grants EPA R809555, NIH EY03139, EY01418, and E501247.)

NEUROTOXICOLOGIC ASSESSMENT OF RATS DERMALLY EXPOSED TO 2,4-D AMINE. J.L. Mattsson, R.R. Albee, and K.A. Johnson. Dow Chemical U.S.A., Toxicology Research Laboratory, 1803 Building, Midland, MI 48640. Sponsor: P.G. Watanabe

Although rats showed signs of systemic toxicity, there were no consistent changes in any performance, electrophysiological, or neuropathological indices to suggest neurotoxicity. Male and female Fischer 344 rats were treated with the dimethylamine salt of 2,4 dichlorophenoxyacetic acid (2,4-D amine) for 2 hours per day, 5 days per week, for 3 weeks. The treatment was applied to the skin of all four legs, from the toes to just above the elbows or knees. The rats were restrained during treatment to prevent licking and the legs were carefully washed after each exposure. Treatment with 235 2,4-D amine caused body weight loss and an excoriating dermatitis. A 12% solution had minimal effects on skin but caused body weight losses, increased grip strength, and increased kidney weights. Rats also were evaluated for neuro-behavioral effects for up to one month post-exposure. Accelerating rod performance, electrophysiologic measurements of caudal nerve and sciatic nerve function, and central and peripheral nervous system pathologic examinations were interpreted as normal. Research supported by Industry Task Force on 2,4-D Research Data.

ACYCLAMIDE REDUCES RETROGRADE TRANSPORT VELOCITY. Matthew S. Miller and Peter S. Spencer. Institute of Neurotoxicology, Deps. of Neuroscience and Pathology, Albert Einstein College of Medicine, Bronx, New York 10461.

Repeated exposure of humans or laboratory animals to monomeric acrylamide (ACMD) results in polyneuropathy of the distal-axonopathy type. Recent studies suggest that small changes in bidirectional fast axonal transport in distal portions of peripheral nerve are associated with ACMD-induced axonopathy. However, it is unclear whether these changes in axonal transport precede, or occur secondary to, axonal degeneration. This study assessed the effects of single doses of ACMD on retrograde axonal transport in male CD-1 mice (20-30 g) using purified iodinated tetanus toxin (TTX) as transport marker. Single doses of ACMD (0-100 mg/kg) produced a dose-dependent decrease in the transport of TTX to the perikarya of sensory neurons in dorsal root ganglia and motor neurons in anterior horn. ACMD was a more potent inhibitor of retrograde transport in sensory axons than motor axons. Substantially greater doses of N,N'-methylenebis-acrylamide were required to alter the axonal transport of TTX. Velocity of retrograde transport was assessed by determining the position of the leading edge of transported TTX at times following single doses of ACMD. ACMD reduced the velocity of TTX transport by up to 75%. No change in neuronal uptake of TTX was detected. It is concluded that single doses of ACMD produce profound alterations in retrograde transport which precede functional or morphologic neuropathy. Supported by NS07063, OH10851 and NS19611.
Monkeys were trained to report detection of a vibratory or an electrical stimulus. In a previous experiment, they were also dosed orally with acrylamide until toxic signs appeared. After recovery (approximately six months after the last dose), three monkeys were dosed a second time with 10 mg/kg/day of acrylamide, 5 days a week, to mimic the regimen of the first dosing period. Two other monkeys received vehicle as in the first exposure. As in the first exposure, electrical sensitivity was not impaired by acrylamide. Vibration sensitivity, however, was reduced in the treated monkeys to an extent comparable to the first exposure. Analysis of covariance showed that the rate of recovery was generally the same after the first and the second exposure.

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BCNU, (1,3-bis(2-chloroethyl)-1-nitrosourea) also known as carmustine, is a lipid soluble anticancer agent. Recent studies have shown that up to 30% of the patients receiving this drug will develop pulmonary fibrosis. A major risk factor for this lung disease is the presence of pre-existing lung damage. Total lung hydroxyproline levels, an index of fibrosis, were not elevated in mice 30 days after treatment with 25, 35, or 50 mg/kg BCNU. Total lung DNA synthesis, measured 2, 4, 7, 10, and 14 days after 35 mg/kg BCNU to quantify repair and indirectly to assess any damage, was not increased compared to control mice. These studies suggest single doses of BCNU near the LD50 dose of 52 mg/kg are not directly toxic to the lung. The ability of BCNU to enhance pre-existing acute lung damage in mice was assessed following treatments with the pneumotoxin butylated hydroxytoluene (BHT). The administration of 35 mg/kg BCNU, 1 day after 300 mg/kg BHT significantly increased total lung hydroxyproline values compared to BHT alone. BCNU inhibited the BHT-induced increase in lung DNA synthesis suggesting the enhancing effect on the development of fibrosis is similar to hyperoxia. However, BCNU treatment increased the susceptibility of mice to oxygen toxicity which could also contribute to the development of fibrosis. Additional studies will determine whether BCNU can enhance the lung damage produced by cyclophosphamide or oxygen. (This work was supported by Grant CH-263 from the American Cancer Society).

**Comparative Effects of Malathion on Plasma Cholinesterase and Behavior in Hens.** P. O. Risinger and P. W. Ferguson. School of Pharmacy, Northeast Louisiana University, Monroe, LA

The domestic chicken has potential for significant exposures to Malathion, an organophosphate insecticide, from contaminated food or airborne sources. These exposures may alter feeding behavior. This study characterized behavioral changes related to plasma cholinesterase activity 5 hours after oral Malathion exposures of either 64 mg/kg, 132 mg/kg or 264 mg/kg to White Leghorn hens. Open-field behavior, tonic immobility, plasma cholinesterase activity and malathion plasma levels were measured for each animal. No gross signs of organophosphate poisoning were observed at any dose. Plasma cholinesterase inhibition (48-54%) was not linearly correlated with malathion plasma levels (4, 12, 24 µg/ml for 64, 132, 264 mg/kg, respectively), however decrease open field behavior was correlated (p < 0.01) with increased plasma malathion. Tonic immobility was not a precise behavioral index of anticholinesterase activity.

Results suggest that in some cases behavioral measurements may be better indices of toxicity than available biochemical measurements.


Bronchopulmonary lavage analysis has been used as a rapid screening test for acute lung injury from inhaled metal salts (Henderson et. al., TAP, 51, 129-35, 1979). We demonstrated the efficacy of lavage fluid analysis for detection of acute pulmonary damage from inhaled metal dusts. Groups of male Fischer 344 rats were exposed 4 hrs to one of five dosage levels of either brass (200, 100, 50, 10, 1 mg/m3) or aluminum (100, 200, 100, 50, 10 mg/m3). At 24 hrs, 14 days and 3 mos post-exposure (PE), rats were evaluated for physiological and histological alterations to correlate with enzymatic and cytological profiles of lavage fluid. At 24 hrs PE, there were dose-related increases in lactate dehydrogenase and protein in lavage fluid of the brass exposed rats, increased pulmonary resistance, acute inflammatory response in terminal airways, and increases in macrophages and neutrophils. All reactions were resolved by 14 days PE. In contrast, aluminum powder produced no alteration in pulmonary function, but elicited persistent changes in enzymatic and cytological lavage fluid parameters with multifocal microgranulomas in lungs and hilar lymph nodes. Bronchopulmonary lavage analysis was useful as an indicator of inhalation hazards of brass and aluminum powders.
229 CHANGES IN PULMONARY CONNECTIVE TISSUE METABOLISM AFTER INHALATION EXPOSURE TO DIESEL EXHAUST.
J.A. Pickrell, R.K. Jones, R.F. Henderson and J.L. Mauberry. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM

Fischer-344 rats and CD-1 mice were exposed to dilute exhaust from a General Motors 5.7 liter diesel engine. Particulate concentration of 0.3, 3.5 and 7 mg/m³ were inhaled for 7 hrs/day, 5 days/wk. Selected endpoints were measured at 12 and 18 months after exposure in both mice and rats, and in rats after 24 months of exposure. No changes were noted in animals exposed to the lowest level of diesel exhaust. At higher exposure levels, progressively increased acid proteinase (170-360% of control levels) at all times reflected lysosomal activity and modified pulmonary architecture. Progressively increased airway hydroxyproline peptides (150-480% of control levels) reflected an increased turnover of pulmonary support tissue. Progressively increased total lung collagen was observed in mice with 6 months and rats after 12 months of exposure (125-200% of control levels). At 12 and 18 months, histologic evidence of thickened pleura and alveolar septa were noted in rats. Lesions in mice were more diffuse and of less magnitude. Rats developed the greater degree of fibrosis, had a greater turnover of pulmonary support tissue and produced more collagen and less elastin, but increased total lung collagen more slowly than did mice which had higher lung burdens of diesel soot. (Research supported by U.S. DOE Contract DE-AC04-76EV01013.)

231 AUTOLYTIC CHANGES IN MICE AS A FUNCTION OF TIME AFTER DEATH. M.R. Osheroff and R.T. Drew.
Brookhaven National Laboratory, Upton, NY

Post-mortem (PM) cellular degradation may interfere with the histopathologic evaluation of tissue reactions to xenobiotics. This study was designed to characterize the temporal autolytic changes in the laboratory mouse. C57BL/6J mice were killed by cervical dislocation and held at room temperature. Mice were necropsied immediately and at 30 minute intervals thereafter. Kidney, heart, lung, liver and thymus were excised and put in 10% buffered formalin. Sections from these tissues were embedded in glycol methacrylate, cut at 1.5 microns and stained with methylene blue-basic fuchsin. Gross and histomorphologic evaluations were monitored for the first 6 hours of PM autolysis. Microscopic examination of the lungs revealed autolytic changes in the conducting airways, alveoli and arterioles at ½ hour PM with progressively increasing parenchymal disintegration at subsequent time points. The changes included pyknosis, karyorrhexis and karyolysis, disappearance of cellular membranes and accumulation of cellular debris within the airways due to interstitial fragmentation and sloughing of individual alveolar and bronchiolar lining cells. These results suggest that xenobiotic-induced changes such as loss of parenchymal integrity and subtle cellular changes may be masked by early autolytic events. Investigators interested in such changes are encouraged to sacrifice moribund animals.

U.S. Dept. of Energy: Contract DE-AC02-76CH00016.

230 DAUNOMYCIN-MEDIATED TOXICITY IN DIPLOID FIBROBLASTS R.M. Arneson and A.P. Autor, Dept. of Pathology, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5

The toxic effects of daunomycin (Da) and other anthracycline antineoplastic agents may be related to their capacity to generate oxygen radicals, particularly the highly toxic hydroxyl radical, via redox cycling. Human fetal lung fibroblasts (MRC-5) were employed to study the effects of Da on diploid cells. Fibroblasts were cultured to confluency in Dulbecco's modified Eagle's medium with 44 mM HEPES plus 10% fetal bovine serum. For experiments, the cells were incubated at 37°C for 6 h in the same medium without serum and with and without Da. Lactic dehydrogenase (LDH) released into the medium (expressed as % of the total cell LDH) was employed as an index of cell damage. LDH release was detectable at 0.1 mM Da with 50% release at 0.3 mM Da. Total cell LDH (i.e., complete cell lysis) was released into the medium in 1.0 mM Da. At 0.3 mM Da, the hydroxyl radical scavenger, dimethyl sulfoxide (5%), inhibited LDH release by 35%. The data also suggest that superoxide dismutase protects cell membrane integrity as well. Thus, oxygen radicals are implicated in the toxic process.

Supported by NIH grants ES00118 and GM12675.


Long-term studies of conducting airway mucus clearance rates preclude the use of sedation. To correlate changes in pulmonary mechanics in a population of unsedated rabbits repeatedly measured for changes in clearance rate, we have developed a pressure plethysmographic method to measure various pulmonary mechanical parameters, e.g., frequency of breathing, tidal volume, minute volume, dynamic compliance and flow resistance. Plethysmograph pressure was measured using a pressure transducer, and esophageal pressure with a fluid filled catheter and transducer. The signals from each transducer were analyzed by a Charles River Data System MF-211 computer. Previously reported panting response of rabbits during unsedated pulmonary function testing (Cutter et al., 1947) was confirmed with this system, with frequencies ranging from 130 to 330 breaths per min. and tidal volumes of 2.7 to 15 ml; the panting response may serve as a tool for study of frequency dependence of compliance. Values for dynamic compliance and flow resistance compared well with those published in the literature. This system is capable of measuring both short term responses of the respiratory system to toxicants and long term changes in pulmonary mechanics, using the same animal as its own control.

Welding and fabrication of molten metal produce high concentrations of ultrafine metal oxides. Inhalation of freshly generated oxide of metals such as \( \text{Zn}, \text{Cu} \) and \( \text{Fe} \) can induce metal fume fever. To examine this phenomenon in animals, we exposed male Hartley guinea pigs to \( \text{ZnO} \) aerosols (CMD 0.05 \( \mu \)m, \( \sigma \) 2.0), generated by the condensation of supersaturated vapors in furnaces at 480°C. Exposure of animals to 5 mg/m\(^3\) \( \text{ZnO} \) for 3 hrs resulted in significant decrease in functional residual capacity (FRC) with only minimal changes in other lung function parameters. Lung weight was unchanged and no gross or morphologic changes were seen. In animals exposed to 5 mg/m\(^3\) \( \text{ZnO} \), 3 hrs per day for 6 days, lung volumes (with exception of total lung capacity), diffusing capacity for carbon monoxide \( \text{(DLCO)} \) and alveolar volume \( \text{(VA)} \) were significantly decreased throughout the 72 hr observation period. Complementary morphologic studies showed increased wet lung weight, alveolar duct inflammation, and elevated \( 3\text{H} \)-thymidine labeling index. (Supported by NIEHS Grant P01-ES02429, and EPA Grant R-809104).


S-sulfonates are formed in vivo by the addition of the hydrated form of \( \text{SO}_2 \) sulfate, across disulfide bonds. One such compound is glutathione-S-sulfonate (GSSGSO\(_3\)), a product of the reaction between sulfate and glutathione disulfide (GSSG). Cysteine-S-sulfonate (CYSOO\(_3\)) could also be formed by the addition of sulfate across disulfide bonds of proteins. We developed a method for simultaneous detection of GSSG, glutathione (GSH), GSSG, and CYSOO\(_3\) using HPLC. The compounds are separated on an anion-exchange column, eluted with a gradient from 100% A (5mm citric acid) to 50% B (200 mm lithium citrate, pH 5.3). Detection is by post-column derivatization with o-phthalaldehyde + 2-mercaptoethanol, yielding a fluorescent product. GSH elutes with a retention time of 11.2 min; GSSG, 25.1 min; CYSOO\(_3\), 25.4 min; and GSSG, 30.5 min; with detection limits of 20 pmol for GSH, 50 for GSSG, 500 for CYSOO\(_3\) and 200 for GSSG. Amino acids elute before GSH. Type II lung cells from rats (L2 cells) and humans (A549 cells) were exposed to 10mM sulfate for 45 min at 37°C. Protein-free fractions were analyzed by the method above. GSSG was not detectable in L2 cells. While it was detected in A549 cells at 1.8 nmol/10\(^6\) cells, GSH concentration was 50 nmol/10\(^6\) cells in A549 cells, 3-fold higher than in L2 cells. No GSSG or CYSOO\(_3\) was found. (Supported by NIH Grants ES02916 and ES07031-06.)


The effects of glutathione s-sulfonate (GSSGSO\(_3\)) on the hepatic and pulmonary glutathione S-transferases (GT) and glutathione reductases (GR) in the rat have been investigated. GSSGSO\(_3\) is a reaction product of glutathione disulfide (GSSG) and sulfate, the hydrated form of sulfur dioxide (SO\(_2\)), an air pollutant. Hepatic and pulmonary GT were studied using 1-chloro-2,4-dinitrobenzene as the substrate. VMax were 2.0 and 0.18 mU/mg/mg protein respectively and KM, 0.082 and 0.098 mM respectively. GSSGSO\(_3\) was found to be a potent competitive inhibitor for both enzymes with Ki of 0.01 and 0.009 mM respectively. Hepatic and pulmonary GR showed VMax of 0.14 and 0.069 mU/mg/mg protein respectively and KM, 0.029 and 0.025 mM respectively. GSSGSO\(_3\) appeared to be a co-substrate of GR from both tissues, with identical VMax but significantly higher KM (0.31 and 0.2 mM respectively) compared to those with GSSG as the substrate. These results suggest that SO\(_2\) may affect detoxification mechanisms, particularly that in the lung, by indirect inhibition, via formation of GSSG, the conjugation reaction of GSH and reactive electrophiles. Although GSSGSO\(_3\) can be enzymatically eliminated by GR, however, such reaction is not favored at low concentrations. (Supported by NIH Grant ES02916.)


Most published studies reporting health effects of \( \text{O}_3 \) exposure present results from animal experimentation. To date, this information has been used primarily in a qualitative manner. Our paper presents a method for using this data base more quantitatively by retrieving data from the animal toxicity literature and combining these with predictions generated by a mathematical model which estimates lung dose subsequent to inhalation of the toxicant. We set up a computerized system into which we entered data from all available studies dealing with health effects in animals following \( \text{O}_3 \) exposure. We used various scenarios for \( \text{O}_3 \) exposure selected from the literature as inputs into the Miller Model to predict \( \text{O}_3 \) dose to precise lung regions for four animal species. According to the Miller Model, the highest dose of \( \text{O}_3 \) in the lung occurs at the transitional zone between airways and alveoli, regardless of animal size or ambient concentration inhaled. Using the Model predictions, we are able to convert exposures described in the literature to specific lung tissue dose. By combining the calculated data with reported health effects from corresponding studies, dose-response curves are developed. (Supported by EPA Contract 68-02-3809.)
NON-CILIATED BRONCHIAL (CLARA) CELL AS THE INITIAL SITE OF 1-NITRONAPHTHALENE INDUCED PULMONARY TOXICITY
D.E. Johnson¹, M.G.I. Riley², and H.H. Cornish². International Research and Development Corp.¹, Mattawan, MI 49071; University of Michigan², Ann Arbor, Michigan 48109

Previous studies have shown that 1-nitronaphthalene (1-NN) induces clinically detectable respiratory distress in rats within 24 hours following a single (100 mg/kg ip) injection. The purpose of this research was to characterize the initial lesion in the lung to more clearly define the mechanism of 1-NN induced pulmonary toxicity. Young adult Charles River CDW rats were injected with 1-NN (100 mg/kg ip). Twenty-four hours later, rats found to be in respiratory distress were killed by carbon dioxide asphyxiation. At necropsy, macroscopic changes in the lungs consisted of a diffuse, irregular red motting visible on the pleural surface and of minimal severity. The lungs were removed and inflated with 10% neutral buffered formalin and fixed in formalin. Paraffin sections were stained with hematoxylin and eosin. Microscopic examination revealed a highly selective non-ciliated bronchiolar (Clara) cell necrosis. Since the Clara cell is a primary pulmonary locus of cytochrome P450 mixed function oxidase activity, these findings suggest that 1-NN induced lung toxicity is due to metabolism of 1-NN in the lung.

SPECIES COMPARISON OF LUNG ANTIOXIDANT LEVELS AND SUSCEPTIBILITY TO O3-INDUCED PULMONARY EDEMA

The effects of 4 hr exposure to different concentrations of O3 ranging from 0.2 ppm to 2.0 ppm were determined in rabbits, guinea pigs, rats, hamsters, and mice. Lavage fluid protein (LFP) concentration 16 hr after exposure (lavage fluid vol/total lung capacity) was used to quantify edema. All species had similar basal levels of LFP. Guinea pigs showed significantly increased LFP at O3 concentrations as low as 0.2 ppm, while other species rarely responded below 1.0 ppm. Basal lung concentrations of vitamin E (VE) vitamin C (VC) and non-protein SH (NPSh) were also determined for the same species and for humans (using frozen tissues taken from surgical patients). Mean VE levels ranged from 8.5 to 30 µg/g wet wt. and were significantly higher in rats (especially females) than in the other species. NPSh ranged from 55 to 90 mg% (wet wt) and was higher in rabbits and guinea pigs. Mean VC levels were highest in mice (45 mg%), while hamsters had the lowest levels (25 mg%). Human lung VC levels (31 mg%) were roughly comparable to those in animals, however, NPSh levels were much lower (8 mg%) and VE levels were slightly lower (8 µg/g). These results suggest that significant species differences in sensitivity to O3 and in lung VC, VE, and NPSh exist. (This abstract does not necessarily reflect EPA policy.)


The exposure of mammals to toxic gases may sufficiently alter lung architecture to affect the deposition efficiency (DE) of aerosols. A technique using a light-scattering, particle-size analyzer to quantitate the DE of monodisperse particles while monitoring the rat's respiratory activity was developed and tested. The difference in the concentration of particles of each size (0.46 μm, 1.09 μm, 2.02 μm and 2.99 μm) between the inspired air and the expired air of the rat is a measure of the DE. Male Sprague Dawley rats (13 weeks of age) were exposed to 1.2 ppm O3 6 hours/day for 2 or 8 days. The rats were anesthetized with 1.5mg/kg urethane, evaluated by pulmonary function and particle deposition efficiency tests, and the lungs subjected to histopathologic examination. The 2 and 8-day O3-treated rats lost 5% of their body weight whereas the control groups gained 1% and 2.5%, respectively. O3-related increases in lung weights were not statistically significant due to the small number of animals. Rats exposed to O3 for 2 days had substantially greater DE compared to controls but no differences were observed in rats exposed for 8 days versus controls. These results of DE will be compared to the results of function tests and histopathologic evaluations. Research supported by the U.S. Department of Energy under contract DE-AC02-76CH00016.


Injury from short-term exposure to ozone (O3) was detected by a simple test of red cell (RBC) filterability. This test measures changes in the ability of the RBC to deform - as occurs during passage through small capillaries. Male CD-1 mice were exposed to 1.0, 0.7, or 0.3 ppm O3 for 4 hours and blood samples obtained by heart puncture. RBC were suspended in Tris-HCl buffer, pH 7.4, containing 10 mg% glucose. After incubation in air for up to 6 hours, the time required for 2.0 ml RBC suspension to pass through a 3 um pore size polycarbonate filter was determined. A significant increase in the 6 hour RBC filtration time for O3-exposed (1.0 ppm) mice over unexposed mice, and a lack of protection by vitamin E were shown. The increases in RBC filtration time for O3-exposed mice appeared to be dose related. Ozone exposure (1 ppm, 4 hours) caused a significant increase in the hematocrit of both vitamin E deficient and supplemented mice. Vitamin E supplementation partially prevented this increase in hematocrit. Measurement of lipid peroxidation by the thiobarbituric acid (TBA) test revealed no significant differences in TBA reactive material between RBC from O3-exposed and unexposed mice. These results suggest that measurement of RBC filterability may be feasible as a clinical test for short-term injury from exposure to oxidant gases. (Supported by EPA Grant R800985.)
241 EFFECT OF NITROGEN DIOXIDE CONCENTRATION ON MEASURES OF EARLY DAMAGE AND EDEMA AFTER EXPOSURE D.J. Guth and R.D. Mavis, Div. of Toxicology, Univ. of Rochester, Rochester, NY 14642

In order to quantitate the response of the lung to various concentrations of NO₂, cell-free lavage fluid from rats exposed to 0, 10, 20, 30, and 40 ppm NO₂ was assayed for several biochemical parameters. The total units of the soluble enzymes lactate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, and glucose-6-phosphate dehydrogenase, and the lysosomal enzyme acid phosphatase (AP) showed a concentration-dependent elevation that was significant (p<0.05) in lavage fluid from rats exposed to 30 and 40 ppm NO₂ and not changed in the 10 and 20 ppm groups. Total protein and sialic acid levels were elevated after 20, 30 and 40 ppm and not changed after 10 ppm. Lavaged cells showed no differences in content of any parameter indicating that free cells are not the source of the increase in lavagable material. Plasma levels of the soluble enzymes were too low to account for the increase in lavagable activity. The increase in protein, sialic acid, and AP could be accounted for by transudation of plasma. Therefore, the source of the lavagable soluble activity is the lung epithelium. These results demonstrate the range of NO₂ concentrations in which measurement of cell damage and fluid transudation are detectable in an acute exposure protocol. (Supported by US DOE Contract No. DE-AC02-76EV03490 and NIH Training Grant No. 5 T32 HL07216-08.)

242 BIOCHEMICAL ASSESSMENT OF ACUTE NITROGEN DIOXIDE TOXICITY IN RAT LUNG. D.J. Guth and R.D. Mavis. Division of Toxicology, University of Rochester, Rochester, New York 14642

In an attempt to quantitate immediate lung response to acute NO₂ exposure (40 ppm for 4 hours), a number of biochemical measurements were made in whole lung homogenates and cell free lavage fluid immediately following exposure. Lactate dehydrogenase (LDH), malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G6PDH), NADPH- and succinate-cytochrome C reductases, acid phosphatase (AP), and N-acetyl-β-D-glucosaminidase activity in the lung homogenate showed no changes in specific activity or total lung content. In the cell free lavage, the soluble enzymes LDH, MDH, G6PDH were increased 1.7, 1.6, 3.7, and 3.0 fold respectively and the lysosomal enzymes AP and NAG were elevated 2.5 and 3.0 times the control level. Lavagable protein and sialic acid were increased to 2.3 and 15 times the control level. The number of free cells was not increased in the exposed group. These results demonstrate biochemical quantitation of direct early damage by NO₂ prior to inflammatory and proliferative responses of the lung. (Supported by US DOE Contract No. DE-AC02-76EV03490 and NIH Training Grant No. 5 T32-HL07216-08.)


To test for fibrogenic interactions between O₃ and silica, pulmonary connective tissue alterations were measured in male, Sprague-Dawley rats. The study was designed as a 2x4 factorial: air or 0.77 ppm O₃ (6 hrs/day, 5 days/week, 7.5 weeks) and instillation of 0, 2, 12, or 50mg silica/rat. Organ/body-weight ratios of lungs and spleen were increased following 50mg of silica but no O₃ effects or O₃-silica interactions were detected. Silica increased the amount of hydroxyproline (HYP), protein, and DNA per lung. If the HYP is normalized to protein, DNA or lung weight the ratios of the silica treated rats are less than that of controls. The ratios of HYP to lung weight and to DNA also responded to O₃ exposure but no silica-O₃ interactions were detected. No differences in lung lysyl oxidase activity were observed. Microscopic examination of lungs revealed a dose-dependent response in the incidence and severity of silica-induced pneumonitis and granulomata, independent of O₃ exposure. Ozone alone produced a low-grade, diffuse inflammatory lesion. Thus, silica induced a dose-dependent fibrosis, evident biochemically and histologically, but was not synergistic with a minimally-effective concentration of O₃. Research supported by the U.S. Department of Energy under contract DE-AC02-76CH00016.

244 OZONE INDUCED CHANGES IN AIRWAY EPITHELIAL PERMEABILITY. P. Doherty Miller, M.W. Warnick and W.O. Amidor, Dept. of Nutrition and Food Sci., Massachusetts Inst. of Technology, Cambridge, MA.

The effect of ozone exposure (O₃) on the permeability of the airway epithelium to horseradish peroxidase (HRP) was studied in male Hartley guinea pigs. Animals were exposed to filtered air (control) or to 1 ppm O₃ for 1 hr, and then received 2 mg HRP in 0.2 ml buffer intratracheally at 2, 8, or 24 hrs post exposure. Blood samples from a carotid artery cannula were taken prior to HRP and at 5 min intervals thereafter to 30 mins post HRP. Plasma levels were determined with an ELISA assay. Increases in HRP were correlated linearly with time over the 30 min period in all groups. At 2 hrs post exposure plasma accumulation of HRP was slower in air exposed animals than in O₃ exposed animals (0.0004 vs 0.0027% of the administered dose per min). At 30 min after HRP instillation the mean plasma level in air exposed animals was 15 ng HRP/ml/100 g body wt., and in O₃ exposed animals, 463 ng HRP/ml/100 g body wt. A recovery toward control levels of permeability was observed 8 hrs post O₃ exposure (rate of accumulation 0.0016% dose per min with a 30 min plasma level of 282 ng HRP/ml/100 g body wt.). At 24 hrs post exposure, mucosal permeability was similar to air exposed controls. The increased permeability of the respiratory mucosal barrier to macromolecules after exposure to ozone may play a role in the reported airway hyperreactivity that occurs after O₃ exposure. (Supported by NHLBI ES 01939)
Using a recently developed method (TAP 59, 451-460, 1983), we have investigated the potency of several aerosols for their ability to abolish the normal increase in tidal volume induced by 10% CO₂ in 20% O₂ and 70% N₂ (CO₂ mixture). Each animal was placed in a whole body plethysmograph and fitted with a head chamber. Tidal volume (VT) was measured by integration of airflow measured by a pneumotachograph attached to the head chamber. First, VT was measured with air flowing through the head chamber and second, while the CO₂ mixture was introduced. Once a plateau increase in VT was observed with the CO₂ mixture, the aerosols were added to this mixture and their effect on VT was measured. Groups of 4 guinea pigs were exposed at various concentrations of each agent and from concentration response linear relationship the concentration of each agent necessary to completely abolish the normal VT increase with 10% CO₂ was calculated. These concentrations, in mg/m³, were as follows for each agent: histamine, 0.7; carbamylcholine, 1; serotonin, 4; propranolol, 20; and sulfuric acid, 50. Supported under N.I.E.H.S. grant 5-ROI-ES02757.

Fischer 344 female rats were exposed nose-only to cigarette smoke generated on a Walton Horizontal Smoking Machine. The rats were lavaged using a standardized procedure at weekly intervals during a 6 week (5 days/week) exposure period. The total number of free lung cells obtained increased in the animals exposed either to smoke from Kentucky 3A1 reference (3A1) cigarettes (52×10⁶ cells/lung at 6 weeks) or to smoke from cigarettes of another American-type tobacco blend (AB) (28×10⁶ cells/lung at 6 weeks). No differences in the number or types of cells (98% macrophages) were seen between sham exposed (rats placed on the smoking machine but exposed to air only) and shibe control animals. The increase in number of macrophages per lung was similar for animals exposed to either 3A1 or AB smoke. A large difference was found in the number of polymorphonuclear leukocytes (PMN) recovered from the lungs of smoke exposed animals. After 6 weeks exposure, 25×10⁶ PMN's were present in the lungs of 3A1 exposed rats while only 6×10⁶ PMN's were present in the lungs of AB smoke exposed rats. Gamma glutamyltranspeptidase levels in the lavage fluid of smoke exposed rats were elevated at all timepoints, but alkaline phosphatase and total protein levels were not elevated.

The alveolar epithelium of the excised bullfrog lung secretes Cl⁻ by an electrogenic, Na⁺ independent process that is reflected by the short-circuit current (Isc). When lobes were mounted in flux chambers and formalddehyde (F) was added in increasing concentrations to the solution that bathed the lumen, a transient rise (10 min duration) in transmural pd (23%), Isc (48%), and conductance (G, 29%) was observed at 10⁻³M. 30 min later pd and Isc had fallen below baseline. Higher F concentrations induced a 70% inhibition of steady-state pd and Isc. The pd, Isc, and G of lobes that were depolarized by cyanide (10⁻⁴M) and dinitrophenol (10⁻⁴M) were increased by 10⁻³M F by 51%, 98%, and 52% of the baseline values before inhibitor pretreatment. When Na⁺ in the bath with metabolic inhibitors was replaced by choline, F produced a similar increase in bioelectric properties. However, when lungs were depolarized by replacement of bath Na⁺ by K⁺, F affected neither pd nor Isc, but G increased. These results suggest that the transient increases in pd, Isc, and G induced by F do not involve energy-dependent transport of Cl⁻ or an increase in the passive entry of Na⁺ into the cell from the luminal solution. Rather, the changes can be explained by an action that raises the K⁺ permeability of the basolateral membranes of the alveolar epithelial cells. Supported by HL16674 and ES07126.

We have shown that bleomycin (BLM) administered intratracheally to rats results in heterogeneously distributed peribronchial disease. Since BLM potentiates the lethality of i.v. BLM in rats, a pilot study was performed in which 30 F-344, male rats were injected with 5 U/kg BLM, exposed immediately to 100% O2 for 40 hrs, and then alternately to air for 48 hrs and O2 for 24 hrs for 2 weeks. Pulmonary function in a subgroup of rats was assessed serially at 4, 10, 18, and 26 wks post-BLM. These tests were used to monitor overt signs of disease. After final assessment, the lungs were prepared for histological and connective tissue analysis. Cohort subgroups terminated at the same time points were similarly evaluated and compared to untreated controls. Diffusion capacity decreased in both serially and singly treated animals at 10 and 18 wks, but returned to control levels by 26 wks. Collagen, elastin, and DNA were elevated at 10, 18, and 26 wks. Light microscopy, however, revealed little abnormality attributable to treatment. These findings are consistent with the initial stages of diffuse interstitial fibrosis and support our continued efforts to develop a relevant rat model of this disease utilizing the BLM-O2 interaction.

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THE ROLE OF OXYGEN AND NADPH AVAILABILITY IN THE POTENTIATION OF PARAOXANTOXICITY BY ASCORBATE. M.R. Montgomery, VA Hospital and Univ. of South Florida, Tampa, FL 33612.

A non-enzymatic reaction occurs when paraquat (Pq) and ascorbic acid (AsA) are combined in a closed system. This redox couple will deplete O2 at 34.9 nmol/min when 10 mM AsA is combined with 1 mM Pq in Krebs-Ringer-phosphate buffer. Catalase (20K units) or superoxide dismutase (5K units) was added to each individually decrease the apparent O2 consumption by 34% and 42%, respectively. When combined, apparent O2 consumption is decreased 64%. DHAS (10%), a hydroxy radical scavenger, stimulates O2 disappearance by 79%. When AsA and Pq are added to a microsomal preparation which is actively metabolizing a xenobiotic substrate, NADPH oxidation rate 34% greater than control is observed when 10 mM AsA and 1 mM Pq are added to a microsomal preparation which is metabolizing aniline. This stimulation is substrate specific no potentiation of the Pq effect is observed when AsA is added to Pq in the presence of microsomal enzymes and ethylmorphine. These data indicate that a complex group of events occur when Pq is combined with AsA: O2 is depleted from the system by reduction to activated Pq and ultimately to water. Simultaneously, the reducing equivalents needed to protect against activated Pq mediated membrane damage (NADPH) are depleted rapidly by an active mixed function oxidase system. Supported by VA Medical Research Funds and NTH Grant ES02846.
253 IN VITRO INHIBITION OF LUNG GSSG REDUCTASE BY BCNU. A.C. Smith and M. Boyd, NIH, Bethesda, MD.

The nitrosourea, BCNU, produces pulmonary damage in a large percentage of tumor patients treated with high doses of this drug. Current in vivo studies in a multi-drug model for BCNU-induced pulmonary damage suggests that the inhibition of lung GSSG reductase by BCNU could possibly be involved in the cellular damage. Lung GSSG reductase, assayed in lung cytosolic fractions, is inhibited by BCNU in vitro. BCNU-induced enzyme inactivation required NADPH and was time and concentration dependent. Exogenously added free sulphydryl compounds, e.g., dithiothreitol and reduced glutathione (GSH), were capable of decreasing the amount of enzyme inactivation during incubation with BCNU. Added substrate, GSSG, completely prevented lung GSSG reductase inactivation by BCNU. These data suggest that the inhibition of pulmonary GSSG reductase by BCNU requires the reduction of the enzyme by NADPH (presumably a disulfide reduction to a free sulphydryl). It is plausible that this essential free sulphydryl group is at or near the active site of the enzyme since added substrate prevents inactivation. Other nitrosoureas, such as CCNU and Me-CCNU, were also capable of inhibiting lung GSSG reductase in vitro whereas streptozotocin and chlorozotocin did not inhibit GSSG reductase. Other sources of rat tissue enzyme responded similarly to BCNU. Liver, heart, and brain, but not kidney, GSSG reductase activities were also inhibited by BCNU in vitro.

254 APPARENT PRODUCTION OF HYDROGEN PEROXIDE BY RAT LUNG ALVEOLAR TYPE II CELLS. R.F. Minchin, A.A. del Campo and M.R. Boyd, NCI, Bethesda, MD.

It is known that incubation of gastrointestinal, cardiac and several other tissues with cerium chloride produces electron-dense deposits within the tissue presumably due to cerium precipitation in the presence of hydrogen peroxide (H$_2$O$_2$). This study utilized an electron microscopic technique first utilized by Briggs et al., (J. Cell Biol. 67: 566) to demonstrate the localized production of H$_2$O$_2$ in polymorphonuclear leukocytes. Incubation of rat lung slices with 1 mM cerium chloride in Tris buffer (pH 7.4) produced highly localized electron-dense deposits that were readily visualized by electron microscopy. The staining was principally associated with the microvilli of the alveolar type II cells and was inhibited by catalase but not superoxide dismutase. Less extensive precipitation was seen in the interstitial space of the alveolar parenchyma. These data suggest that the rat lung type II cells are capable of producing considerable quantities of H$_2$O$_2$. X-ray microanalysis confirmed the presence presence of cerium in the deposits localized on the type II cells, although energy lines corresponding to phosphorus were also associated with the precipitate. This observation suggests that at least some of the precipitate may be the highly insoluble salt cerium phosphate which can arise from the presence of alkaline phosphatase, an enzyme known to be localized on the surface of type II cells.


Chlorphentermine (CP) is well known to cause pulmonary phospholipidosis. However, the mechanism of drug-induced phospholipidosis is not well understood. In the present study, the effect of CP on incorporation of 14C-choline was studied in the rat lung. Sprague-Dawley rats were treated for 4, 7 and 14 days with CP (50 mg/kg/day; po). Pair-fed controls received the saline, and their food and water consumption were restricted to match the consumption of the CP-treated rats. Lung slices (0.5 mm) were incubated for 60 min and phospholipids were extracted. Total phospholipid content was increased 2-fold by CP treatment. A significant increase in the amount of phosphatidylcholine (PC) was observed in the lungs of 7 and 14 days CP-treated (52.5 and 64.7 µg/mg dry lung, respectively) in comparison to the free-fed controls (27.0 µg/mg dry lung). The rates of 14C-choline incorporation into PC were significantly decreased in the lungs of the 4, 7 and 14 days CP-treated rats (62.3, 66.3 and 47.1%, respectively) in comparison to the pair-fed rats (44.1%) in comparison to the free-fed controls (78.2%). Since the content of PC was significantly increased in spite of apparently reduced synthesis, it appears that the degradation of PC is significantly inhibited more than offsetting the depressed synthesis. (Supported by HL-20622.)

256 ANILINE INDUCED METHEMOGLOBINEMIA AND HEMOLYSIS AS A FUNCTION OF EXPOSURE CONCENTRATION AND DURATION. B. A. Burgess, T. P. Pastoor and G. L. Kennedy, Jr. E. I. du Pont de Nemours and Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, P. O. Box 50, Newark, DE

Modifying work schedules to shorter shifts necessitates evaluation of appropriate exposure limits. This study was undertaken to provide insight to set such limits for aniline. Groups of 14 male rats each were exposed to concentrations of either 0, 10, 30 or 90 ppm of aniline for one of 6, 6, 12 or 12 days daily. Exposures were 5 days a week for two weeks. Methemoglobin (MhB) levels were measured daily; hematology and pathology were evaluated after the tenth exposure and after 14 days recovery. Body weights and clinical signs were unaffected. Ten ppm was a no-effect concentration at all exposure durations. A hemolytic response (decreased RBC count, splenic congestion, and hemoglobin deposition) was seen at 30 and 90 ppm (at 3, 6 and 12 hrs). Hemolysis was accompanied by compensatory increases in MCV and MCHB. Fourteen days post exposure, spleens were nearly normal at 30 and 90 ppm, and MCV and MCHB remained elevated at 90 ppm. MhB levels plateaued after 4 exposures, remained at a steady-state concentration to the 10th exposure, and decreased to normal 14 days post exposure. All effects were predominantly concentration, not time, dependent. Concentration appears to be the primary determinant to use in setting exposure limits for aniline.
The localization of (14-C) acetaldehyde (AA) in nasal mucosa of rats following inhalation exposure was investigated by autoradiography. F-344 rats (3/week) were exposed to 1500 p.p.m. of AA for 10, 30, and 60 min. in a nose-only chamber. The heads were prepared for autoradiography immediately after exposure, and 50-micron transverse, step sections were cut. Sections were placed against x-ray film at -20°C for 6, 4, or 2 weeks. The resultant autoradiograms indicated very little deposition of radiolabel in the olfactory tissues even after 60 minutes of exposure. Most of the radioactivity was associated with areas lined by respiratory epithelium especially that of the ventral meatus, maxillary turbinate and free margin of the naso-turbinate. In the sections including the ethmoid turbinates (mainly olfactory epithelium), only the naso-pharynx and the maxillary recesses contained radiolabel. Histochemical staining of aldehyde dehydrogenase activity in these same tissues showed that radiolabel was localized in areas with high enzyme activity. Because the 14-C localization did not correlate with reports of AA induced lesions in the olfactory epithelium, the distribution of radiolabel may be attributed to nasal mucus clearance mechanisms and/or metabolism of AA to nontoxic compounds in the respiratory epithelium.

The subchronic inhalation toxicity of N-methylformamide (MMF) was evaluated in male rats. Urine samples were analyzed for MMF to assess the possibility of using the urine as a biological monitor of worker exposure. Groups of 15 rats were exposed 6 h/day, 5 days/week for 2 weeks to atmospheres containing either 0, 1, 2, 3, or 6 p.p.m. of MMF. Rats exposed to 0.32 and 0.97 mg/L showed weight depression during the exposure period. Following 10 exposures, rats in the 0.32 and 0.97 mg/L groups had increased serum cholesterol concentrations. Rats exposed to 0.97 mg/L also had decreased serum urea nitrogen concentrations and serum alkaline phosphatase activities, and increased serum GPT and GGT activities. Livers in rats exposed to 0.32 and 0.97 mg/L were enlarged; hepatocytes had pale cytoplasm, an increase in the number of mitotic figures, and cytoplasmic lipid vacuolation. Thirteen days after exposure, clinical chemistry parameters showed complete recovery. Histologic examination showed complete recovery in hepatic tissue of rats exposed to 0.32 mg/L and partial recovery in rats exposed to 0.97 mg/L. MMF was excreted in a dose-dependent fashion in the urine. The target organ of MMF was the liver; 0.12 mg/L was a no-observed-effect level under the conditions of this study.

Dimethylacetamide (DMAC) is a widely used industrial solvent. Potential human exposure is primarily through inhalation and skin contact. Subchronic inhalation toxicity was evaluated in male and female rats exposed to DMAC vapors for 6 hours a day at concentrations of either 0, 100, 288, or 622 p.p.m. The initial high concentration of 641 p.p.m. was reduced to 622 p.p.m. after the first day because of the presence of an aerosol in the inhalation chamber. The latter exposure concentration was terminated after 4 days with 3 rats dead and 2 in extremis. The remaining groups were exposed for 5 days a week for 2 weeks. Histologic examination of the 622 p.p.m. group immediately after exposure showed liver hypertrophy and necrosis, lymphocytic depletion in the thymus and spleen, and hypocellularity in the bone marrow. In the 288 p.p.m. group, liver hypertrophy was observed. Two weeks later, the liver hypertrophy was still evident in the 622 and 288 p.p.m. rats along with testicular atrophy. No histological effects were seen in liver or testes in the 100 p.p.m. group. Histological evidence of nasal irritation was seen in all groups immediately after exposure and 2 weeks later. Immediately after exposure there was a dose-related increase in blood cholesterol in all test groups with recovery in 2 weeks.
3-Chloropentafluoropropene (PFAC) is a highly toxic gas with an approximate lethal concentration of 27 ppm. Subchronic toxicity was evaluated in male rats. Groups of 10 rats were exposed 6 hours/day, 5 days/week for 2 weeks to atmospheres containing either 0, 0.5, 1.5 or 5.4 ppm. Urinalysis, clinical chemistry and pathologic evaluations were performed at the end of the exposure period and 13 days later. During exposure, rats in the 5.4 ppm group exhibited salivation, dry red nasal and oral discharges, and rapid and labored breathing. Rats in this group also had significant body weight depression during the exposure period. Following 10 exposures, rats in the 1.5 and 5.4 ppm groups had increased red cell mass and increased hemoglobin concentrations. Rats exposed to 5.4 ppm also had increased hematocrits, more alkaline urine, and enlarged, heavy lungs with acute inflammation of alveolar tissue, hemorrhage, and replacement by fibrous connective tissue. Thirteen days later there was a marked reduction of acute inflammation in the lungs with only focal areas of slight alveolar fibrosis. Other changes were absent at this time. The significant effect of exposure to PFAC was on the lungs and it occurred at a concentration of 5.4 ppm.

263 ACUTE AND SUBCHRONIC INHALATION TOXICITY OF p-NITROBENZYL CHLORIDE IN RATS. L. A. Kinney, B. A. Burgess, H. C. Chen and C. L. Kennedy, Jr., E. I. du Pont de Nemours and Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, P. O. Box 50, Elton Road, Newark, DE

P-Nitrobenzyl chloride (pNBC) is a crystalline solid with a high vapor pressure. Because exposure to airborne pNBC is possible, its inhalation toxicity was studied. A 4-hour ALC in male rats is 280 mg/m³. In the subchronic study, rats were exposed 6 hours/day, 5 days/week for 2 weeks to either 0, 7.1, 18 or 53 mg/m³. Exposures at 53 mg/m³ were discontinued after the 7th exposure since deaths occurred. Rats were given pathological, urinalysis, and chemical examinations after the last exposure and after 2 weeks observation. Immediately following exposures, all exposed rats showed a slight increase in red blood cell mass. The 18 and 53 mg/m³ rats exhibited depressed growth, increased liver enzyme concentrations, decreased thymus weight, and nasal turbinate irritation. The 53 mg/m³ rats showed gasping, distended gastrointestinal tract, atrophic thymus, and left heart hyperplasia. Except for nasal turbinate irritation at both 18 and 53 mg/m³, and left heart hyperplasia at 53 mg/m³, all effects were reversible. The only effect seen in the 7.1 mg/m³ group was a slight increase in red blood cell mass which recovered in 2 weeks. Exposure to higher concentrations produced damage to the lymphoreticular and respiratory systems and systemic effects which appear to be reversible.

A TWO-WEEK SUBCHRONIC INHALATION TOXICITY STUDY ON TERACOL® 1000 IN RATS. B. A. Burgess and L. A. Kinney, E. I. du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, P. O. Box 50, Elton Road, Newark, DE

Teracol® 1000 is a polytetramethylene ether glycol used in polyurethane products to improve flexibility, water resistance, and hydrolytic stability. At room temperature, Teracol® 1000 is a waxy, white solid with an undetectable vapor pressure. Groups of 10 male rats were restrained and exposed nose-only to aerosol concentrations of either 0, 90, 290 or 1000 mg/m³ for 6 hr/day, 5 days/week for 2 weeks. Urinalysis, clinical chemistry and pathologic evaluations were done after the last exposure and 14 days later. Two rats exposed to 1000 mg/m³ were sacrificed in extremis. After the 10th exposure, rats exposed to 290 and 1000 mg/m³ exhibited increased RBC count, Hct and Hb, lymphocytic depletion, and necrosis of the spleen and thymus. Rats exposed to 1000 mg/m³ also exhibited depressed body, spleen and thymus weights, and acidic urine. Marginal increases in lung and liver weights and serum BUN were noted in all exposed rats. Fourteen days later, the only effects noted were elevated high level liver weights and serum GPT. In view of Teracol® 1000's negligible vapor pressure and the mild, reversible changes seen at 90 mg/m³, Teracol® 1000 poses little or no hazard by the inhalation route.

EFFETS OF MULTIPLE EXPOSURE TO AEROSOLIZED DIESSEL FUEL. S. Lock, Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN

The military use aerosolized diesel fuel (DF) as a visual obscuring during field exercises. This presentation reports on the effects of multiple exposures of Sprague-Dawley rats to comparatively low DF aerosol concentrations. Animals of both sexes were exposed for 4 hours/day, twice/week over a period of 13 weeks to DF aerosol concentrations of 0 to 1.5 mg of particulate/L. Mass median diameter of the particulates was 0.5 μm (eg 1.4). Body weight and food consumption were recorded weekly. Animals not sacrificed after the last exposure had the same parameters measured during a 2 month recovery period. Exposure to DF aerosol influenced growth rate, the early exposures having the most pronounced effect. Four days after the last exposure 12/12 from each group were used for a variety of end points, the other half of each group being held for 2 months prior to testing. Measurements included the number of lavaged alveolar free cells, pulmonary function, startle reflex, blood cell counts, organ weights, clinical chemistry and histopathology. There were minimal effects on lavaged pulmonary cell numbers and pulmonary function. Lung histopathology was not significantly altered. There were some slight changes in organ weight as a result of exposure. No other important effects were observed. (Sponsored by USABRL and ONR, DOE, contract W-7405-eng-26 with UCC)

Acute and subchronic inhalation toxicity studies were conducted with 2-methyl-1,2-dibromo-3-chloropropane (MDCCP) vapor. The six-hour LC50 values (95% confidence limits) were 255 (192-339) ppm for F-344 rats and 227 (171-300) ppm for B6C3F1 mice. For animals that died on study, cause of death was not determined but animals evidenced respiratory distress. In the subchronic study, groups of male F-344 rats were exposed to MDCCP vapor for 6 h/week, 5 days/week, for 10 weeks at concentrations of 50, 25, 10, 5, and 0 ppm. No treatment-related clinical signs of toxicity or changes in body weight gain were noted during the course of the study. In situ teratological measurements taken weekly throughout the 10-week exposure period showed no differences between exposed and control animals. Gross and histopathological examination of high dose and control animal tissues indicated no treatment-related lesions. The absence of teratological toxicity at levels up to 50 ppm of MDCCP is an interesting contrast with effects reported for the structurally similar pesticide, DBCP, known to elicit teratological effects at exposures of 1-10 ppm.

266 PREDICTIVE PHARMACOKINETIC MODELS FOR INHALED JP-10, BENZENE, AND TOUENE FROM IN VITRO DATA. J. P. Murphy, M. E. Andersen, M. L. Gargus, and H. J. Clewell, Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB OH

A physiological model has been used to predict the inhalation kinetics of vapors based on biochemical and solubility properties determined in vitro. In this paper we apply this approach to JP-10 (C14H10O), a candidate Cruise Missile fuel, benzene (C6H6), and toluene (C6H5CH3). Tissue partition coefficients for JP-10, BENZ, and TOL and tissue metabolic constants for JP-10 metabolism (Vmax and Km) were calculated by via-equilibration methods. Metabolic constants for BENZ and TOL were obtained from the literature. JP-10 tissue:air partition coefficients were determined for blood (620), fat (10139), liver (534), kidney (1024), and lung (24); metabolic constants were determined only for liver, kidney, and lung. Vmax for these 3 tissues were, respectively, 29, 3, and 11 nmol/gmin tissue/min. These in vitro values were used with the physiological model to predict kinetic behavior in a 500 ppm JP-10 exposure. Predicted behavior was compared to laboratory data obtained with 200-220 g male rats exposed to 500 ppm and bled serially from indwelling catheters both during and after the exposure. Predicted kinetics of BENZ and TOL were compared to literature data. There was good agreement between predicted curves and experimental data in all three cases.

267 EVALUATION OF SUBCHRONIC TOXICITY OF o-NITROCHLOROBENZENE. R.S. Kair, F.R. Johannsen, G.J. Levinskas, Monsanto Co., St. Louis, MO, J.B. Terrill, Bio/dynamics Inc., East Millstone, NJ o-Nitrochlorobenzene (ONCB) is a chemical intermediate used for the synthesis of various industrial chemicals. To evaluate the subchronic toxicity of this compound, three groups of 15 male and 15 female Sprague-Dawley rats were exposed to ONCB vapor 5 hrs/day, 5 days/week for 4 weeks at target concentrations of 10, 30, or 60 mg/m3. A control group of 15 animals/sex was exposed to room air. Chamber concentrations were determined 4 times a day using a U.V. spectrophotometer. Parameters monitored in this study included: observation for signs of toxicity, body weights, ophthalmoscopic exam, hematology and clinical chemistry. At necropsy, selected organ weights were recorded and over 35 tissues/animal were examined microscopically for all control and high-exposure level animals.

No mortality was observed in this study. Mean body weights of all groups were comparable to controls. Animals exposed to the mid and high concentrations of ONCB showed a significant increase in blood methemoglobin levels, a significant decrease in hemoglobin, hematocrit and red blood cell counts. Spleen and liver weights (absolute and relative to body weight) were significantly increased for these two groups. No unexpected gross or microscopic tissue changes were observed in animals exposed to ONCB at levels up to 60 mg/m3.


Even though exposure to the high boiling coal liquids is known to produce adverse biological effects in rodents, the effects of middle boiling range materials have not been determined. To further this database for materials derived from the solvent refined coal (SRC) II process, Fischer-344 rats and CD-I mice were exposed to an aerosol of middle distillate (MD; boiling range, 350-550°F) at concentrations of 0.73, 0.20, 0.04, or 0.00 mg/L. Particle size ranged between 1.4 and 2.0um MMD. Rats exposed to the high dose lost weight during the first week of exposure, failed to gain weight during the exposure period and then regained weight to the level of controls by 4 weeks post exposure; mice followed the same trends except that the fraction of body weight lost at the beginning of the exposure was greater than for rats. One hundred percent of the rats and 55% of the mice from the high dose group survived the exposure. By the end of the exposure, liver and kidney weights were increased and thymus and ovary weights decreased relative to controls. Blood samples collected at the end of the exposure from high exposure group rats had decreased levels of Hgb and RBC and increases in reticulocytes relative to controls. Data from this study suggest the need for subchronic and chronic studies with MD. (Supported by the U.S. Dept. of Energy Contract No. DE-AC06-76RLO-1830).
269 MEASUREMENT OF N-ACETYL-P-AMINOPHENOL AS AN INDICATOR OF BODY LOAD AFTER SUBCHRONIC EXPOSURE OF RATS TO ANILINE. T. P. Pastoor and R. A. Burgess. E. I. du Pont de Nemours and Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, P. O. Box 50, Elkon Road, Newark, DE Sponsor: G. L. Kennedy, Jr.

Groups of male rats were exposed to airborne concentrations of either 0, 10, 30 or 90 ppm of aniline for either 3, 6, or 12 hours daily for two weeks. After each day's exposure, urine was collected from four rats from each group. In a separate experiment, urine from rats dosed orally with $^{14}C$-aniline was enzymatically hydrolyzed and extracted with acetonitrile. Seventy-seven percent of the urinary radioactivity was extracted into the organic phase, and chromatographed as a single peak containing 86% of the applied radioactivity. The peak was identified as N-acetyl-p-aminophenol (N-APAP; 4-aminophenol). Urine samples from the subchronic exposures were enzymatically hydrolyzed, extracted, and the total amount of N-APAP measured by gas chromatography. The total amount of N-APAP excreted within each exposure concentration was the same. The difference in the total amount excreted between exposure concentrations (10, 30, and 90 ppm) was approximately 2- to 3-fold. Using N-APAP as an indicator showed that the body-load of aniline and aniline metabolites had plateaued by the end of the 3-hour exposure, remained at a steady-state concentration through the end of the 12-hour exposure, and was correlated with exposure concentrations.

270 ACUTE AND SUBCHRONIC INSULATION TOXICITY OF ANHYDROUS TRIMETHYLAMINE IN RATS. H. A. Kinney, B. A. Burgess and H. C. Chen. E. I. du Pont de Nemours and Company, Inc., Haskell Laboratory for Toxicology and Industrial Medicine, P. O. Box 50, Elkon Road, Newark, DE Sponsor: G. L. Kennedy, Jr.

Anhydrous trimethylamine is a flammable, colorless gas with a distinct fishy odor used as an intermediate in industrial processes. Since trimethylamine is readily airborne, its inhalation toxicity was studied. A 4-hour ALC for trimethylamine in male rats is 3500 ppm. In the subchronic study, groups of 10 rats were exposed 6 hours/day, 5 days/week for 2 weeks to either 0, 74, 240, or 760 ppm. Rats were given pathologic, urinalysis and clinical chemical examinations after the 10th exposure and after 2 weeks recovery. After 10 exposures, all exposed rats showed dose-dependent nasal cavity and turbinate irritation ranging from very mild to severe. The 240 and 760 ppm rats exhibited a slight increase in red cell mass and decreased kidney weights. The 760 ppm rats alone showed dehydration, depressed growth, mild emphysematous alveoli, increased lung and heart weights, and decreased spleen and thymus weights. After 2 weeks recovery, the slight nasal irritation at the lower levels persisted but was less severe in the 760 ppm rats. All other effects were reversible. The only effect in the 74 ppm group was very mild nasal irritation which persisted for 2 weeks. Exposure to higher concentrations produced reversible lung damage and systemic effects.

271 METHODOLOGY FOR TOXICOLOGICAL EVALUATION OF CIGARETTE SMOKE AFTER NOSE-ONLY INHALATION IN MICE AND RATS. C. J. Henry, W.C. Hall, R.M. David, C.J. Stone, Microbiological Associates, Bethesda, MD and Lorrillard Research Center, Greensboro, NC

Tobacco smoke has been characterized in a series of short-term toxicological tests. Fischer 344 female rats and B6C3F1 female mice were exposed nose-only to cigarette smoke generated under standard conditions on a Walton Horizontal Smoking Machine. Cigarette smoke doses varying in concentration and length of exposure were used to determine the maximum tolerated dose (MTD) which could be given during one 140 minute exposure period. The MTD was found to be the same for rats and mice. When tested on naive animals, the MTD of gas phase smoke was twice the MTD of whole smoke. Using the single-day whole smoke MTD, mice and rats were evaluated in 6-week repeated dose studies to determine the effects of smoke on body weights, lung weights, carboxyhemoglobin levels, respiratory tract histopathology, serum clinical chemistry and hematology and lung mitotic indices. Striking differences were observed between smoke-exposed rats and mice. Squamous metaplasia of the nasal cavity and larynx, hypertrophy and hyperplasia of the airway epithelium were observed in rats but not mice. Pigmented alveolar macrophages in the lung and serum neutrophils were increased in both species of smoke-exposed animals. No increase in the lung mitotic index was observed in either species and body weight changes were less than 10% over the 6 week period.

272 COMBINED EFFECTS OF CARBON MONOXIDE AND HEAT STRESS ON FIXED RATIO SCHEDULE PERFORMANCE IN RATS. M.M. Preache and P.S. McGilie III Research Institute, Chicago, IL Sponsor: B.S. Levine

The effects of carbon monoxide and high environmental temperature (30.5°C) were examined in adult male Sprague Dawley rats trained to lever press on a chain fixed ratio-fixed ratio schedule for food presentation. Separate groups of rats were exposed to CO for 75 min sessions at concentrations of 0, 200, 450 and 700 ppm; performance of the FR20-FR30 schedule was evaluated during the last hr of the session. For each of two CO exposure sessions, half of the rats in each group were exposed to 30.5°C and the remaining animals were tested at 24°C (ambient temperature). Carboxyhemoglobin (COHb) levels were determined for rats exposed to 450 and 700 ppm at both temperatures. At ambient temperature, CO disrupted performance only at 700 ppm. Exposure to 30.5°C disrupted performance under all exposure conditions. Comparable effects were observed in both 0 and 200 ppm groups (performance decrements of 55% of baseline). At 450 ppm in combination with 30.5°C performance was decreased to 43% of baseline. The greatest decrease was seen with the combination of 700 ppm and 30.5°C, a decrease to 30% of baseline values. COHb values determined at the end of exposure were comparable under both conditions. (Supported by U.S. Army Medical Bioengineering R and D Laboratory under Contract NO. DAMD17-80-C-0182)
Pregnant or lactating Sprague-Dawley rats or Hartley strain guinea pigs were exposed via a nose-only inhalation technique to diluted cigarette smoke for a time period up to 10 minutes (rats) or 3.3 minutes (guinea pigs) twice or thrice daily for 7, 10, or 14 days. The dams, their fetuses and pups were euthanized and tissues (lung, liver and kidney) were removed and postmitochondrial fractions (6-9) were prepared for the analysis of aryl hydrocarbon hydroxylase (AHH) activity. In the rat, smoke components (SC) did transfer via the milk as shown by an increase in hepatic AHH in the 14-day pup. Evidence was obtained for the placental transfer of SC in the rat when placenta and fetal lung and liver showed significant induction of AHH. In the guinea pig, SC did not appear to cross the placental barrier, nor were present in breast milk in concentrations sufficient to induce AHH in fetal and pup tissues. However, the guinea pig studies need to be repeated at the higher levels of smoke exposure employed in the rat studies. On account of current interest in comparative toxicology, these studies should be extended to other species, such as the Syrian golden hamster, a more widely used animal model in tobacco smoke carcinogenesis.

Rats exposed nose-only to cigarette smoke once a day for 10 min. had blood COHb levels averaging 3.7% after exposure and voided 2.7 µg of nicotine daily in the urine. A series of assays using S-15 hepatic enzymes from rats exposed to smoke for 3, 4, 8 and 16 weeks showed that: (a) these reactions mediated by the monoxygenase enzymes were consistently increased over that of control animals, but the only significant increase (p<0.05) was in AHH activity in rats exposed 16 wks. (b) 1-naphthyl glucuronoyl transferase activity was unaltered, and (c) DNB glutathione S-transferase activity was decreased after 16 wks. Similar results were obtained with rats exposed to smoke for 17 wks (once/day for 6 wks, then twice/day for 11 wks) even though the 2/day exposures did indeed double the quantity of smoke inhaled; blood COHb, 8.1%, and urinary nicotine, 5.7 µg/24hr. Liver glutathione content was reduced by 25% in rats exposed for 16 and 17 wks. Neither pentobarbital sleep nor zoxazolamine paralysis time was affected. These results show that smoke exposure at the levels and duration tested did not have a profound effect on hepatic enzyme activity in rats but they do suggest that GST-dependent detoxification reactions could be compromised considerably in the heavy smoker. (Supported in part by THRI Grant No. 45014).

Simultaneous exposure to CO and HCN occurs in fires, but their relative contribution to morbidity and mortality is often unclear. Acute toxicity, carboxyhemoglobin (COHb) and blood cyanide concentrations were studied in rats exposed 30 min to CO and HCN. For CO the 30-min L(t)50 was 155,510 mg min/cu m; the mean COHb for lethal exposure was 75% (range 70-84%) and for survivors 34% (range 25-60%). For HCN the 30-min L(t)50 was 4521 mg min/cu m; the mean blood cyanide in dead rats was 239 (range 197-305) µg/dl and for survivors was 120 (range 10-253) µg/dl. For 30:1 (CO:HCN) mixtures toxicity was less than additive, possibly indicating antagonism between CO and HCN; blood analyses showed CO toxicity was most significant. For lethal exposures COHb levels were all >66% (mean 73%); blood cyanide averaged 121 (range 57-143) µg/dl. With 12:1 (CO:HCN) mixtures, toxicity also appeared less than additive, but blood analyses indicated both HCN and CO contributed to lethal toxicity. For dead rats the mean COHb was 52 (range 31-65) %, and the mean blood cyanide was 183 (range 120-235) µg/dl. With 1:1 mixtures toxicity was mainly from HCN. For dead rats COHb levels were <40% and the mean blood cyanide was 251 (range 172-315) µg/dl. These findings show that the differential contribution of CO and HCN to toxicity varies with their relative and absolute atmospheric concentrations.

Rats exposed nose-only to cigarette smoke once a day for 10 min. had blood COHb levels averaging 3.7% after exposure and voided 2.7 µg of nicotine daily in the urine. A series of assays using S-15 hepatic enzymes from rats exposed to smoke for 3, 4, 8 and 16 weeks showed that: (a) these reactions mediated by the monoxygenase enzymes were consistently increased over that of control animals, but the only significant increase (p<0.05) was in AHH activity in rats exposed 16 wks. (b) 1-naphthyl glucuronoyl transferase activity was unaltered, and (c) DNB glutathione S-transferase activity was decreased after 16 wks. Similar results were obtained with rats exposed to smoke for 17 wks (once/day for 6 wks, then twice/day for 11 wks) even though the 2/day exposures did indeed double the quantity of smoke inhaled; blood COHb, 8.1%, and urinary nicotine, 5.7 µg/24hr. Liver glutathione content was reduced by 25% in rats exposed for 16 and 17 wks. Neither pentobarbital sleep nor zoxazolamine paralysis time was affected. These results show that smoke exposure at the levels and duration tested did not have a profound effect on hepatic enzyme activity in rats but they do suggest that GST-dependent detoxification reactions could be compromised considerably in the heavy smoker. (Supported in part by THRI Grant No. 45014).

In vitro metabolic activity of hepatic enzymes from rats exposed to cigarette smoke. M.J. Graziano and H.W. Dorough, Dept. of Entomology and Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40546.

Increasing numbers of workers are working novel workshifts in which the exposure period is longer and the recovery period is shorter than for a standard workshift. Depending upon the half-life of the encountered chemical, workers on novel workshifts might be expected to accumulate the toxicant. In this study, carboxyhemoglobin (COHb) formation and elimination in rats and mice exposed to an 8 hr/day, 5 day workweek at dichloromethane (DCM) concentrations of 200, 500 or 1000 ppm were compared. The effect of the novel workshift was insignificant as determined by the COHb level after 1st day's exposure, immediately prior to 2nd day's exposure, after the last workday's exposure and 2 or 3 days after the last exposure. The half-lives of COHb and DCM in blood were measured. The relatively short half-lives of COHb and DCM indicate that neither COHb nor DCM would be present for prolonged periods after DCM exposure ceased. Treatment with SKF-525A or CoCl2 did not affect the half-life of DCM, suggesting that DCM is rapidly exhaled. This study indicates that for compounds like DCM, with a short half-life and readily reversible biological effect, no increased toxicity would be expected in workers on novel workshifts.
A STEADY-STATE GENERATION AND INHALATION EXPOSURE SYSTEM FOR PLASTICS PYROLYSIS PRODUCTS. A. Carson and A. Vinegar, University of Cincinnati College of Medicine, Kettering Laboratory, Cincinnati, OH Sponsor: P.B. Hammond.

An apparatus was designed and constructed for producing steady-state plastics pyrolysis exposure for periods up to one hour. In addition, the dose of the exposure inhaled by the animals was quantified. The generation/exposure system has been tested using polystyrene as a standard material. Pyrolysis is carried out at a surface temperature of 600°C. Net air flow is constant (10L/min.). Total aerosol mass and particle size distribution are measured for a range of dilution using a cascade impactor. Rats are exposed, in a head-only fashion. Inhaled dose is quantified by integrated flow, body plethysmographs. Frequency response of the volume measuring system is tested by a computer generated flow input signal (2-128 Hz).

Total respirable aerosol masses (µg/m³) for 15 minute samples are: 0.997±0.0057 (MMAD=0.7μm); 0.290±0.0350 (MMAD=0.7μm); and 0.1219±0.0135 (MMAD=0.6µm), for 100%, 30%, and 10% theoretical dilutions, respectively. Amplitude response of the plethysmographs is 1.0 ± 0.012. Phase response (s) is linear with respect to frequency, r=0.999.

These data support the use of this system for toxicologic testing requiring (1) quantification of the inhaled dose and (2) generation of steady-state pyrolysis exposure over a dilution range of at least two log units.

Supported by a grant from Owens Corning Fiberglas.


The use of stain, and soil resistant treatments of home furnishings is becoming increasingly widespread. Data are not available as to how these treatments affect the toxicity of materials during thermal decomposition. The acute inhalation toxicity of the combustion products of Nylon 6 (N6), fluoropolymer-finished Nylon 6 (FPN6) and Douglas fir (DF), as a reference standard, was investigated using the NBS test method and measurements of incapacitation by the leg-flexion avoidance method. The LC50 values determined for N6, FPN6 and DF were 88.2, 92.2 and 31.6 mg/L, respectively (flaming) and 110.1, >100 and 32.8 mg/L, respectively, (nonflaming). In both modes, DF evolved large quantities of CO and incapacitated animals at all sample concentrations; lethality occurred during exposure (flaming) and during and post exposure (nonflaming). Flaming combustion of the two nylon evolved less CO than DF and incapacitated animals mainly at high sample concentrations; lethality occurred mostly post exposure (N6) or during exposure (FPN6). Nonflaming combustion of the nylons generated small quantities of CO and toxic effects (incapacitation, lethality) were minimal (N6) or did not occur (FPN6). The results indicate that, in either mode, the toxic potencies of the two nylons are comparable and are less than that of DF.

PATHOLOGICAL INDICES OF PTFE FUME INIOXATION: DEPENDENCE ON THE METHOD OF THERMAL DECOMPOSITION. K. P. Lee and S. J. Williams, E. I. du Pont de Nemours and Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, P.O. Box 50, Elkon Road, Newark, DE

The toxicity of polytetrafluoroethylene (PTFE) decomposition products is dependent upon the condition of heat application. Pathological studies were designed to assess differences in regional damage in the respiratory tract as a function of burning conditions. Rats in Group I were exposed for 30 min. to 0.075 mg/L PTFE fume formed at 700°C in a cup furnace. One hr. p.e. rats exhibited marked alveolar edema and pulmonary hemorrhage with minimal changes in the airways. Rats not sacrificed died within 8 hours p.e. Rats in Group II were exposed for 30 min. to PTFE fume formed at 700°C in a CH4 flame. These rats exhibited extensive degeneration and desquamation of airway epithelium with slight interstitial but no alveolar edema in the lung. Rats exposed to 5.5 mg/L or 12.5 mg/L of PTFE fume formed in a 700°C acetylene flame (Group III) exhibited dose-related changes in the respiratory tract similar to but more extensive than changes observed in Group II rats. Degeneration and desquamation of the respiratory epithelium were evident in Group III rats. All rats not sacrificed in Groups II and III survived and all tissue damage was reversible by 14 days p.e. These data support earlier observations of the dependence of PTFE fume toxicity on the method used to decompose the polymer.


The effect of exposure to FA on the FA concentration of the blood was determined. Eight male F-344 rats were exposed to 14.4 ± 2.4 ppm of FA for 2 hr, and blood was collected immediately after exposure. FA concentrations in the blood were determined by GC/MS (Biomed. Mass Spectrom. 9, 347 (1982)). The blood of eight rats unexposed to FA was collected and analyzed in the same manner. Measured FA concentrations (µg/g of blood) were: controls, 2.24 ± 0.20; exposed, 2.25 ± 0.20 (mean ± S.D.). FA concentrations in human blood were determined by analysing samples of venous blood collected before and after exposure of six human volunteers (4 M, 2 F) to 1.9 ± 0.1 ppm of FA for 40 min. Average FA concentrations (µg/g of blood) were: before exposure, 2.61 ± 0.48; after exposure, 2.77 ± 0.71 (mean ± S.D.). In neither experiment was there a statistically significant effect of exposure on the average FA concentration of the blood. However, human subjects differed significantly with respect to their blood FA concentrations, and significant differences (either an increase or a decrease) were found between the FA concentrations of the blood taken before and after exposure from some of the subjects, suggesting that blood FA concentrations may vary with time.
In some combustion product toxicity tests, polytetrafluoroethylene (PTFE) has been shown to be much more toxic than other common materials. These studies were conducted to determine the conditions under which the toxicity of PTFE combustion products is expressed. A modification of the NBS exposure system was used in which materials could be heated either in a cup furnace or above a small gas flame. At 700°C, the products formed from PTFE in the gas flame are at least 850 times less toxic to rats than those formed in the cup furnace. The initial products formed at 700°C under either condition (flame or cup furnace) are identical but disappear rapidly in the presence of continuous heat. If the cup furnace is removed 1 min. after the sample is added the toxicity of the products is again low. However, when heat from either the cup furnace or from a small secondary flame is applied continuously to the initial products formed from PTFE high toxicity is observed. Also, the presence of smoke from external hydrocarbon sources reduces the toxicity of PTFE combustion products by a factor of 15 to 1000. An understanding of the kinetics of the atmospheric reactions by which highly toxic species are formed from PTFE could be a determinant in assessing its hazard under real fire conditions.

Chrysotile asbestos, quartz, forsterite (an olivine), and tantalum particles were cryogenically ground and separated by size to yield test particles of low dispersivity in the one micron size range. The physicochemical characteristics of these ground and separated minerals were determined through use of X-ray photoelectron spectroscopy, energy dispersive X-ray analysis, X-ray diffraction analysis, neutron activation analysis and scanning transmission electron microscopy. It was found that the grinding and separation procedures did not alter the mineral composition or the trace element composition and preserved the crystalline structure. Further, investigation of the electrokinetic properties of these dusts by electrophoretic quasieleastic light scattering was performed. The small size dispersivity of these samples facilitated use of this technique for the determination of the apparent electrokinetic charge and estimation of surface charge density. It is suggested that analyses such as these be an integral part of studies of the toxicologic properties of such minerals.

Male and female Fischer 344 rats and B6C3F1 mice were exposed to 0, 25, or 75 ppm ethyl acrylate vapor 6 hrs per day, 5 days per week, for up to 27 months. Additional groups of rats and mice were exposed to 225 ppm for 6 months, and then held without further exposure for 21 additional months. There was no indication of an oncogenic response in either rats or mice. However, there were treatment-related changes in olfactory portions of the nasal mucosa in rats and mice in all exposure groups. A follow-up study in which Fischer 344 rats and B6C3F1 mice were exposed to 0 or 5 ppm ethyl acrylate for 24 months revealed no treatment-related changes in the nasal mucosa.

Previous studies have shown that repeated inhalation of 10,000 ppm toluene over several days potentiated the accumulation of liver triglycerides (LTG) and antagonizes the hyperlipemia seen after 4 wks of ETOH consumption. To further characterize this interaction, rats were given orally either a single dose (4.5 g/kg) of ETOH or an equivalent volume of saline. 90 min later, half of the rats were exposed to 10,000 ppm toluene for 50 min periods with 30 min of room air between each period. Blood samples were periodically taken by suborbital sinus puncture and analyzed for ETOH 23 hrs after adm. Tissue samples were collected for analysis. Neither plasma triglycerides nor relative liver wt. were significantly affected by any treatment. LTG conc. was elevated in rats receiving either ETOH alone or ETOH plus toluene, but there was no signif. diff. between these two treatment groups. The mean blood ETOH conc. of the rats receiving both toluene and ETOH dropped to zero 5 hrs after ETOH adm., but increased again within 2 hrs after this time. In rats receiving only ETOH, this gradual rise in ETOH levels 5-8 hrs after adm. was not noted. These results indicate the increase in LTG observed after a single dose of ETOH is not potentiated by short term toluene exposure and toluene alters the normal elimination of ETOH. (Supported by NIH Grant 1T 32ES07039).
Chlorinated propane are used as industrial chemical intermediates, extraction solvents and degreasing agents. The purpose of this study was to evaluate the comparative toxicity of 3 such materials in rats by the most likely route of industrial exposure. Each of three chlorinated propanes (1,2,3-Trichloro-alpha; 1,2,3-Tetra-r; 1,1,2,2,3-Penta-r) was administered as a vapor to groups of 15 male and 15 female Sprague-Dawley rats at target concentrations of 50, 15, 5, 1.5 and 0.5 ppm. Exposures occurred 6 hrs/day, 5 days/week for 13 weeks. Parameters investigated included: physical observations, survival, body weights, hematology, clinical blood chemistry, urine analysis, organ weights, necropsy and microscopic evaluation of over 40 tissues/animal. An increased incidence in lacrimation and mucoid nasal discharge were observed in most treatment groups. Significantly reduced group body weights were noted at levels of 15 and 50 ppm TCP only. No effects considered related to treatment were observed in survival, hematology, clinical chemistry or urinalysis with any of the 3 chlorinated propanes tested. Changes in organ weights paralleled microscopic findings in the same tissues. Histopathologic alterations were noted at target concentrations at and above 5 ppm TCP (liver, lung, spleen), TCP (liver) and PCP (kidney).

The effects of inhalation of combustion products of 95% red phosphorus and 5% butyl rubber (RP/BR) mixture used as an obscuration is being investigated in rats, under conditions approximating potential tropo exposure. The aerosols are produced by extruding and burning RP/BR in specially designed generators. In exploratory experiments rats exposed to 0.5, 1, 2 or 3 mg/liter of RP/BR aerosol for 30 min to 4 hrs showed significant decreases in pulmonary bactericidal activity and significantly decreased total cell counts in lung lavages. Subsequent range finding studies showed that single 1-hr exposure to 3 mg/liter (maximum generator capacity) aerosol resulted in 25% mortality, whereas exposure to 2.6 mg/liter resulted in 6% deaths. After multiple exposure for 1 hr/day on 5 consecutive days to 1.5, 2.0, 2.5 and 3.0 mg/liter mortality, during a 19-day observation period, ranged from 5 to 90% with an LC50 value estimated at 2.3 mg/liter. Repeated exposures were conducted to examine the relationship between aerosol concentration, frequency and duration of exposures, recovery time and the role of these factors on the various biologic response parameters. (Supported by the U.S. Army Medical R&D Command, Contract No. DAMD-78-C-2121.)
SUSCEPTIBILITY OF VARIOUS STRAINS OF MICE TO CEPHALORIDINE NEPHROTOXICITY. Dale A. Pasino, Katsuyuki Miura and Jerry B. Hook. Department of Investigative Toxicology, Smith Kline & French Laboratories, Philadelphia, PA

Marked species and sex differences have been observed in the nephrotoxicity to the cephalosporin antibiotic cephaloridine (CPH). When attempting to develop a mouse model for CPH nephrotoxicity based on histological examination, significant strain differences were observed. To investigate these findings further, male and female C57BL/6J, BALB/c, CD-1, CFW, CBA/J, and DBA/2 mice were given either 4 or 6 g/kg of a 30% solution of CPH s.c. and killed 48 hs. later. The ability of renal cortical slices to accumulate the organic ions p-aminohippurate (PAH) and tetraethylammonium (TEA), changes in blood urea nitrogen (BUN) and kidney-to-body weight ratios were used to assess renal function. CPH produced dose-dependent nephrotoxicity in C57BL female mice. After 6 g/kg CPH, PAH and TEA slice-to-medium ratios were reduced by 70% and 49%, respectively; BUN was elevated 10-fold. The same dose given to CFW females had no effect. BALB/c, CD-1, CBA/J and DBA/2 females showed intermediate signs of toxicity. Male mice of all strains showed little or no signs of toxicity. CPH nephrotoxicity has been correlated with the uptake and retention of CPH within the tubular cell. These data suggest possible differences in transport, cellular concentration and/or metabolism of CPH may exist among strains and between sexes of mice.


Hexachloro-1:3-butadiene (HCBD) is a highly specific nephrotoxin which causes acute damage to the rat kidney. The metabolism of HCBD has been investigated in order to explain its organ specific toxicity. The major hepatic metabolite of HCBD has been shown to be a conjugate with glutathione. No evidence was found for cytochrome P-450 metabolism of HCBD. The glutathione conjugate was excreted in bile, partially degraded, reabsorbed, and excreted through the kidneys. Both the glutathione conjugate and its mercapturate derivative were potent nephrotoxins when dosed orally to rats, causing renal damage identical to HCBD itself. Bilateral cannulation of rats prior to a dose of HCBD completely protects against kidney damage, demonstrating that the glutathione conjugate of HCBD is implicated in HCBD mediated kidney damage. The cysteine conjugate derived from this metabolite has been shown to be activated in kidney slices by the enzyme s-laspe to give ammonia, pyruvate and a reactive thiol which causes kidney damage as measured by a decrease in organic ion transport. Evidence for the structure of this reactive thiol has been obtained by the identification of a sulphenic acid metabolite of HCBD in vivo.

CORRELATION OF THE COVALENT BINDING OF 1-(2-CHLOROPHENYL)-5-(TRANS-4-METHYLCYCLOHEXYL)-1-NITROSUREA (MeCCNU) WITH THE RENAL SLICE UPTAKE OF p-AMINOhippuric ACID (PAH) IN PRETREATED F344 RATS. R.A. Kramer, M.G. McNemar and W.R. Boyd, NIH, Bethesda, MD.

The experiments described herein address the role of metabolism and of carbenylation and alkylation in mediating the nephrotoxicity of MeCCNU. Pretreatment with piperonyl butoxide decreased the covalent binding, in kidney, of MeCCNU labeled either within the alkylation (Clle-14C-MeCCNU) or carbenylation (Chx-14C-MeCCNU) region of the molecule and ameliorated the MeCCNU-induced decrease in kidney slice PAH uptake. Methylicholanthrene pretreatment decreased the covalent binding of Chx-14C-MeCCNU, but not of Clle-14C-MeCCNU, and also was protective against the MeCCNU-induced decrease in PAH uptake. On the other hand, depletion of tissue glutathione by bithionol suicide decreased the covalent binding of Clle-14C-MeCCNU, however increased covalent binding by Chx-14C-MeCCNU and produced an even greater decrease in PAH uptake than MeCCNU alone. Phenobarbital pretreatment had no effect on the covalent binding of Chx-14C-MeCCNU nor the renal slice accumulation of PAH. These data suggest that carbenylation by Chx-14C-MeCCNU may be responsible for the decrease in renal slice PAH uptake. Moreover, metabolism may play some role in determining the nephrotoxicity of MeCCNU.


Tetrafluoroethylene monomer (TFE) is known to be nephrotoxic to the rat kidney. We have studied the mechanism of toxicity in the rat both in vivo and in vitro. TFE is metabolised by rat liver fractions to a conjugate formed by the addition of glutathione across the double bond without the liberation of F- ion. The reaction is catalysed by a microsomal glutathione transferase. The equivalent cysteine and cysteineglycine conjugates have been found in rat bile in vivo following a 6hr exposure to 6000ppm TFE. No evidence has been found for cytochrome P-450 metabolism. The chemically synthesised cysteine conjugate was nephrotoxic when dosed orally to rats, the renal damage being identical to that caused by TFE, suggesting that this hepatic metabolite is responsible for the observed toxicity of TFE. Experiments in vitro have shown the cysteine conjugate to be a substrate for the renal enzyme s-laspe. Incubation of the conjugate with s-laspe produced ammonia, pyruvate and a reactive fragment which had a marked effect on ion transport in kidney slices. Our results indicate that TFE is metabolised by a single metabolic pathway in the liver to give a GSH conjugate which is degraded and further activated in the kidney to a toxic metabolite.

Uranyl fluoride has been shown to induce diuresis and glucosuria in guinea pigs. To determine whether this toxicant impairs tubular reabsorption, renal clearance measurements were made on control guinea pigs and on those which had been injected i.p. with 2.5 mg uranyl fluoride/kg 48 hr prior to clearance measurements. All animals were anesthetized during experiments. The following results (mean ± S.D.) were obtained from control (C) and dosed (D) animals: glomerular filtration rate (GFR, ml/min/g kid) 0.85±0.21 (C), 0.097±0.039 (D); osmolar clearance (ml/ml glomerular filtrate) 0.048±0.029 (C), 0.179±0.041 (D); fractional water reabsorption (% of filtered load) 96.36±1.31 (C), 85.94±4.73 (D); reabsorption rate of glucose (umole/min/g kid) 5.78±2.66 (C), 0.533±0.520 (D); fractional glucose reabsorption (% of filtered load) 99.97±0.02 (C), 49.72±37.59 (D); filtered load of glucose (umole/min/g kid) 5.79±2.66 (C), 0.919±0.308 (D). It appears that exposure to uranyl fluoride decreases GFR and impairs solute reabsorption. Apparently, the glucosuria observed is due to decreased glucose reabsorption even though the filtered load is reduced. Supported in part by NIH Training Grant No. ES07026.

295 CHARACTERISTICS OF MERCURY ACCUMULATION BY RAT RENAL CORTICAL SLICES. A. Blacker and R.J. Richardson, Toxicology program, School of Public Health, The University of Michigan, Ann Arbor, MI 48109-2029.

Accumulation of mercury (Hg) by rat renal cortical slices was studied by preincubation of slices for 30 min in 5 ml of Krebs-Ringer bicarbonate (KRB) buffer and rinsing them off with 1 ml of KRB containing 1 μg HgCl₂ plus or minus substances being evaluated for their effect on Hg uptake. Hg accumulation was increased up to 3-fold by the addition of endogenous or exogenous sulfhydryl (SH) compounds to the incubation medium or by decreasing preincubation and incubation temperatures from 37° to 25° or 0° C. GSH and GSSG levels in slices incubated at 25° or 0° were 2-3 times higher than slices incubated at 37° C. Addition of 3mM GSH to the preincubation medium increased Hg accumulation at 37° to the level observed for slices incubated at 0°, but without concomitant elevation of GSH or GSSG levels in the slices. Addition of 10μM DNP to the medium decreased Hg accumulation up to 40% at 37° but had no effect on uptake at 25° C. Slices accumulation of Hg was not saturable over a media concentration range of 0.1 to 500μM HgCl₂. Hg accumulation appears not to depend directly upon metabolic energy but to depend upon diffusible SH groups. (Supported in part by NIEM Training Grant 5T32 ES 07062).


Previous studies (Woods, J.S. & Fowler, B.A. J. Lab. Clin. Med. 90:266, 1977) have shown that prolonged exposure of rats to methyl mercury elicits a pronounced increase in urinary uroporphyrin (Uro) levels, consistent with dose and duration of exposure. This finding suggests a principal effect of Hg on uroporphyrinogen decarboxylase (UD), which decarboxylates uroporphyrinogen during heme biosynthesis. The present studies measured the effects of Hg in vivo and in vitro on hepatic and renal UD to assess the relative contribution of liver and kidney to Hg-induced uroporphyrinuria. Male rats, treated IP with Hg as HgCl₂ (0.75 or 1.5 mg/kg) 18 hrs prior to sacrifice, demonstrated a pronounced decrease in renal UD to 54 and 37% of control at 0.75 and 1.5 mg/kg, respectively, whereas liver UD was unaltered at either dose level. In studies in vitro Hg (0.01 or 0.1 mM) inhibited renal UD to 73 and 11% of control, respectively, whereas liver UD was inhibited to only 85 and 61% of control at those dose levels. By contrast, lead acetate, which does not elicit uroporphyrinuria, did not inhibit either hepatic or renal UD in vivo (20 mg/kg) or in vitro (1 mM). These results suggest that Hg-induced uroporphyrinuria is largely associated with inhibition of renal rather than hepatic UD. The kidney may thus constitute the principal source of increased urinary Uro elicited during Hg exposure.
CHARACTERIZATION OF A MURINE STRAIN DIFFERENCE IN RENAL EXCRETION OF MERCURY. K.M. Mulder and P.J. Kostyniak. Department of Pharmacology and Therapeutics, SUNY at Buffalo, School of Medicine, Buffalo, N.Y. Sponsor: T.H. Clarkson

Previous work in this laboratory identified a murine strain difference in excretion rates for methylmercury (MM). This difference was accounted for by an enhanced urinary excretion of MM in the CFW strain, while fecal excretory rates for MM were similar in both strains. In the present studies, MM distribution between plasma and red cells was measured in both strains of mice. No strain difference was observed either in vivo or in vitro conditions. Glutathione, the major non-protein thiol within most cells, has a high affinity for MM and appears to be involved in the biliary excretion of MM. In an attempt to determine whether differences in glutathione levels play a role in the strain variation in renal excretion of MM, urine and plasma glutathione levels were measured in both strains of mice. A significant difference in urinary glutathione levels was observed (CFW mice: 4.7 ± 0.3 mmol/mg creatinine, CBA/J mice: 2.8 ± 0.2 mmol/mg creatinine). Plasma levels in CFW and CBA/J mice were 30.0 ± 2.7 μM and 11.4 ± 1.3 μM, respectively, a difference of approximately 3-fold. These differences in glutathione levels between the strains may contribute to the enhanced renal excretion of MM in the CFW strain. This work was supported by grants GM25359 and GM07145.  

EFFECT OF PROXIMAL TUBULAR FUNCTION ON RENAL EXCRETION OF MERCURY. W. K. Sewell, Investigative Toxicology Department, I-64, Smith Kline & French Laboratories, 709 Swedeland Road, Swedeland, PA 19479.

Since the susceptibility of the proximal tubule to peroxidative insult is unclear, the effect of various prooxidants on tubular function was examined. Renal cortical slices from male, CD rats were incubated (90 min; 37°C; 100% O₂) in a phosphate buffer (pH 7.4) containing 75 μM PAR, 20 μM TEA, 0.5 mM lactate and 2.5 mM glutamine. FeSO₄/ascorbic acid (Fe/ASA: 0.4 mM/1.0 mM or tert-butyl hydroperoxide (TBHP: 0.5, 1.0, or 2.0 mM) were used as prooxidants. Proximal tubular function was estimated by slice PAH and TEA accumulation (S/M ratio). Malondialdehyde (MDA) evolution was used to estimate peroxidation. After 90 min, controls generated 22±5 mmol MDA/g tissue compared to 48±20 mmol MDA/g in Fe/ASA-treated slices. TBHP increased MDA evolution to a lesser extent (2.0 mM TBHP: 19±20 mmol MDA/g tissue) yet TBHP at 1.0 and 2.0 mM reduced PAH (44% and 65%) and TEA (36% and 56%) accumulation. Fe/ASA did not alter PAH uptake although TEA uptake was depressed (34±2). The poor correlation between MDA generation and tubular dysfunction may reflect differences in the spectrum of secondary peroxidation products induced by TBHP and Fe/ASA within the cells of the proximal tubule.


The ability of acute chromium (sodium dichromate) poisoning to produce a nephropathy was evaluated in adult Sprague Daley rats using renal function tests and histopathology. Animals were exposed to single chromium doses of 20, 40 or 80 mg/kg s.c., followed by two consecutive 24 hour urine collection periods. The efficacy of selected therapeutic agents to protect against the acute nephrotoxic effects of chromium was also evaluated using chelators, valence enhancers and SH-group protectors, including vitamin K, dimercaprol, dithiolethrol, ascorbic acid, EDTA and prostaglandin E₂. Acute administration of 20 or 40 mg/kg Na₂Cr₂O₇ produced a marked acute proteinuria, glycosuria, phosphaturia, enzynuria, a severe electrolyte imbalance, morphologic damage and an increased kidney weight in a dose-dependent manner. Eighty mg/kg Na₂Cr₂O₇ usually resulted in death within 24 hours of administration. Of the therapeutic agents tested, only vitamin C appeared to offer a significant degree of protection from the acute renal effects of chromium. Ascorbic acid lowered the mortality rate of the 80 mg/kg group from 7/8 to 5/8. The animals exposed to 20 and 40 mg/kg chromium plus vitamin C (150 mg/kg), while still exhibiting signs of acute renal failure, showed functional and structural effects that were significantly less severe than in the corresponding positive control groups. Ascorbic acid may therefore have value as an emergency therapy immediately after occupational chromium exposure, i.e. spills, splashes, etc.

POSTRADIATION HYPOTHALAMIC BLOOD FLOW IN PRIMATES. L.G. Cockerham, T.F. Doyle, and D.J. Hampton. Physiology Department, Armed Forces Radiobiology Research Institute, Bethesda, MD.

Early transient incapacitation (ETI) is the complete cessation of performance during the first 30 minutes after radiation exposure and performance decrement (PD) is a reduction in performance ability in the same time frame. Supraethal долo of radiation have been shown to produce ETI and/or PD in a variety of species. One possible explanation for the ETI/PD phenomena is the directly correlated drop in blood pressure that occurs postradiation. In an attempt to elucidate mechanisms underlying the radiation-induced ETI/PD phenomena, primate regional cerebral flow was measured by the hydrogen polarographic technique, both before and after exposure to gamma radiation. Systemic blood pressures and hematocrits were determined simultaneously. Data obtained indicated that 100 Gy, whole-body, gamma radiation produced a 41% decrease in systemic mean blood pressure 10 min postradiation followed by a total decrease of 65% by 60 min postradiation. Blood flow in the super optical nucleus of the hypothalamus exhibited a postradiation decrease. This decrease in postradiation regional cerebral blood flow began within 10 min after radiation and lasted for at least 60 min when it was almost 40% lower than preradiation levels. Postradiation hematocrits showed an initial increase 15 min after radiation followed by a decrease to below preradiation levels by 60 min postradiation. This study indicates that postradiation regional brain blood flow decreases with postradiation hypotension and may be responsible for ETI/PD.

Of various toxicological mechanisms of whole organs, two main groups can be recognized – one resulting from deleterious alterations in metabolic patterns (e.g., excessive production of reactive metabolites) and the other due to inefficient microcirculation leading to deranged pattern of substrate and product transport (e.g., local ischemia). We report recent evidence supporting the latter mechanism.

Livers from Wistar male rats were perfused via the portal vein with Krebs-Henseleit buffer (O2/CO2 = 95/5; 37°C; constant flow non-recirculating). When the hepatic nerves were electrically stimulated (20 V; 20 Hz; 20 nsec), the hepatic O2 uptake decreased by 20%, and the portal pressure and glucose output increased by 15% and 20%, respectively. The perportal and perivenous oxygen tensions of surface area measured using a miniature O2 electrode (platinum wire = 50 μm diameter) decreased almost to zero with a transient rise in perivenous O2 tension by 100–200 mm of Hg lasting about 1 minute. Infusion of 0.1 μM norepinephrine led to similar results except that the hepatic O2 uptake increased by 15%. Both stimuli caused local ischemia as revealed by uneven staining of the liver by trypan blue infusion. Any chemicals causing similar circulatory defects can be hepatotoxic.

EFFECT OF BUTYLATED HYDROXYANISOLE ON DOXORUBICIN-INDUCED TOXICITY. A.B. Combs and O. Tabora. College of Pharmacy, University of Texas, Austin, TX

Doxorubicin (DOX), an anthracycline antibiotic, is one of the most effective anticancer drugs, but is associated with cardiotoxicity. Peroxidation of lipid membranes due to DOX-induced free radical formation is one of the possible mechanisms. Many antioxidants have been shown to reduce DOX toxicity. The phenolic antioxidant 2(3)-1-butyl-4-hydroxyanisole (BHA) is a widely used food additive, and its antioxidant properties suggest the possibility of it having a protective action against DOX-induced lipid peroxidation. The effect of BHA pretreatment upon the survival of mice given three different acute ip doses of DOX (13.1, 18.19 mg/kg) was examined. BHA was administered in two ip doses of 150 mg/kg each, 18 hr apart. The last dose was given 6 hr before DOX. BHA did not protect against the acute toxicity of DOX. In fact, there was increased lethality when BHA was used. To determine whether the enhanced toxicity was due to enzyme induction, the effect of BHA followed by the administration of P-450 inhibitors such as SKF525A and cimeti didine upon DOX toxicity was evaluated. No protection resulted from the use of enzyme inhibitors and SKF525A increased the lethality in these animals.


Isoproterenol (ISO), a β-adrenergic agonist, produces ventricular fibrillation (VF) and myocardial lesions in rats. The myocardium of ISO-pretreated rats develops a resistance to further lesion-inducing effects of subsequent challenges. Cyclic AMP has been considered to play a major role in β-adrenergic agonist-induced myocardial necrosis as well as in the genesis of VF. We examined the VF threshold and the responsiveness of the myocardium to cAMP formation in ISO-resistant and sensitive male Sprague-Dawley rats. Seventy-two rats were pretreated sc with ISO at 50 μg/kg for 2 consecutive days and were challenged 10 days later with a single injection of graded doses of ISO (0.1 to 27.0 mg/kg sc), and electrocardiograms were recorded. The incidence of VF and death was significantly lower in resistant rats at all the challenge doses, e.g., at 1 mg/kg ISO, mortality was 17% in resistant rats vs 80% in controls. In another experiment, hearts from similarly treated rats were used for cAMP determinations by a radioimmunoassay procedure. The basal myocardial cAMP levels and the levels after in vitro ISO stimulation were not significantly different between ISO-and saline-pretreated rats. These data indicate that the mechanism for the altered myocardial sensitivity or increased VF threshold in ISO-resistant rats appear to involve factors other than cAMP.


The formamidine pesticide chlordimeform causes cardiovascular effects, and the amitraz metabolite U-40481 is vasoactive in vitro (Pestic. Biochem. Physiol., 12: 109, 1979). The in vivo cardiovascular effects of U-40481 were, therefore, studied in anesthetized dogs. Injections were made through a cannula in the left femoral artery into the aortic bifurcation and the right femoral arterial blood flow and arterial P.P. at the bifurcation were monitored. Low U-40481 concentrations (≤3 mg/kg) decreased femoral artery blood flow while 10 mg/kg U-40481 increased it. Pretreating the dogs with either propranolol, atropine, or a combination of pyribenzamine and cimetidine did not block the U-40481 effects. Phenoxbenzamine pretreatment, however, blocked the U-40481 effects on femoral blood flow. U-40481 did not block the cardiovascular effects of ACh or histamine, but reversed the responses to epinephrine and markedly potentiated the isoproterenol effects. Thus, the cardiovascular effects of U-40481, in low doses seem to result primarily from β-adrenoceptor stimulation and, with higher doses, from α-adrenoceptor blockade and potentiation of β-responses. (Supported in part by Grant 804973 from the Environmental Protection Agency).
305 EFFECT OF ACUTE DOXORUBICIN (ADRIAMycin) INTOXICATION ON HEPATIC AND CARDIAC SULPHHYDRYL REDUCING EQUIVALENTS. K.B. Wallace, Dept. of Pharmacology, Univ. of Minnesota-Duluth, Duluth, MN. Sponsor: W.R. Hewitt

The effect of an acute intoxicating dose of doxorubicin on both hepatic and cardiac glutathione and other soluble sulphhydryl reducing equivalents was examined in rats. The concentration of reduced non-protein sulphhydryl groups (NPSH) in liver decreased by 35% between 4 and 12 hrs following a single bolus injection (15 mg/kg, i.p.) of doxorubicin. This was accompanied by a 3-fold increase in soluable mixed disulfide concentration which persisted for 24 hrs. In contrast, no significant depletion of GSH or accumulation of oxidized glutathione (GSSG) was observed in liver within 24 hrs of drug administration. Cardiac NPSH was diminished only transiently at 4 hrs following drug treatment. Furthermore, doxorubicin-induced changes in cardiac GSH, GSSG and mixed disulfide concentrations were less substantial. The data indicate that doxorubicin-induced oxidation of sulphhydryl groups to the corresponding mixed disulfides is not exclusive to the glutathione regulatory system and fails to account for the cardiotoxicity of the drug.

(Supported in part by a PMA Foundation Research Starter Grant).

307 STUDIES ON THE POSSIBLE ROLE OF KEY GLUCONEOGENIC ENZYMES IN THE STARVATION-LIKE SYMPODmetry IN RATS EXPOSED TO 3,3'4,4'-TETRACHLOROXYBBZINE. M.T.S. Heila, B.B. Kreamer, and L.P. Cugliettii, Env. Tox. Ctr. and Dept. of Entomology, Univ. of Wisconsin, Madison, WI.

A starvation-like syndrome has been reported in rats exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and the isosteric 3,3',4,4'-tetrachlorooxazobenzene (TCAB), a trace contaminant found in several chloroalumline-based herbicides. This study was conducted to examine the possible role of key gluconogenic enzymes in the expression of this peculiar syndrome. Male Sprague-Dawley rats were given multiple i.p. doses (25 mg/kg of TCAB, and sacrificed at predesignated time points. In addition to animals fed ad libitum, pair-fed animals were included as controls. The key gluconogenic enzymes examined were: phospho-enolpyruvate carboxykinase (PEPCK), pyruvate kinase (PK), pyruvate carboxylase (PC), fructose 1,6-diphosphatase (FDPase), and glucose-6-phosphatase (G-6-Phase). Although there was no statistically significant differences between the control and treatment groups in liver weight for the 5-week study period, TCAB induced pronounced changes in the levels of gluconogenic enzymes in addition to cytochrome P-450. The altered gluconogenic enzyme profile is different from animals subjected to food deprivation. Thus, it is possible that changes in the gluconogenic state of treated animals may be partially responsible for the observed body weight loss and subsequent death.

(Supported in part by NIH grant RO1-E001737)

306 ALTERATIONS IN FATTY ACID COMPOSITION OF PHOSPHOLIPIDS IN RAT HEART MUSCLE DURING WEIGHT LOSS AND INCREASED RESISTANCE TO CARDIOTOXICITY OF ISOPROTERENOL. S. Gudbjarnason*, V. Whittle*, P. Sjaastad and T. Balazs, Food and Drug Admin., Washington DC and Science Institute, U. of Iceland*

The fatty acid profile of myocardial phospholipids was examined in relation to weight reduction and development of increased resistance to the cardiotoxicity of isoproterenol. Sprague Dawley male rats of 500 g BW are very sensitive to the cardiotoxicity of isoproterenol (LD50 = 0.6 mg/kg sc versus 900 mg/kg in small rats), frequently developing ventricular fibrillation that leads to death within 1 hr. Rats of 500-700 g BW (N=12) fed 4-5 g of commercial feed per day for 2 weeks lost 15-20% of their body weight, had increased resistance to cardiotoxicity of isoproterenol (LD50 900 mg/kg) and showed an extensive modification of the fatty acid composition in major phospholipids of heart muscle. Phosphatidylinoline (20:4n6) and stearic acid (18:0) were significantly increased, replacing Timoleic acid (18:2n6) and palmitic acid (16:0). In phosphatidylethanolamine, docosahexaenoic acid (22:6n3) was increased significantly and replaced 18:2n6. Changes in membrane composition or availability of competing polyunsaturated fatty acids in the heart may influence the cardiotoxicity of isoproterenol.

308 IN VIVO ADMINISTRATION OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN RESULTS IN AN ALTERATION OF THE SURFACE CHARACTERISTICS OF HEPATIC MEMBRANES. D.W. Brewer, B.Y. Madhukar, and P. Matsumura, Pesticide Research Center, Michigan State University, E. Lansing, MI 48824

Canaliculi rich plasma membrane fractions were prepared from male Sprague-Dawley rats (150-200 g) treated i.p. with a single dose of either 25 µg/kg TCDD or an equal volume of acetone/sol. After 10 days post treatment electrophoretograms revealed many plasma membrane proteins to be significantly reduced from those of control animals. Measured binding and enzymatic activities indicated many to be significantly lower than controls. Na/K-, Mg-, Ca-ATPase and gamma glutamyl transpeptidase levels were 59, 61, 56 and 74 % of controls, respectively. Protein kinase activity, determined in the absence and presence of c-AMP, was 267 and 374% control, respectively. At 10 days after dosing epidermal growth factor, insulin, and Concanavalin A bound 18, 134 and 77% control, respectively. All of the altered enzymatic activity and receptor binding capacities followed a dose-response pattern, and all were significantly altered at 2 days after dosing. Over the course of the treatment period significant differences were noted at 20 days post treatment and levels in surviving animals at 40 days were not significantly different from controls. From these results it is concluded that administration of TCDD to laboratory rats results in alterations of the hepatic plasma membrane. If these membrane perturbations occur at critical sites in critical organs, symptoms of TCDD's toxicity may be explained. (Supported by research grant TSOI863 from NIEHS.)
IN VIVO ADMINISTERED 2,3,7,8-TETRACHLORODIBENZOP-DIOXIN (TCDD) REDUCES EPIDERMAL GROWTH FACTOR (EGF) BINDING TO RAT HEPATIC PLASMA MEMBRANE. B.V. Madhukar, D.W. Brewster, D.W. Bombiek and F. Matsumura. Pasteur Research Center, Michigan State University, E. Lansing, MI.

As a part of a concerted effort to recognize various effects of TCDD on hepatic plasma membrane functions, we examined the changes in EGF binding characteristics following in vivo exposure of male Sprague-Dawley rats to TCDD at a single dose (1 to 115 μg/kg). The results indicate that EGF binding was markedly inhibited by in vivo treatment of TCDD. Significant decrease (P < 0.05) in binding was observed at 10 and 20 days after treatment at 25 μg/kg. A dose-related response in binding was evident. Scatchard analysis of the binding data indicated that the number of high affinity binding sites decreased in the membranes from TCDD-treated rat livers compared with those from untreated controls. The change in the affinity of the receptors was not significant (Kd 2.4 x 10^-11 M/liter in TCDD-treated membrane vs 2.7 x 10^-11 M/liter in the control). The results further show that the loss of EGF binding was correlated with the severity of TCDD intoxication as judged by the reductions in body weight. In vivo exposure of rats with other compounds known to either bind with the liver cytosolic Ah receptor and/or induce hepatic monooxygenases did not elicit a similar response of EGF binding with the exception of Aroclor 1242 (a polychlorinated biphenyl mixture). It is concluded that the TCDD-caused reduction in EGF binding of the hepatic plasma membrane may be related to its tumor promoting action. (Supported by research grant no. ES01963 from NIEHS.)

INHIBITION OF MITOCHONDRIAL RESPIRATION BY ACETAMINOPHEN: A NEW MECHANISM OF ACETAMINOPHEN HEPATOTOXICITY. L.Y. Cheng and S. Ji, Dep. of Pharmacology and Toxicology, College of Pharmacy, Rutgers University, Piscataway, N.J. 08854
Sponsor: R. Snyder

We have recently observed that acetaminophen (AA) inhibits O2 uptake by the isolated perfused rat liver maximally up to 60% with a half-maximal AA dosage of 5 - 10 mM. To check whether this inhibition was due to a real inhibition of mitochondrial electron transport or only an apparent inhibition resulting from a flow redistribution within the liver lobes, we isolated mitochondria according to published procedures and measured the rate of mitochondrial O2 uptake in the presence and absence of AA. AA (25 mM) inhibited the state 3 respiration completely and the state 4 and 2,4-dinitrophenol - uncoupled respiration by 40 - 50%.

Based on these observations, we conclude that AA is hepatotoxic at least partly due to its ability to inhibit mitochondrial production of ATP and equivalent intermediate forms of energy that may be required to protect cells from injuries. For example, mitochondrial ATP and other forms of energy (high-energy intermediate, membrane potential, etc.) may be essential to maintain the cytosolic level of reduced glutathione for cellular defence mechanisms. The AA-induced inhibition of mitochondrial respiration will weaken such defence mechanisms, thus contributing to its hepatotoxicity.

STRUCTURAL REQUIREMENTS FOR CYTOPROTECTIVE AGENTS IN GALACTOSAMINE-INDUCED HEPATIC NECROSIS. J.R. Macdonald, A.J. Gandolfi, I.G. Sipes, Dept Pharmacology/Toxicology and Anesthesiology, Univ of Arizona, Tucson, AZ.

Administration of cystamine 12 hr after galactosamine (G) reduces subsequent hepatic necrosis without preventing Ca2+ accumulation. Since increases in free cytosolic Ca2+ rather than total cellular Ca2+ may be the critical event leading to cell death and because the reduced form of cystamine, cysteamine, can chelate Ca2+, we investigated whether cysteamine and structurally-related compounds could reduce G-induced hepatic necrosis. Cysteamine (C), N-acetylcysteamine (NACe), S-methylcysteamine (SMCe), penicilamine (PEN), taurine (T), 2-aminomethylisothiouronium bromide (AET), chloropropamide (CP), and N-acetylcysteine (NAC) were administered to male S/D rats at doses of up to 1.5 mM/kg ip and EDTA at doses up to 0.75 mmol/kg ip 12 hr after G (400 mg/kg, ip). GPT activity, histology, and hepatic Ca2+ content were assessed 24 hr after G. Compounds with a free sulphydryl and an amine on the adjacent carbon, C and PEN, or NACe and NAC, which can be deacylated to yield this structure all reduced necrosis by more than 50%. All non-sulphydryl sulfur-containing compounds were not protective. EDTA, a non-sulphydryl Ca2+ chelator, was protective. None of the compounds prevented G-induced increase in total hepatic Ca2+. The results suggest that the mechanism of cytoprotection in this model may be Ca2+ chelation since the ability to chelate free Ca2+ is common to these protective agents. (NIH T-32-ES07091 and AM 16715).

COMPARISON OF THE PROTECTIVE ACTION OF IMMUNOPOTENTIATORS TO CCl4 HEPATIC INJURY. R.C. Lind, A.J. Gandolfi, Department of Anesthesiology, University of Arizona, Tucson, AZ.

Protection from CCl4 hepatotoxicity has been reported in rats by pretreatment with various immunopotentiators [endotoxin (ET), zymosan (Z), and levamisole (L)] and has been attributed to RES stimulation (ET and Z) or antioxidant properties (L). In addition, ET has been reported to decrease hepatic cy P-450 levels. A study was undertaken to determine the specific specificity of protection and to demonstrate if the hepatoprotective action of these immunopotentiators is due to a reduction in P-450. Male S/D rats received increasing doses (0.05-5.0 mg/kg IP) of ET or Z over 6 days. Others received L (25 mg/kg PO) over 7 days. On day 8, rats were either terminated for measurement of P-450 levels or given CCl4 (0.5 ml/kg PO). ET and L caused 30 and 50% reductions in hepatic P-450 respectively while Z had no effect. SGPT levels 24 hr post-dosing with CCl4 indicated no protection from ET and Z. [SGPT=161±108 (ET), 55±27 (Z) vs 56±22 (controls)]. L protected 4 of 5 rats (SGPT=31±12). In female S/D rats ET had a protective effect from 1.0 ml/kg CCl4 PO in 4 of 5 rats [SGPT=36±4 (ET) vs 69±19 (control)]. Z and L afforded no protection [SGPT=101±11 (Z) and 122±6 (L)]. Contrary to previous reports, not all the immunopotentiators protected against CCl4 hepatotoxicity. In fact, the efficacy appears to be related to the sex of the rat. Studies are underway to discern if the protection is linked to the ability to bioactivate CCl4. (NIH AM 16715).
313 BROMOZENZENE TOXICITY AND METABOLISM IN ISOLATED HEPATOCYTES D.A. Bankovic and R.E. Bullinge
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To assess the role of catecholic metabolites in bromoberzone (BB) hepatotoxicity, metabolism and toxicity studies were conducted with isolated rat hepatocytes. Incubation of cells from phenobarbital (PB) treated rats with 3 mM 3B for 2 hrs (10 cells/ml, 5 mM incubation) yielded 3.3 mM metabolites. The distribution of metabolites was 1-, 2-, and 3-bromophenol, 95%, 3%, 10%, respectively; 2,3- and 3,4-bromobenzendiol, 11% and 34%; 3,4-bromocatechol, 31%; and bromomethoxy catechol, 3%. Although intracellular glutathione (GSH) concentration was decreased to 6% of control value, and significant covalent binding of radioactivity to protein occurred (1.85 nmol/mg), no significant leakage of lactate dehydrogenase was observed.

BB toxicity was markedly enhanced by reduction of the hepatocyte concentration to 2 x 10^5 cells/ml, and by extension of the incubation time to six hours. Under these conditions BB caused approximately 50% LDH leakage at a concentration of 3 mM in hepatocytes from untreated rats, and at 1 mM in hepatocytes from PB treated rats. GSH depletion and covalent binding were also potentiated by PB. Under these specific conditions of incubation, isolated hepatocytes in suspension are an appropriate system for relating the toxicity and metabolism of BB.

Supported by NIHES grant ES02886.

315 EFFECTS OF IN VIVO MODULATION OF GLUTATHIONE (GSH) LEVELS BY L-2-OXOTHIAZOLIDINE-4-CARBOXYLATE AND DL-BUTHIONINE SULFOXIMINE ON PRECOCE II INDUCED HEPATOTOXICITY. S. K. Duddy and M.T.S. Hilsa, Envion. Tox. Ctr. and Dept. of Entomology, Univ. of Wisconsin, Madison, WI 53706

DL-buthionine sulfoximine (BSO) has been reported to reduce hepatic GSH levels in vivo; L-2-oxothiazolidine-4-carboxylate (OTC) has been reported to increase hepatic GSH levels. The effects of BSO and OTC on hepatotoxicity of the anti-juvenile hormone precocene II (P-II) were examined in male Sprague-Dawley rats. In Exp. 1, animals received 333 mg/kg BSO ip at hrs 0 and 6, and 100 mg/kg P-II at hr 2. In Exp. 2, animals received 400 mg/kg OTC ip at 6, 9, 13 hrs, and 175 mg/kg P-II at hr 1. Appropriate controls were included. Necropsies were performed 24 hrs after P-II treatment. Alterations in SGOT, SGPT levels and liver histology were used as indices of response. BSO alone produced no significant toxicity; P-II caused a slight elevation in SGOT, but did not elevate SGPT or cause necrosis. BSO+P-II produced severe necrosis and increased SGOT and SGPT levels dramatically. OTC elevated SGOT and SGPT by 110% and 180%, respectively, but caused little necrosis. P-II elevated SGOT and SGPT by 5200% and 3500%, respectively; OTC+P-II increased these enzymes by less than 300% and produced minimal necrosis. These results suggest hepatic GSH is of great importance in mediating P-II induced hepatotoxicity. (Supported by USDA Hatch Grant # 2730 and the Coll. of Ag. & Life Sci., UW-Madison)

316 ASPIRIN EFFECTS ON FERRETS USED AS A MODEL FOR REYE'S SYNDROME. R.H. Cray, D.G. Robertson, D. Beshoukhi, and F.A. de la Iglesiasi, Depts. of Environmental and Ind. Health and Pediatrics, Univ. of Michigan, and Path. and Exper. Toxicology, Warner-Lambert/Parke-Davis, Pharm. Res., Ann Arbor, MI

This study was conducted to evaluate subcellular organelle changes in hepatocytes from ferrets following administration of aspirin alone or with subsequently induced viral infection. This treatment has been described as an animal model for Reye's Syndrome. Eight week old male ferrets were used. Aspirin 50 mg/kg was administered to group A animals on day 1 and twice daily for the next two days, then fasted overnight. On the 4th day, ferrets were given food and sacrificed 6-7 hours later. Group V-A animals were infected with influenza B virus and then treated, fasted, and sacrificed as in group A. Liver tissue was processed for morphometric analysis. Significant increase occurred in cell volume of V-A, from 5612 to 6656 um3. No change in volume or number of mitochondria was observed in A or V-A. Peroxisomes in A were reduced by 25%, while the individual peroxisome volume or the total volume fraction in V-A were reduced by 21% and 36%, respectively. Lipid droplets were increased 10- and 20-fold, in A and V-A, respectively. These studies indicate that a combination of viral infection and aspirin potentiate the increase of lipid in the cells, reduce the volume fraction of peroxisomes in ferret hepatocytes.

Glutathione (GSH) is a critical substrate for the GSH-S-transferases, which have been shown to inactivate potentially toxic substances in mammalian tissue. Differences exist in the longitudinal distribution of these enzymes in male reproductive tissue from testis to vas deferens. The purpose of this study was to determine the effect of Ca and BSO on the levels of GSH in the male rat reproductive system. Cd dosage (0.75 mg/kg) which does not produce characteristic testicular necrosis depresses GSH to 35% of control following 3 daily ip injections. GSH was reduced to 70% of control after 3 days at 1 mg/kg, 0.5 mg/kg did not significantly depress GSH. Administration of BSO, an inhibitor of GSH synthesis, resulted in lowered GSH at 4 and 8 hours. GSH was assayed by the o-phthalaldehyde spectrofluorimetric method. Histological examination of testes from 0.5 and 0.75 mg/kg specimens did not show the marked necrotic effect observed at 1 mg/kg. Histochemical localization of GSH by staining with mercury orange reveals differences in GSH content between segments of the tract as well as within individual tissues. Cd and BSO are able to depress GSH in selected segments of the male reproductive system, and this depression may increase the possibility of tissue damage caused by reactive metabolites. (Supported in part by USPHS Grant ES02084).


ORDRAM, a rice herbicide, has been shown to affect male fertility in mice and rats, but not in rabbits or nonhuman primates. This study was conducted to determine the no-effect level and reversibility of the male fertility effect in rats exposed by inhalation. In phase I, adult male Sprague-Dawley rats were exposed to 0.0, 0.1, 0.6, 1.8, or 4.0 mg/m³, 6 hr/day, 5 days/wk for 13 weeks. Impaired male rat fertility, measured as preimplantation losses following mating with unexposed females, occurred after 4 weeks of treatment at all exposure levels above 0.1 mg/m³. After 12 weeks exposure, decreased male fertility was present only at 4.0 mg/m³, indicating that some reduced fertility present at 4 weeks was transitory. Seven weeks after completion of exposure, male fertility in all exposure groups was comparable to control. ORDGRAM exposure did not cause treatment-related changes in serum reproductive hormone levels or testicular morphology. In phase II, the no-effect level was more precisely defined by exposing male rats to 0.0, 0.07, 0.16, 0.30, 0.64, or 1.6 mg/m³, 6 hr/day, 5 days/wk for 4 weeks. Male rat fertility was impaired and increased abnormal sperm from the cauda epididymides were observed at 0.64 mg/m³ and higher. Thus, the no-effect level for impaired fertility in male rats after 4 weeks exposure was 0.3 mg/m³.


The role of peroxidases in the activation of carcinogens such as benz(a)pyrene and DES via radical mechanism is well documented. However, the changes in peroxidase activity due to pregnancy are largely unexplored.

In the present study, peroxidase activity was measured with guaiacol as the substrate in uterine extracts of nonpregnant CD-1 mice and in uterine, placental and fetal extracts of pregnant CD-1 mice on Days 12, 16, and 18 of gestation.

The uterine peroxidase activity in nonpregnant mice (N=10) was high and rapidly declined to only 0.2% at Day 18 of pregnancy (N=6). Moreover, this activity in pregnant mice was significantly p<0.05 greater on Day 12 than on Day 18 of gestation by at least 8-fold. In contrast to this, fetal peroxidase activity increased 30-fold between Days 12 (N=6) and 16 (N=3) of gestation, while the activity in placental extracts did not vary significantly (p<0.05) during the same gestational period.

These results suggest that peroxidase activity in the mouse uterine-fetal-placental unit undergoes dramatic shifts during pregnancy. Future research will evaluate different xenobiotics as: (1) potential substrates, and (2) inducers of peroxidases from these tissues.

320 DETECTING CHEMICALS HAZARDOUS TO DEVELOPMENT WITH ARTEMIA NAUPLLI. R.B. Sleet and K. Brendel, Dept. of Pharmacology, Arizona Health Sciences Center, Tucson, AZ. Sponsor: I.C. Sipe.

Efforts have begun to establish test subjects other than the intact pregnant mammal, that may serve as a rapid teratology screen. We examined developing Artemia nauplli in vitro transcribing instar 1 to determine their potential for indicating chemicals as potential developmental hazards and thus prioritizing them for more extensive in vivo testing. Several criteria important for assessing the Artemia system’s potential for screening are the ability to: 1) collect homogeneous populations of instar I nauplli, 2) characterize intermediate development quantitatively, and 3) show development-related differentials in nauplial vulnerability to various teratogen. Homogeneous nauplial populations are hatched in a flow-through hatching system and transferred to cold storage at 4°C. At 4°C the nauplli are in a quiescent state with little physical (body length, body water volume) and biochemical (DNA, protein levels) change. Intermediate developments of instar I nauplli at 25°C, which was characterized by drinking activity, body elongation, volume, DNA and protein metabolism, was greatest between 6 and 24 hrs. Development-related differentials in nauplial vulnerability were shown by comparing LC50 or EC50 (phototactic response) estimated for each compound during development. Nauplli appear useful as an initial screening organism for in vitro developmental toxicity testing. NIHST Training Grant ES 07091.

Previously, EE was shown to produce decreased sperm counts and increased percent of abnormal sperm following an acute exposure in male rats. The present study correlated the seminiferous defects with histopathological lesions in the testes associated with repeated EE exposure. Adult, Long-Evans hooded male rats were intubated with 936 mg/kg or water for 5 days/week for 6 weeks. Semen evaluations were conducted on a weekly basis on semen samples recovered from the genital tract of mated females. No differences were seen until Week 5, at which time there was a decrease in sperm counts and an increase in abnormal morphology. By Week 6, 3 males from the EE group were azospermic and the rest had severely depressed sperm counts. A decrease in motility was also seen at this time. At Week 7, there were scattered lesions in the testes, primarily at the spermatocyte stage. By Week 6, the testes were in maturation arrest with only spermatogonia and early spermatocytes present. These results agree with data from our previous study, confirming the sensitivity of the spermatocyte stage. Moreover, repeated EE exposure does not markedly change the temporal pattern of effects observed with high dose acute exposure. Supported by NIOSH R01-OH-01272 and NIHS ES-07073.


Chemicals may interfere with a variety of phenomena associated with the development of functional spermatogenesis. We have examined sperm production rate (SPR), epididymal sperm reserves and transit time, and percentage motile, swimming velocity, morphology, and nuclear resistance to swelling in sodium dodecyl sulfate (SDS) of sperm from the cauda epididymis in F344 rats. Groups (n = 15) of rats at 6, 8, 10, 15, and 20 weeks of age were studied to investigate the suitability of these indicators for detecting toxic effects, and to establish the age at which rats mature. Mean SPRs (10^6 sperm/rat/day), as measured by enumerating spermatids in testicular homogenates, were 223, 225, 417, 494, and 457 at 6, 8, 10, 15, and 20 weeks, respectively. Epididymal sperm reserves and transit time also increased with age. The remaining indicators did not significantly change with age. No correlation was found between SPR and either testis weight or epididymal sperm reserves at 10, 15, or 20 weeks of age. These results suggest that male F344 rats should be at least 10 weeks of age at the start of reproductive toxicity studies, and that testis weight and epididymal sperm number are unreliable indicators of sperm production. The low variability among adult rats in the indicators of testicular and epididymal functioning examined in this study suggests that they should be sensitive measures of male reproductive toxicity.

323 THE EFFECT OF ORNIDAZOLE ON FERTILITY AND SPERM MOTILITY IN RATS. R. Michael McClain and John C. Downing. Department of Toxicology and Pathology, Hoffmann-La Roche Inc. Nutley, NJ 07110.

Reproductive studies were performed in rats with ornidazole (2-chloromethyl-2-methyl-1-nitro-1-imidazolidethanol), a compound with trichomonacidal activity, at dosages of 0, 25, 100 and 400 mg/kg/day. Infertility was observed at the high-dose, which is 15 times the human use dose, but not at lower dosages after 81 days of treatment for male rats. Subsequent studies revealed that male fertility was affected, that the testes and epididymides were histologically normal, and that the effect was reversible within several days after the cessation of treatment.

A detailed study of male fertility and sperm function was performed using 20 control and 20 rats treated with 400 mg/kg/day for 4 weeks during which fertility was assessed by weekly matings. Sperm production and function were assessed in one-half the rats while the reversibility of effects after a 2 week recovery period was assessed in the remaining half. Male rats were killed by the 2nd week of treatment. After 4 weeks of treatment testes weights, spermatid reserves, epididymal sperm counts, sperm morphology and sperm viability were similar in treated and control rats. A quantitative assessment of sperm motility using a dark-field photomicroscope with a stroboscopic light source revealed that motility was markedly inhibited. Although the percentage of non-motile sperm was not substantially increased in treated rats, the vigor of tail movement was markedly decreased resulting in decreased sperm velocity. Restoration of fertility and normal sperm motility were observed in the recovery rats assessed 2 weeks after cessation of treatment.

It is concluded that ornidazole at a high dosage of 400 mg/kg/day produces infertility in male rats by inhibiting sperm motility. These effects are rapidly reversible after the cessation of treatment.

324 REPRODUCTIVE EFFECTS OF EXPOSURE TO SODIUM CHLORITE IN LONG-EVANS RATS. B.D. Carlton, A.H. Bassan, D.L. Habash, M.K. Smith. Battelle Memorial Institute, Columbus, OH, and USEPA, HERL, Cincinnati, OH. Sponsor: G.L. Fisher

Chlorite is by-product of the proposed alternative water disinfectant, chlorine dioxide. The reproductive effects of sodium chlorite administered ad libitum in the drinking water were studied using Long-Evans rats. Animals received 0-140 ppm for a total of 66 - 76 days. Males were dosed for 56 days and females for 14 days prior to breeding and throughout the 10-day breeding period. Following breeding the males were necropsied and evaluated for sperm parameters and reproductive tract histopathology. Females were maintained on dosed water throughout gestation and lactation. Pups and adult females were necropsied at weaning on postnatal day 21. Histopathology, sperm parameters, and hematology were evaluated in additional groups of male rats receiving 0, 100, or 500 ppm sodium chlorite. No differences were observed between control and chlorite-exposed rats when fertility, viability, litter size, developmental landmarks, histopathology, hematology, sperm motility, or sperm count were evaluated. In both sets of male rats a significant (p<0.05) dose-dependent decrease in sperm progressive movement (µm/sec) was observed. In addition, a significant (p<0.01) increase in abnormal sperm morphology was also observed. Supported by EPA Project No. CR-810301. This abstract does not represent the policy or opinion of the USEPA.

The genotoxic effect of inhaled methyl chloride (MeCl), a known testicular toxin, was assessed in a dominant lethal test (DLT) using male F-344 rats. Four groups of 40 males were exposed to 0,1000 or 3000 ppm MeCl 6 hr/day for 5 days or received an ip injection of triethylencelamine (TEM; 0.2 mg/kg) on the afternoon of day 5 as a positive control. After a 2 day recovery period, a F-344 female was caged with each male weekly for 8 weeks. Necropsy of females was 17 days after the first day of cohabitation. Exposure to 0 or 1000 ppm caused no change in any DLT parameter measured. Exposure to 3000 ppm resulted in sperm granulomas in one or both of the epididymis of 39% of the males. TEM caused increases in both dead implants/total implants (D/T, up to 0.98) and preimplantation loss (PI loss, up to 7.3/female) only at weeks 1 to 5 post-exposure (PE), indicative of a strong dominant lethal effect and subsequent recovery. 3000 ppm MeCl caused slight increases in D/T only at weeks 1 and 2 PE (0.17 and 0.33 respectively), and increases in PI loss (up to 9.1/female) during all 8 weeks. Pregnancy rate was decreased at every week PE, ranging from 28-78% (control range = 83-95%). We conclude that MeCl is an inducer of dominant lethal mutations in mature sperm exposed in the vas deferens and epididymis.


In a dominant lethal test using male F-344 rats, 3000 ppm of inhaled methyl chloride (MeCl) increased post-implantation loss in weeks 1 and 2 post-exposure (PE), and increased pre-implantation (PI) loss and decreased pregnancy rate up to 8 weeks PE. We examined the cause of these changes in males. Male rats exposed to 0,1000 or 3000 ppm MeCl 6 hr/day for 5 days or after a single ip injection of 0.2 mg/kg triethylencelamine (TEM). Five males per treatment were examined each week for 8 weeks PE. In the 3000 ppm-exposed males the testis/body weight ratio was decreased to 88% of control by week 3 PE and declined to 50% of control at week 8. In sperm isolated from the vas deferens, motility was decreased to 70% of control at 1 week PE and cell number was decreased to 52% of control by week 2. Both parameters continued to decline throughout the 8 wk observation period. Testicular spermatid count at week 8 (a measure of spermatogonial survival) was 22% of control levels and was decreased as early as 2 weeks PE, suggesting destruction of more mature spermatogenic stages. No effect of exposure to 1000 ppm MeCl or TEM was seen on any parameter measured. Thus, the increased PI loss and decreased pregnancy rate in females bred to 3000 ppm exposed males was due primarily to failure of fertilization because of lowered sperm number and motility.


Ital-Italian disease in Japan, characterized by extensive skeletal demineralization and attributed in part to cadmium (Cd) ingestion, occurred primarily in postmenopausal women with a history of multiple childbiths. To examine effects of Cd, diet, and reproductive history on the etiology of this disease, 3 groups of female mice (260-340 mice/group) were given diets containing 0.25, 5, or 50 ppm Cd. At each Cd level, mice received either a sufficient diet (+) or an "Ital-Italian household" diet (-) (deficient in certain vitamins, minerals, and fat). One-half of the females were bred for 6 consecutive pregnancy/lactation cycles; remaining females were nonpregnant controls. Reproductive data were recorded and mean values per cycle were compared, since changes were the same for each successive cycle. On the + diet, fertility (+), litter size at birth (LS), and pup survival during lactation (PS) were the same for all 3 Cd levels; pup mass at weaning (PM) decreased 25% with 0 ppm Cd. On the - diet, 50 ppm Cd (compared to 0.25 ppm) caused a decrease in F (37%), LS (18%), and PM (42%), but not PS; at 5 ppm Cd, no decreases occurred. At all 3 Cd levels, exposure to the - diet (compared to the + diet), caused decreases in F (12-48%), LS (16-33%), PS (8-20%), and PM (41-54%). (Work supported by the U.S. Department of Energy under contract No. W-31-109-ENG-38.)

EFFECTS OF DIBUTYL PHTHALATE (DBP) ON THE REPRODUCTIVE SYSTEM OF TWO STRAINS OF YOUNG MALE RATS. J.A. Ward, H. Zenick, W.W. Wilfinger, R.J. Niemehuis. University of Cincinnati College of Medicine, Cincinnati, OH.

DBP causes testicular atrophy in young male rats. The primary lesion is thought to lie within the Sertoli cell. Although the histopathology has been well-documented, the hormonal response to this insult has not been systematically examined. This study was designed to examine endocrine status after subchronic exposure to DBP. Twenty-two day old Sprague Dawley (SD) and Long Evans Hooded (LEH) rats were dosed (p.o.) for 30 days with 0,500, or 1000 mg/kg/day for DBP. At sacrifice, serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and plasma testosterone (T) were determined. Zinc levels were measured in one testis, the other was prepared for histological evaluation. Bone dependent increases in LH and FSH were observed. No alteration in plasma T was evident. Tissue zinc concentrations were depressed in the high-dose groups only, and were lower in treated SDs than in LEHS. Histology showed seminiferous tubular degeneration and hyperplasia of interstitial tissue, primarily in the high-dose group. The hormonal and morphological patterns are indicative of compensated primary gonadal failure, with normal basal pituitary function. These results suggest that loss of intra-gonadal endocrine regulatory mechanisms might contribute to the mechanism by which DBP exerts its toxic effect on the testis. This research was funded by NIOSH Grant R01-0901272/NEHS ES-07073.
Adult female minks were fed diets that contained 2.5 or 5.0 ppm 2,4,5,2',4',5'- or 2,3,6,2',3',6'-hexachlorobiphenyl (HCB), 0.1 or 0.5 ppm 3,4,5,2',4',5'-HCB or 2.5 ppm Aroclor 1254 for one month prior to breeding through parturition. All mink fed 0.5 ppm 3,4,5,2',4',5'-HCB died within 60 days, while those fed 0.1 ppm failed to mate and showed 50% mortality after three months exposure. Only one stillborn kit was whelped in the Aroclor 1254 group. No adverse reproductive effects were observed in the animals fed 2,3,6,2',3',6'- or 2,4,5,2',4',5'-HCB. Aroclor 1254 caused a significant elevation in cerebellar and hypothalamic norepinephrine (NE) concentrations and a significant elevation in hypothalamic dopamine (DA) concentrations. Cerebral DA was elevated by 0.1 ppm 3,4,5,2',4',5'-HCB while NE concentrations were significantly elevated by 5.0 ppm 2,3,6,2',3',6'-HCB in the midbrain and by 5.0 ppm 2,4,5,2',4',5'-HCB in the medulla.

As part of a comprehensive safety assessment program, a Three Generation Reproduction Study was conducted in Charles River CD-1 CD-1 rats with monosodium cyanurate (CA) administered ad libitum at 400, 1200, and 5375 ppm in drinking water. One control group received tap water adjusted to pH 7.4 at 25°C and a Na control group received a solution of tap water containing 0.056 ppm of sodium bicarbonate. Each treatment level consisted of 12 males and 12 females. For mating, 1 male was cohabitated with 2 females for 7 days. Any females failing to exhibit evidence of mating after the initial 7 days were replaced with randomly selected males from the same treatment group. Treatment was initiated at 36 days of age for the 0 parents and continued for a minimum of 100 days prior to mating. Females were rested for 14 days after the lactation period before being mated for a second litter. The only change attributed to treatment were slight increases in mean male body weight and average female water consumption throughout the first generation at the high dosage. No biologically significant differences in fertility indices, length of gestation, litter size, pup survival, or body weights at parturition or throughout lactation were observed between treated and control groups. A concentration of 5375 ppm of CA had no effect on reproductive or litter parameters in the first generation of the study.

The influence of selected teratogenic agents on fetal cerebral ventricular size was determined following in utero exposure. Treated pregnant golden hamsters were given single intraperitoneal injections of one of the following on day 8 of gestation: chlormethadione (28.5 mg/kg); amitriptyline HCl (70.3 mg/kg); Na arsenite (21 mg/kg); acetanilide (1600 mg/kg); or Na salicylate (1100 mg/kg). Control animals received injections of saline only. Fetuses were recovered on gestation day 15 following maternal sacrifice. Fetal cerebral ventricular size was determined using a binocular dissecting microscope equipped with a calibrated ocular micrometer. The width of each of these ventricles was measured in coronal head slices located approximately 2 mm behind the eyes. A trend toward increased mean ventricular size was apparent in all experimental groups. A value of 150 μm was chosen as corresponding to the nearest measurement of the saline-treated control mean fetal cerebral ventricular size. A trend toward increased frequency of fetuses with mean fetal cerebral ventricular size greater than 150 μm was apparent for each of the experimental groups compared with controls. Thus, teratogenic agents may produce more subtle effects upon the developing CNS rather than overt hydrocephalus.
LEARNING PERFORMANCE OF OFFSPRING OF RHESUS MONKEYS EXPOSED TO LOW LEVELS OF 2,3,7,8-TETRA-CHLORODIBENZO-P-DIOXIN (TCDD). S. L. Schantz, and R. E. Bowman, University of Wisconsin Primate Laboratory, Madison, WI. Sponsor: R. E. Peterson

Learning performance of 6 rhesus monkeys born to TCDD-exposed mothers did not differ from that of 7 controls. TCDD-exposed mothers were fed a diet containing 5 parts per trillion TCDD for 7 mo. prior to breeding and during gestation and nursing. Offspring were weaned at 4 mo. At 14 mo. they were tested on 4 discrimination-reversal learning problems in the Wisconsin General Test Apparatus (WGTA). Each problem consisted of an acquisition phase followed by a series of reversals. A spatial problem and a spatial problem with color and shape present as irrelevant stimulus dimensions were followed by problems in which color and shape became the relevant stimulus dimensions. At 20 mo. each monkey was tested on delayed spatial alternation which consisted of a series of trials in which the correct location alternated between right and left on each trial. Intertrial delays of 5, 10, 20, and 40 sec. were used in counterbalanced order. There were no significant differences (p >.05) between TCDD-exposed and control offspring on any of these learning tests. Following weaning, TCDD-exposed mothers were maintained on the TCDD diet for 2 additional years and bred again, along with their controls. Learning performance of offspring from this second breeding round is currently being evaluated. (Supported by NIH grant 5-T32-ES07015-09).


Cyclopiazonic acid (CPA) is a mycotoxin produced by fungal species of the Penicillium and Aspergillus genera. It is isolated from meats and from agricultural commodities such as corn and peanuts. To assess the potential effect of CPA on pregnancy and fetal development, Fischer 344 rats were dosed daily with 0, 1, 5, or 10 mg CPA/kg B.W. on either days 8-11 or days 12-15 of pregnancy (p = 0.05) decreases in feed consumption by high dose dams of both groups. Body weights at term of dams receiving the two highest doses in both groups tended to be less than controls. One high dose rat in each group died prior to term and signs of toxicity were observed in other high dose animals. Remaining dams were killed on day 21. Compared to controls, there were no significant differences in pup weights, or percentage of resorptions or fetal deaths. Partially descended testes was the most common soft tissue aberration. Significant differences in skeletal development included retardation of ossification of cervical vertebrae (days 12-15) and caudal vertebrae (days 8-11) in the medium and high dose groups. Retardations in fetal development were, therefore, the most common manifestations of embryotoxicity. The potential of CPA to cause malformations is low since signs of maternal toxicity, including death, were observed in the high dose group.


We evaluated the effects of maternal administration of vitamin A acetate on pup development and behavior. Vitamin A acetate was administered by oral gavage to pregnant rats (N=10/treatment) on gestation days 6-19 at doses of 25,000, 50,000 or 100,000 IU/kg/day. Male and female pups from dams that received 100,000 IU/kg/day showed a significantly reduced live birth index but no anomalies. Twenty-four and 48 hour survival indices were also significantly reduced. The mean pup body weight change at 100,000 IU/kg/day was significantly reduced at days 1-3, 3-7 and 21-42. Pinna detachment and eye opening were significantly delayed in male pups from all three dose groups and in female pups from the 50,000 and 100,000 IU/kg/day groups. Incisor eruption was significantly delayed in male and female pups from the 25,000 and 50,000 IU/kg/day groups. The following showed no treatment effects: dam mean weight change, length of gestation, total litter size, surface righting, cliff avoidance, negative geotaxis, swimming development, open field activity and operant learning ability. No gross or histopathologic changes were attributed to treatment. Maternal administration of vitamin A acetate produced neonatal death, decreased survival and a delay in physical development. Reflex development, motor coordination and operant learning were not affected.


Forty male F-344 rats exposed by inhalation to 1500 ppm of methyl chloride for 10 weeks had a 10 to 20% body weight gain depression (BWGD) and did not produce any litters when bred to methyl chloride exposed or unexposed females. Of 40 males exposed to 475 ppm, 28 did not produce litters and had a significantly lower mean body weight than the 12 males that did produce litters (246 ± 15 gms vs. 260 ± 10 gms). Ten weeks post exposure all groups had similar mean body weights. Some 1500 ppm males regained fertility, and the 475 ppm males were as fertile as control males. To determine if BWGD alone had an effect on reproduction, the groups of F-344 rats were fed either ad libitum or a restricted diet (BWGD: 215 males, 162 females). In 70% (56) of the control animal matings females had copulation plugs and 60% (48) had litters. Only 16% (13) of the BWGD group females had plugs and no litters were born. After a 10 week recovery period (both groups fed ad libitum), there was no significant difference in body weight or in fertility between the groups. Therefore, BWGD alone is sufficient to impair reproduction in rats. The interpretation of reproduction studies conducted at doses which cause BWGD may be confounded by this problem.
Sodium Omadine®, I, is a broad spectrum antimicrobial used in metalworking fluids and as a preservative in cosmetics. I was applied topically to the shaved back at 0, 0.5, 1.5, 3.0, and 7.0 mg/kg/day in a cream (Aquaphor®) from gestation days 6-15 to pregnant Charles River CD rats. Triamcinolone Acetonide, II, was used as a positive control given at 0.03 ml/rat/day. Rats were fitted with an agar collar during the treatment period to prevent oral ingestion of the test articles. Dams were sacrificed on gestation day 20 and the location and numbers of viable and non-viable fetuses, early and late resorptions, implantations and Corpora lutea were recorded. All fetuses were individually weighed, sexed and examined for external, visceral and skeletal malformations. The dose of 7.0 mg/kg/day of I was excessive producing maternal body weight losses and 20% mortality. Doses of 3.0 mg/kg/day of I produced erythema without maternal or fetal toxicity. Minimal skin irritation was noted in the positive control and 0.5 mg/kg/day group. Cleft palate, omphalocele, fetal anasarca and small genital tubercle were the most frequent anomalies observed in the II group. I given topically throughout organogenesis at 3 mg/kg/day in the rat was not teratogenic.

Omadine® MDS, a pyridine-N-oxide derivative, is intended for use in cosmetics and shampoos. Previous studies have established that Omadine® MDS is not teratogenic when applied dermally to rats and rabbits. In this study, pregnant rats were dosed daily with 0, 10, and 30 mg/kg on gestation days 6 through 15. Pregnant rabbits were dosed with 0, 1.5 and 5 mg/kg on gestation days 6 through 18. Blood samples taken during and after dosing were analyzed by HPLC for 2-methylsulfonfylpyridine (2MSP), the persistent metabolite. In rats, peak plasma levels attained on gestation day 10 were 470 ng/ml in the 10 mg/kg group (no-effect level for maternal toxicity) and 1500 ng/ml in the 30 mg/kg group (slight maternal toxicity). In rabbits, peak plasma levels attained on gestation days 12 and 15 were 155 and 148 ng/ml at 1.5 mg/kg (no-effect level for maternal toxicity) and 513 and 573 ng/ml at 5 mg/kg (slight maternal toxicity). The determination of 2MSP plasma levels is useful in monitoring the potential toxic effects of pyridines once the relationship between the plasma level and toxic manifestation is established.

The present study examined responsiveness to conditional and unconditional stimuli following neonatal chlordone exposure, both separate from and within an avoidance paradigm. A control for the dimethylsulfide vehicle was included. On day 4 postpartum, the offspring of Fischer-344 dams received a subcutaneous injection (20 µl) of water, DMSO, 0.2 mg, or 1.0 mg of chlordone dissolved in DMSO. Body weights during preweaning and early postweaning periods were slightly, but significantly, lower (3%) in both sexes following the high dose of chlordone. DMSO did not alter body weight. In an automated clinch-jump task chlordone increased shock sensitivity of males and decreased shock sensitivity of females. On a multiple measure emergence task chlordone treatment decreased all test latencies. Chlordone also produced alterations for both acquisition and reversal (retention) of a two-way avoidance task. None of these behavioral changes suggested an effect of chlordone when comparisons were made to the water, rather than DMSO, vehicle. These findings indicate that changes in nonassociative factors must be considered in evaluation of associative processes following toxict exposure and that because DMSO may alter behavioral reactivity, controls for its use as a vehicle may be an important variable in toxicological studies.
Carbon disulfide (CS₂) was evaluated for developmental toxicity after subcutaneous administration in New Zealand White (NZW) rabbits on gestational days (gd) 6-15 at 0, 25, 75, or 150 mg/kg, and in CD rats on gd 6-15 at 0, 100, 200, 400 or 600 mg/kg. At sacrifice (gd 30, NZW; gd 20, CD) all fetuses were examined for gross, visceral, and skeletal malformations. In rabbits, significant maternal toxicity and increased maternal liver weight were observed at 75 and 150 mg/kg. The percent of resorptions/litter was significantly increased at all dose levels, but the percent of fetuses malformed was significantly increased only at the highest dose. In CD rats, dams had significantly reduced gestation body weight gain at all dose levels. Mean fetal weight was reduced significantly in the 200, 400, and 600 mg/kg litters, but there were no significant differences in the incidence of malformations or resorptions at any dose level. Thus, CS₂ treatment resulted in significantly increased prenatal mortality but not malformations in rabbits at a dose which was not toxic to dams (25 mg/kg). In rats, all doses of CS₂ were toxic to the dam, and only the 200 mg/kg dose and above produced fetal toxicity in the form of reduced fetal weight. Supported by NCTR/NTP Contract No. 222-80-2031(C).


Terbutaline sulfate (T) and prenatal P₂ adrenergic agonists, are used to delay premature labor. Adverse cardiac effects in the offspring of treated mothers have been reported. In addition, excessive prenatal catecholamine exposure has been implicated in the etiology of hypertrophic cardiomyopathy (Perllof, Am. Heart J. 101: 219-226, 1981). We investigated the myocardial effects in the offspring of 3 groups of pregnant Sprague-Dawley rats (N=12/group) that were given T (2 mg base/kg sc), P₂ (2 mg/kg sc), or saline twice daily (AM & PM) from 14-21 days of pregnancy, and of another 3 groups (N=7-8/group) that were given T (2 mg base/kg), P₂ (2 mg/kg), or saline twice daily from 18-21 days of pregnancy; offspring of the latter 3 groups received 20 mg/kg sc of the same drug given to their mothers once daily from 1-4 days of age. Electrocardiograms were recorded for determination of heart rate at intervals from 2 to 212 days of age on representative rats from each group. Five to 12 offspring from each group were killed at 1, 5, 9, 37, or 150 days of age. Heart rates showed no differences for any group. Morphological examination of hearts at the above ages revealed no effects attributable to treatment. Hearts of newborn treated and control groups showed areas of disorderly arrangement of myocytes in the ventricles up to 9 days of age.


Recent advances in the area of infant cognition have provided new insights into the capabilities of neonates. Investigations of cross-modal ability and recognition memory have been used to evaluate the cognitive processes of human infants. Our studies have detailed the development of cross-modal ability, pattern recognition and object permanence in macaques. Macaque infants demonstrate cross-modal matching (oral-visual) within the first 30 days of life and later prefer more complex visual stimuli. Preference for novel visual stimuli has been reported by 30 days of age while object permanence develops by 50 days of age. We have used these procedures to examine the cognitive functioning of infants exposed in utero to methylmercury. Exposed offspring showed a significant delay in the development of object permanence. No differences were found between control and exposed offspring on cross-modal matching (oral-visual), but exposed offspring did not exhibit a visual preference for complex stimuli as did the controls. Results from assessments of early perceptual-cognitive functioning may provide a sensitive index for a teratogenic response. NIAMS ES-00677 and ES-07032.


Pregnant Sprague-Dawley rats were administered 0.6 or 6 mg/kg polybrominated biphenyls (PBB) in a peanut butter vehicle daily from day 6 of gestation through day 24 postpartum. Offspring of these dams were assessed for response to drug-induced stimulation of spontaneous locomotor activity following a specified accommodation period. Essentially no differences in stimulation of activity were observed between control and treated male offspring following intraperitoneal injection of d-amphetamine, scopolamine or fenfluramine. Female offspring of PBB-treated dams were significantly less active than controls following d-amphetamine injection, particularly during portions of the estrous cycle characterized by high estrogen levels. Female offspring of treated dams were significantly more active than controls following scopolamine injection; activity was specifically enhanced during diestrus. Essentially no differences in stimulation of activity were observed between control and treated female offspring following fenfluramine injection. These results indicate that female rats are more susceptible than males to the behavioral disruption produced by perinatally administered PBB. This behavioral disruption may result in part from deficits in dopaminergic and cholinergic, but not serotonergic, systems. (Supported by NIAMS grant ES02783).
Ethylene glycol monoethyl ether (EGEE) has been shown to produce embryolethality and terata when administered throughout gestation. The purpose of the present study was to determine if EGE administered over limited intervals during organogenesis would produce terata in the absence of either maternal toxicity or fetal mortality. Sperm-positive (day 0) Sprague-Dawley rats were gavaged with either water on days 7-15 (control), or 200 mg/kg EGEE on days 7-9, 10-12, 13-15, or 7-15 of gestation and sacrificed on day 20. The number of live and dead implants were counted. Live fetuses were weighed, measured for crown-rump length, and examined for gross, visceral, and skeletal anomalies. EGEE administration on days 7-15 resulted in a significant decrease in maternal weight gain and an increase in prenatal mortality, with neither of these effects observed with any short-term dosing interval. However, EGEE administered over 3-day intervals did produce a decrease in fetal weight in all treatment groups, with variable effects on fetal length. Short-term EGEE administration produced cardiovascular and skeletal anomalies. The incidence of cardiovascular anomalies in the 7-9, 10-12, 13-15, and 7-15 groups was 5, 11, 1, and 24%, respectively, with none observed in controls. Therefore, this study demonstrated that EGEE can, in the absence of maternal toxicity or fetal mortality, produce fetal weight reduction and malformations when administered over short periods of gestation.

Cassava, which contains the cyanogenic glycoside linamarin, is a staple food for 450-500 million people in 26 tropical countries. Previously, we found that pregnant hamsters given a large oral dose of linamarin on day 8 of gestation produced offspring with skeletal defects and encephalocoeles. In this study, groups of pregnant golden hamsters were fed diets consisting of 50% cassava meal, 20% Purina lab chow throughout gestation. Several different low-cyanide (sweet) and high-cyanide (bitter) varieties of cassava were studied. One group of pregnant animals was fed 100% Purina lab chow. A second control group was fed a special low protein diet closely resembling cassava in nutritional value. All animals were kept in metabolism cages throughout pregnancy. Food consumption was monitored and thiocyanate concentrations in plasma and urine were measured. Respiratory and malformations were not significantly increased in the cassava-fed groups. However, fetuses from cassava-fed dams had significantly lower body weights and showed evidence of retarded skeletal ossification when compared to lab chow controls. These differences are probably associated with the poor nutritional value of the cassava diets rather than any direct embryofetotoxic effect.
Neonatal rats were intra-gastrically given either lead acetate (50 mg/kg) or an equal molar solution of sodium acetate at days 6, 9, 12, 15, and 18 postpartum. At 52 days of age each animal began a 13-day training sequence to develop maze running skills. Animals within each treatment group, i.e., lead exposed (Pb), control vehicle (CV), and control nonhandled (CN) were assigned to either the Latent Learning (LL) or Open-Field (OF) testing group. The former individually explored a maze containing a symmetrical pattern while satiated, the latter were exposed to OF apparatus devoid of barriers. All animals were then food deprived and appetitively tested in the LL maze. Pb, CV, and CN animals naive to the maze did not differ in maze performance. In comparison, both the CV and CN animals that previously experienced the maze committed fewer errors. Pb treated animals within each testing group did not differ and showed no evidence of LL. No main effects of Pb exposure were observed in either OF activity or shock threshold response.

This study explored potential long-term alterations following neonatal triethyl lead (TEL) exposure. Accordingly the offspring of Fischer-344 dams were administered a s.c. injection (20 μl) of either distilled water, 4.5-, or 9.0-mg/kg TEL. The fourth pup of each sex was an undernourished control animal. All animals showed intensive ambulation during an initial activity test (day 90) in a large dark chamber. Repeated activity testing (days 96 & 103) found that control animals habituated to this test environment; however, TEL-treated females remained hyperreactive to these stimuli as indicated by their failure to show between session habituation. Within a small well-lighted chamber, where the predominant response of the rat is freezing, the high dose TEL females displayed a spectral analysis profile (day 92) of decreased body movements, also suggestive of a hyperreactive response to the test environment. Auditory startle evaluations (day 94) demonstrated greatly increased (100%) responsiveness to an 8 KHz tone by TEL animals of both sexes. Thus, TEL-induced alterations were observed in a variety of behavioral tests which, when considered with respect to environmental determinants of the species-specific defensive reactions of the rat, indicate that hyperreactivity represents a long-term consequence of neonatal TEL exposure.

Three rhesus monkeys were exposed to a high pulse of lead acetate soon after birth and low chronic levels for the rest of the first year after birth. At six years of age they showed a significant deficit on delayed spatial alternation (DSA) performance. This deficit persisted over extended training. We have tested the effects of drugs affecting the cholinergic and dopaminergic neurotransmitter systems on this lead-induced disorder. Both of these systems are involved with memory processing and are affected by lead intoxication. Dopaminergic drugs haloperidol, sulpiride and amphetamine have been used, but they did not significantly affect the lead-induced DSA deficit. The cholinergic antagonist scopolamine decreased DSA percent correct performance in the lead-exposed and control groups but did not show any selective effects on the lead treated group. We are presently testing the effects of chronic L-DOPA therapy on these monkeys' performance. Manipulating these systems may add insight into the mechanisms of lead-induced cognitive disorders, provide a means to increase the sensitivity of the behavioral test for lead toxicity and possibly provide leads for therapeutic treatments.

Three day old (d.o.) HA/ICR mice received 10mg S. marcescens LPSW ip and organ weights and Cu concentrations were compared to those found in mice receiving only physiological saline. LPSW did not affect body weight between 2 and 28 d.o. At 7 d.o., treated pups had lower thymus weight (p<0.01) and larger livers (p<0.01) and spleens (p<0.05). Thymic, spleen and brain Cu concentrations were unaffected by LPSW. Liver Cu was decreased (p<0.05). At 14 d.o. only liver weight was increased (p<0.05) in treated mice. No differences existed in tissue Cu concentrations. At 28 d.o., spleen weight was higher (p<0.05) in treated mice. Tissue Cu concentrations were not different in treated and control mice. Early life exposure to LPSW, an endotoxin changed body composition and transiently diminished the liver Cu concentration. Altered Cu metabolism may be involved in LPSW-induced changes in body composition.

(Supported by NIH grant # ES0 1062.)
In rodents, postnatal exposure to Cd can produce brain lesions, alterations in activity, and impaired performance on discrimination tasks. Postnatal day 1 (PN1; birth = PN0) s.c. exposure to CdCl₂ altered the suckling and nest-seeking behavior of Long Evans rat pups (Newland et al., Teratology, 27:65-66A). We now report that PN1 s.c. CdCl₂ injection (0.1,3.6 mg/kg) causes an increased incidence of brain hemorrhage and hydrocephalus, changes in functional tests on PN27, and differences in the acquisition and maintenance of operant behavior in adulthood. Hemorrhage was observed at 3 mg/kg Cd (17/27) and 6 mg/kg Cd (17/11) on PN2 but not later. Hydrocephalus, defined by dome-shaped heads and confirmed by histology, was observed at 3 mg/kg (2/14) and 6 mg/kg (11/24) by PN21. The hydrocephalic rats were abnormal in the PN21 testing of rod-hang, cliff avoidance, landing foot spread and gait. Acquisition of operant responding by non-hydrocephalic rats was altered by Cd: response rate increases were seen at 3 mg/kg, while at 6 mg/kg Cd more training was required and lower response rates were observed. Thus, acute Cd exposure as early as PN1 can induce acute brain hemorrhage, hydrocephalus and long-term behavioral deficits in non-hydrocephalic animals. ( Funded by ES 01248 and ES 07026)

Sodium nitrite administered in the drinking water to Long-Evans rats during pregnancy and lactation severely affected the development and viability of their offspring. Pups were born from late day 0 gestation on 2 gm/l or 3 gm/l NaNO₂ in tap water gained 45% of day 0 weight by day 21 gestation as compared to 55% for controls. Water consumption of treated dams was about 80% of controls. At littering there were no significant differences in litter size, pup birthweight or sex ratio. Thereafter, pups of treated dams gained less weight, progressively became severely anemic and began to die by the third week postpartum. By day 21 postpartum mortality of pups from dams drinking 2 gm/l and 3 gm/l NaNO₂ was 10% and 34% respectively and 2% for controls. Mean body weights of survivors were 75% and 55% of control. Hemoglobin levels and RBC counts were drastically reduced. Gross chylous lipemia of serum and fatty degeneration of the liver were noted. Microscopic evaluation of blood smears and bone marrow aspirates indicated severely decreased erythropoietic activity. Pups born to untreated dams and cross-fostered onto treated dams showed intermediate effects. Pups born to treated dams and cross-fostered onto untreated dams were not significantly affected. (This abstract does not necessarily reflect EPA policy).

Previous research has indicated that AAF requires biotransformation by a P450-dependent monooxygenase system in order to produce neural tube abnormalities in cultured, day 10 rat embryos (Faustman-Watts et al., Teratology 27:19-28, 1983). In the present study, the extent of endogenous embryonic bioactivation of AAF was examined by pretreating pregnant rats with MC or vehicle alone on day 8 of gestation. Transplacentally exposed embryos were explanted into culture media containing AAF and also into control media. A 60% increase in malformations was seen with MC-pretreated embryos as compared to vehicle-exposed embryos. When carbon monoxide was included in the culture system, the incidence of these malformations was dramatically reduced. MC-pretreated embryos cultured in media without AAF did not exhibit these malformations. The presence of carbon monoxide in the culture bottles did not alter normal embryonic development. These interesting findings are consistent with the hypothesis that day 10 rat embryos possess P450-dependent enzyme systems capable of catalyzing the conversion of AAF to dymorphogenic intermediates. Supported by NIH grants DH-04839.

The effect of age, chronic lindane treatment, genotypic and phenotypic differences on metabolism of lindane by female (YS x VY) F1 hybrid mice were investigated after 6, 13, 26, 52 and 82 weeks of treatment. This study is part of a collaborative investigation with NCTR to determine whether enzyme activity and/or metabolite profiles are correlated with the incidence of neoplasms in inbred mice. Twenty-four hours prior to sacrifice both controls and mice fed 160 ppm lindane in their diet were dosed p.o. with 18 mg lindane (containing 60 μCi [14C] lindane/kg). Total excreted radioactivity was significantly reduced in the treated mice compared to their corresponding controls. While contributing to a significant reduction in the excretion of urinary metabolites, increasing age also plays a role in the elevated fecal excretion of radioactivity. The viable yellow (AV/a) and pseudoagouti (AV/a) mice had significantly higher lindane dehydrogenase activity than the black (a/V) mice. Age and treatment induced changes in the excretion of 9 lindane metabolites were observed. Results from this study indicate that chronic lindane treatment exacerbates the elevated tissue storage and impaired excretion of lindane and its metabolites which occur with increasing age in female (YS x VY) F1 hybrid mice.


A single oral dose of 50 mg (6.68 μCi/kg) of [14C]-TOCP was administered in corn oil to the male rat. Following administration, the animals were held in individual metabolism cages which were connected to two traps, one containing Amberlite XAD-4 to collect volatile organic chemicals and the other to trap 14CO2. Three animals were sacrificed at various time points and the tissues, gastrointestinal tract contents, daily excreta and trap contents were analyzed for radioactivity. No radioactivity was detected in the CO2 or Amberlite XAD-4 trap. Approximately 94 and 35% of the radioactivity were excreted in the urine and feces, respectively within five days following administration. The highest concentrations of radioactivity were observed with the routes of excretion, i.e., the gastrointestinal tract parts, its contents and the urinary bladder, particularly at the earlier time points. Among other tissues, liver contained the highest concentration of radioactivity followed by kidney. Plasma and red blood cells also contained high concentrations of radioactivity. Among neural tissues, the cerebral cortex contained the highest concentration of radioactivity at 12, 24, and 48 hrs following application. TOCP and its metabolites were separated and identified by high performance liquid chromatography and liquid scintillation counting. (Supported in part by NIEHS ES02717 and NIOSH OH00823).


The disposal and metabolism of the selective herbicide, RO-NEET, (S-ethyl, N-ethyl, N-cyclohexyl thiocarbamate) in male cynomolgus monkeys following a single dose of 10, 40, or 150 mg/kg [ring 14C] RO-NEET were studied. Excretion of 14C was rapid with 75% of an oral dose (160 mg/kg) being excreted in 24 hrs. Total recovery of 14C at 12 hrs. was approximately 88% (86% urine, 2% feces). RO-NEET was extensively metabolized prior to urinary excretion. In total, 10 of the 25 metabolites isolated from monkey urine were identified (75.3% of total urinary 14C). Urinary metabolites were separated by TLC and identified by MS. Approximately 27.9% of the urinary 14C was identified as N-ethylcyclohexylamine. Other metabolites included 3-(N-ethylamino)cyclohexan-4, 4-(N-ethylaminocyclohexanol (13.1%), and cyclohexylamine (1.5%). RO-NEET was also found to undergo ring hydroxylation followed by glucuronidation. The conjugated ring hydroxylated metabolite of RO-NEET comprised approximately 26.5% of the urinary 14C. No unmetabolized RO-NEET was found in urine of monkeys administered a single 160 mg/kg dose of RO-NEET. A proposed metabolic pathway for the biotransformation of RO-NEET in monkeys is presented.


Male cats were treated dermally with 50 mg (5.8 μCi/kg) of [14C]TOCP applied on a preclipped area of the back of the neck. Three animals were sacrificed at 0.5, 1, 2, 5, and 10 days following application. Earlier, we reported the absorption, metabolism, and excretion of [14C]-TOCP in the male cat. This study is the continuation of this work which describes the differential disposition of TOCP and its metabolites in various tissues and the bile and feces. Tissue extracts were analyzed by HPLC along with authentic standards. TOCP was the most predominant compound in the brain, spinal cord, and sciatic nerve, while the liver, kidneys, and lungs contained mostly metabolites. The major metabolite in the liver, kidneys, and lungs was α-hydroxybenzoic acid followed by di-α-cresyl hydrogen phosphate. The half-life of TOCP in the tissues ranged from 2.2 to 4.8 days with the longest half-life being observed in the sciatic nerve. Bile contained mostly metabolites with α-cresyl dihydrogen phosphate as the major compound, whereas feces contained TOCP as the predominant compound. This observation may suggest that the radioactivity present in the feces could have resulted from additional mechanisms such as filtration through the gastrointestinal tract rather than excretion through the bile alone. (Supported in part by NIEHS ES0 2211 and EPA Grant No. 00-02-3279).
361 IN VITRO DETOXIFICATION OF METHYLPARATHION (MeP) BY HUMAN PLACENTAL AND FETAL LIVER GLUTATHIONE S-TRANSFERASES (GSTs). L.L. Radulovic, J.L. LaFerla, W. C. Emptage, and A. P. Kulkarni. Toxicology Program, Dept. of Environ. and Industrial Hlth., The University of Michigan, Ann Arbor, MI.

The kinetic parameters of human feto-placental GSTS-mediated metabolism of MeP were investigated. The cytosolic GSTS from term placenta obtained from non-smokers was purified to homogeneity by Sepharose 4B-GSH and hydroxyapatite column chromatography. Fetal liver GSTS was partially purified by affinity chromatography. Using I-Chloro-2,4-dinitrobenzene as an alternate substrate, placental and fetal liver GSTS exhibited about 400 and 60 fold purification, respectively. GSTS activity in these enzyme preparations towards MeP was assayed by four different methods: (1) GSH depletion; (2) MeP disappearance by HPLC; (3) dnmethylparathion (DmMeP) formation by HPLC; and (4) radiometrically using 14C-ring labelled MeP. Both enzyme preparations metabolized MeP to a single product, DmMeP, via O-dealkylation. Fetal liver GSTS activity ranged from 30-122 nmoles DmMeP/min/mg, as compared to 0.7-9.2 nmoles DmMeP/min/mg for partially purified pre-term placental GSTS and 2.4-7.5 nmoles for term GSTS. Using HPLC method, the fetal liver exhibited an average Km of 0.49 mM for MeP and a Vmax of 301 nmoles DmMeP/min/mg. With varying concentrations of GSH, a Km of 0.16 mM and a Vmax of 67 nmoles DmMeP/min/mg were obtained.

362 A PHYSIOLOGICAL BASED MODEL FOR PARAQUAT (PQ) PHARMACOKINETICS IN THE RAT. M.S. Dey, W.L. Hayton*, and R.J. Krieger. Veterinary Medicine, University of Idaho, Moscow ID and *College of Pharmacy, Washington State University, Pullman, WA

Kinetic analysis of PQ disposition has been based on classical compartmental models which have no physiologic or anatomic reality. The ability to predict tissue PQ cons after exposure is important to evaluate potential outcome and treatment. A physiological based model of PQ kinetics is described using the NONLIN computer program. The model predicts PQ disposition in 10 tissues: blood, injection site, heart, brain, liver, kidneys, lungs, GI tract, spleen and remaining carcass. Female SD rats (280g) were SC injected with a pneumotoxic, non-nephrotoxic dosage of PQ (72 umoles/kg). Blood (0.2 ml) was serially sampled between 1 min and 7 days. At 6-7 days times and tissues were analyzed for 14C-PQ. The model accurately predicts rapid absorption (ka=3.5x10^-3 min^-1) from the injection site with 99% of the dose absorbed by 4 hours. Peak PQ cons were achieved in the brain, blood, heart, and liver 20 min. after injection while the lungs, kidneys, spleen, and carcass achieved peak levels at 40 min. Peak PQ cons were achieved in the liver with lower cons in the lungs, spleen, blood, heart, liver, carcass, and brain, respectively. The present model accurately predicts individual tissue PQ cons which facilitates study of the effects of chemicals and treatments on PQ disposition. (Supported in part by NIH Biomedical Research Development Grants 1 S08 RR09703-01A2 and 2 S07 RR07170.)


Tridiphane (2-[3,5-dichlorophenyl]-2-(2,2,2-trichloroethyl)oxirane) (TRID) is intended for use in combination with atrazine and/or cyanazine for the control of annual grasses and certain broadleaf weeds in crops such as corn and sorghum. The purpose of this study was to determine the pharmacokinetics and metabolism of 14C-TRID in Sprague-Dawley rats and B6C3F1 mice. Following a single oral dose of 1 or 160 mg/kg to rats and 5 or 150 mg/kg to mice, TRID was almost completely absorbed (99%), extensively metabolized (about 5% or less excreted as parent compound) and subsequently excreted via the feces or urine in both species. The half lives for excretion of C from the body ranged from 11 to 17 hours. The predominant metabolites for both species were identified as 1,1,1-trichloro-3,4-dihydroxy-3-(3,5-dichlorophenyl)butane (DIOL) and the glucuronide conjugate of the DIOL. Disproportionate increases in the areas under the plasma concentration-time curves for rats, and quantitative changes in metabolite profiles in mice were observed at the higher dose levels suggesting that the fate of TRID was dose-dependent in both species.


The in vivo biotransformation of GS to the active cholinesterase (ChE) inhibitor GO was studied to determine the relationship between plasma GO levels and toxicity in the rat. The kinetics of GS in the blood and brain were measured along with GO in the plasma and brain. These results were related to plasma and brain ChE inhibition and signs of toxicity. Lethal and sublethal doses of GS were given i.v. to male rats. Brain GS exceeded blood levels at all times and doses. GS exhibited similar elimination profiles from both compartments at both doses. No brain or plasma ChE inhibition occurred at 1 min after dosing although brain and blood GS were maximal. The rate of onset and degree of toxicity corresponded with the rate of plasma and brain ChE inhibition and GC accumulation. A sublethal dose caused plasma and brain GO to gradually peak at 15 and 20 min, respectively, followed by a decline. Recovery of the rat was associated with a reactivation of plasma ChE, but no brain ChE reactivation occurred. A lethal dose caused a rapid increase in brain and plasma GO. Brain GO peaked at 4 min while plasma GO continued to increase until 10 min. Brain and plasma GO remained near maximum until death. At both doses plasma GO always exceeded brain GO after 1 min. These results indicate that GS was most likely produced by hepatic activation of GS, is responsible for toxicity. (Supported by grant from NIH ES01381)
Carbosulfan (CS), a new N-methyl carbamate insecticide, has lower mammalian toxicity than its parent compound, carbofuran (CF). CF is a primary metabolite of CS and the present study characterizes the relationship between plasma CS and CF concentrations and erythrocyte acetylcholinesterase (AChE) inhibition. Female Sprague-Dawley rats (225-315g) were given intravenous (80-640 µg/kg) or oral (540-2030 µg/kg) dosages of (14C-carbonyl)carbosulfan (0.4 ml/kg propylene glycol). Animals were eviscerated at 4, 30 or 240 min. AChE activity was measured using a radiometric assay and values were compared with pretreatment AChE activity. Ethyl acetate extracts of plasma were subjected to thin layer chromatographic analysis and CS and CF were quantitated. Reduction in AChE activity was better correlated (r=0.97) with plasma CF than in plasma CS (r=0.75). Peak plasma CS and CF were measured at 4 min after i.v. exposure and at 30 min after oral dosing. Carbsulfan is rapidly metabolized to carbofuran. The observed decrease in AChE activity is directly related to plasma carbofuran concentration. (Supported in part by NIH Biomedical Research Development Grant 1 SO8 RR09073-01A2 and 2 SO7 RR07170).

**Pathway of Carbamate Ester Hydrolysis in Rats.**

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Ester hydrolysis of insecticide carbamates is a major route of metabolism. However, it is unclear whether there is direct hydrolysis of the carbamate [ROOC(NHCH₃)] or of oxidative metabolites formed by cytochrome P450 enzymes. The present study, propoxur [2-isopropoxyphenyl-0-C (O)NHCH₃] was given orally to rats whose P450 enzymes were induced with phenobarbital (PB), or inhibited with piperonyl butoxide (PB), and the hydrolysis of the carbamate to expired 14CO₂ compared to control animals. If hydrolysis did proceed through an oxidative intermediate, then, it was expected that hydrolysis would be increased by PB and decreased by PB. Such was not the case; hydrolysis rates were similar for all animals, with about 30% of the dose hydrolyzed in 12 hr. Urinary radiocarbon, which consists of 14C-carbonyl-labeled oxidative metabolites, was increased 20% by PB and reduced 52% by PB. Hepatic microsomal preparations, PB-induced, which oxidized 50% heptachlor to its epoxide (oxidase activity) and hydrolyzed 25% malathion to its monoacid (carboxylesterase activity) hydrolyzed less than 5% propoxur. The data suggest that carbamate ester hydrolysis is not dependent upon prior metabolism of the compound by the cytochrome P450 enzymes and, thus, indicate that direct enzymatic hydrolysis, perhaps extrahapatic, is the predominant pathway. (Supported by EPA Grant No. R805143).
DOSE-DEPENDENT PHARMACOKINETICS OF THE HERBICIDE BUTHIDAZOLE IN RATS AND MICE. Y.H. Atallah, and C.C. Yu, Research Department, Velcich Chemical Corporation, Chicago, IL 60611

The pharmacokinetics and metabolism of a new herbicide (buthidazole) is reported in correlation to the toxicity of the compound. The fate of the compound in rats and mice follows a one compartment open model system. However, the kinetics is markedly dose-dependent because of limited capacity for metabolism and elimination. Multiple dosing at high levels showed that buthidazole induced its own metabolism and excretion. The dose-dependent fate and the induction of metabolism and excretion by multiple dosing of buthidazole precludes evaluation of buthidazole toxicity at low dose levels by extrapolation from high dose levels (>1000 ppm in diet). The correlation of dose-dependent metabolism and pharmacokinetics and toxicity of buthidazole supports the conclusion that the maximum tolerated dose for toxic effects that coincide with several rate limited processes in rats and mice is between 100 and 1000 ppm dietary concentration.

THE RELATIVE TOXICITY OF A METABOLIZED VERSUS NON-METABOLIZED PCB CONGENERS, C.D. Milne and S.D. Aust, Department of Biochemistry & Center for Environmental Toxicology, Michigan State University, East Lansing, MI 48824

3,4,3',4'-Tetrabromobiphenyl (TBB) and 3,4,5,3',4',5'-hexabromobiphenyl (HBB) both produce a toxicity similar to that produced by TCDD. However, the tetrabromobiphenyl congener induces its own metabolism whereas the hexabromobiphenyl is not metabolized. The time course of induction of liver microsomal aryl hydrocarbon hydroxylase (AHH) activity was determined in immature rats administered a single dose (21.3 μmol/kg) of either TBB or HBB. Microsomal AHH activity was maximal at day 2 and then steadily decreased in TBB treated rats, whereas it remained induced in rats administered HBB. The relative affinities for TBB binding to the cytosolic TCDD receptor were found to be ten times that for HBB. In a following experiment, the total number of cytosolic and nuclear binding sites were determined over time in livers from rats administered a single dose (21.3 μmoles/kg) of either HBB or TBB. The level of the receptor in the cytosol returned to near starting level in rats administrated TBB. Metabolism of the TBB congeners results in decreased AHH activity and the reappearance of the TCDD receptor in the cytoplasm. Toxicity is decreased without sustained occupation of the receptor by TBB. AHH induction or receptor affinity assays can therefore overestimate toxicity of polyhalogenated aromatic hydrocarbons if the chemicals can be metabolized at a significant rate. Supported by NIH Grant ES02786.

PCBs AND PBEs: BIOLOGIC AND TOXIC EFFECTS ON C57BL/6J AND DBA/2J MICE: L.W. Robertson, A. Parkinson, S. Bandiera, I. Lambert, J. Merrill and S. Safe, Dept. of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843

Pretreatment of genetically inbred responsive C57BL/6J and non-responsive DBA/2J mice with several coplanar and monochlorinated biphenyls (PCBs) and polychlorinated biphenyls (PCBs) clearly illustrated the differential responses of these animals to toxic halogenated aromatics. All the compounds tested induced hepatic microsomal benz(a)pyrene hydroxylase in the responsive C57BL/6J mice, however, most of the halogenated biphenyls were inactive in the DBA/2J mice. Comparable results were observed for halogenated biphenyl-mediated thymic atrophy in the two strains of mice. Two compounds, 3,3',4,4'-tetrabromobiphenyl and 3,3',4,4',5'-pentachlorobiphenyl, induced benz(a)pyrene hydroxylase and caused thymic atrophy in both strains of mice at dose levels of 750 and 1500 μmol/kg respectively. However, it was apparent that the non-responsive DBA/2J were much less susceptible to the toxic effects of the two halogenated biphenyls. These results were similar to those observed for 2,3,7,8-tetracloro dibenzo-p-dioxin and support the proposed receptor-mediated mechanism of action for the toxic halogenated aromatics. (Supported by N.I.H. Grant No. ES02786.)

REACTIVE METABOLISM AND HEPATOTOXICITY OF HALOTHANE IN AROCLOR 1254 PRETREATED RATS. S. J. Waters, A.J. Gandolfi and J.G. Signes, Dept of Anesthesiology, University of Arizona, Tucson, AZ

Typical models of halothane (1,1,1-trifluorobromo- chloroethane) hepatotoxicity employ phenobarbital induction and hypoxic exposures to promote reductive metabolism. Reactive intermediates formed by the reductive pathway are thought to initiate the events that are ultimately expressed as hepatic necrosis. We examined halothane induced following Aroclor 1254 induction and hypoxic exposures. Male Sprague-Dawley rats (200-300g) were induced with Aroclor 1254 (PCB) by daily doses of 50 or 100 mg/kg for 4 days or a single oral dose of 500 mg/kg. These protocols produced cytochrome P-450 levels of 1.94±0.9, 2.06±0.05 and 2.6±0.02 mmole/mg protein, respectively. Induced animals were exposed to 1% halothane, 45% O2 for 2 hr. Elevated S/GTT levels determined 24 hr post-exposure were positively correlated to extent of induction, with values of 51±9, 87±4 and 139±8, respectively. Cytolobular necrosis was observed with light microscopic examination. Serum F- levels, indicative of reductive metabolism of halothane, determined immediately post-exposure ranged from 4-14 μM (similar to serum F- levels from phenobarbital induced halothane hypoxia exposure, i.e. 9-21 μM) with higher values correlated with increased induction. These data show PCB induction allows the reductive metabolism of halothane even under hypoxic conditions and may suggest a unique induction of microsomal drug metabolizing enzymes. (NIH AM16715).
374 METABOLISM OF PBB CONGENERS BY RECONSTITUTED MONOXGENASE SYSTEMS, R. Voormam, R.A. Mills, J.A. Bumpus, L.A. Morehouse, and S.D. Aust, Department of Biochemistry and Center for Environmental Toxicology, Michigan State University, East Lansing, MI 48824

The planar congeners 3,4,5,3',4',5'-hexabromobiphenyl (345-HBB) and 3,4,3',4'-tetrabromobiphenyl (34-TBB) both induce cytochromes P450 NIF-B and P450 NIF/SF-G. In earlier metabolism studies we showed that 345-HBB was not metabolized while 34-TBB could be metabolized in rats. The in vitro metabolism of 34-TBB by liver microsomes from 34-TBB treated rats was correlated with time-dependent changes in the microsomal content of the isozymes P450 NIF-B and P450 NIF/SF-G. The metabolism of several pure PBB congeners including 2,3,3',4',4'-tetrabromobiphenyl (2334-TBB) and 34-TBB was studied using reconstituted monooxygenase systems. The isozymes P450 NIF-B, P450 NIF/SF-G, and P450 NIF/B were purified from rats treated with phenobarbital, 345-HBB and used to reconstituted monooxygenase activity. 2334-TBB and 34-TBB can be metabolized by both P450 NIF-B and P450 NIF/SF-G. 2334-TBB was also metabolized by P450 NIF-B whereas 34-TBB was not. This supports our earlier conclusions that congeners metabolized by 3-methylcholanthrene-induced microsomes must have adjacent non-halogenated ortho and meta carbons on at least one biphenyl ring whereas congeners metabolized by phenobarbital-induced microsomes must have adjacent non-halogenated para and meta carbons. 2334-TBB satisfies the requirements for metabolism by both types of cytochromes. Supported by NIH Grant ES02786.

375 POLYCHLORINATED DIBENZOFURANS: SYNTHESIS AND QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS: S. Bandiera, T. Sawyer, M. Romkes, G. Mason, L. Safe and S. Safe, Dept. of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX, 77843.

The development of two new synthetic reaction sequences has resulted in the synthesis and characterization of twenty-five pure polychlorinated dibenzofuran (PCDF) isomers and congeners. Quantitative structure-activity relationships (QSAR) within this series of compounds were determined by comparing their aryl hydrocarbon hydroxylase (AHH) induction potencies (EC50) and cytosolic receptor protein binding affinities (ED50). The most active compound was 2,3,4,7,8-pentachlorodibenzoferan followed by 2,3,7,8-tetrachlorodibenzofuran. The most active PCDF congeners were all substituted at the lateral 2,3,7,8-positions and most of these compounds have previously been shown to be highly toxic to rodents. The QSAR studies also confirmed an excellent correlation between AHH induction potencies and receptor binding affinities and provides further support for the receptor-mediated mechanism of action for the PCDFs. (Supported by N.I.H., Grant No. ES02937.)

376 APPLICATION OF BIOASSAYS FOR THE ANALYSIS OF TOXIC HALOGENATED AROMATICS: M.A. Denomme, S. Bandiera, T. Sawyer, G. Mason, E. Keys and S. Safe, Dept. of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX, 77843, Dept. of Chemistry, University of Guelph, Guelph, Ontario, Canada MIGZI

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and related toxic halogenated aromatics bind to a hepatic cytosolic receptor protein and are potent inducers of aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin 0-deethylase in rat hepatoma H-4-II-E cells in culture. Both of these in vitro assays have been used to estimate the 2,3,7,8-TCDD equivalents in the following mixtures: 1) the polychlorinated dibenzofuran (PCDF) and dibeno-p-dioxin (PCDD) fractions from incinerator fly ash; 2) PCDFs and PCDD extracts from commercial chlorinated phenols; 3) commercial and reconstituted polychlorinated biphenyl (PCB) mixtures; 4) mixtures containing 3,3',4',4'-tetrachlorobenzene and 5) reconstituted PCDD and PCDF mixtures. The AHH/EROD induction assays routinely gave lower 2,3,7,8-TCDD equivalents in most samples compared to the receptor assay. However, reconstituted studies suggest that for PCDFs and PCDDs the effects are additive. The utility of these methods as rapid bioassay screening methods will be discussed. (Supported by N.I.H. Grant No. ES02937.)
377 EVIDENCE FOR AN ADDITIONAL PATHWAY OF POLYCHLORINATED BIPHENYLS (PCBs) METABOLISM IN DOG LIVER MICROSONES. R.G. Schnellmann and L.G. Sipes, Dept. Pharmacol. & Toxicol., College of Pharmacy, University of Arizona, Tucson, AZ 85721.

PCBs with adjacent unsubstituted meta- and para-positions are readily metabolized through an arone oxide. However, the rate of elimination of hexachlorobiphenyls (HCB) in the dog increased with the number of unsubstituted meta-positions. These results suggested the dog metabolized these compounds differently than other species. This study compared the metabolism of 2,2',3,3',4,4'-HCB (236-HCB), 4,4'-dichlorobiphenyl (4-DCB) and 2,2',4,4',5,5'-HCB (245-HCB) by human and dog liver microsomes. Each 14C-PCB was incubated with NADPH and microsomal protein. Metabolite formation and covalent binding of PCB-equivalents to microsomal protein were determined by solvent extraction. Metabolites were identified by HPLC. The Vmax of 236-HCB vs 4-DCB in the human was 51 vs 1.2 pmoles/mg/min and 13 vs 73 in the dog. Only the dog metabolized 245-HCB. More HCB was covalently bound to microsomal protein than 4-DCB in both the dog (48 vs 31 pmoles/mg/20 min) and human (19 vs 3 pmoles/mg/20 min). The ratio of 4,4'-dichloro-3-biphenylol to 3,4'-dichloro-4-biphenylol was greater in the dog 5:1 vs 3:1. Since the in vitro metabolism of these compounds are similar in the human, monkey, and rat, these results suggest that the dog has an additional pathway of PCB metabolism either not found in other species or found only to a limited extent. (Supported by ES-07091.)


The preferential association of PCB congeners with liver proteins is a result of PCBs binding to cytosolic macromolecular components. The cellular uptake, partitioning and binding of 14C-4-chlorobiphenyl (4-DCB), 2,2',3,3',6,6'-hexachlorobiphenyl (236-HCB) and 2,2',4,4',5,5'-hexachlorobiphenyl (245-HCB) was examined in isolated hepatocyte suspensions derived from male SD rats (200 g). Congeners (10 μM) complexed with albumin were incubated in the presence of both hepatocytes (46 x 10^6 cells) and the lipid extracted from 4 x 10^6 cells at 0°C until equilibrium, 4 h. The partitioning of the congeners between the lipid (2-3 nmols) and aqueous (0.23 nmols) compartments was less than total cellular uptake; 2.2 nmols for 4-DCB and 236-HCB and 8.4 nmols for 245-HCB; suggesting a substantial binding of congener to cellular proteins. Cytosol prepared from PCB-hepatocyte incubations at 37°C was analyzed by HPLC using a Spherogel TSK SW 3000 column. Distinct radioactive, UV-absorbing peaks resulted: 2-3 peaks, 4-DCB; 2 peaks, 236-HCB and 2 peaks, 245-HCB representing 55, 49, and 43% of the radioactivity, respectively. The early eluting peaks represented binding of congener to high mw macromolecules and possibly albumin (mw 300,000-69,000). Inhibition of PCB metabolism by cell disruption resulted in the loss of several late eluting peaks for 4-DCB and 236-HCB. Binding of congener and metabolites was by hydrophobic association.


Oral hexadecane (Hex) was shown to decrease the body burden of lipophobic compounds in several species. The Hex-effect appeared to be species dependent. Final conclusions were pending because of varying experimental conditions. In this study the effect of Hex (5% w/w) on the disposition of 14C-HCB (1.5 mg/kg) was examined under identical conditions in rats and rabbits. Urine and feces were collected daily. Blood was obtained periodically. 55 days after dosing, the animals were sacrificed and HCB was determined in serum, erythrocytes, heart, muscle, lungs, liver, kidney, spleen, fat, skin, and brain as well as in excreta. The half-life of HCB calculated from daily fecal excretion was similar in the two species examined (rat: 24 d; rabbit: 32 d). Hex treatment increased the daily fecal excretion about 3- to 4-fold, resulting in correspondingly reduced half-lives of HCB (rat: 8 d, rabbit: 12 d). 8 weeks of Hex treatment reduced HCB concentration in fat (major tissue storage) of rabbits about 5-fold and of rats about 13-fold compared to controls. In general, fat concentration paralleled other tissue concentrations and blood in treated as well as untreated animals. Data indicate no major difference between herbivores (rabbit) and omnivores (rat) concerning hexadecane facilitated decontamination.


The effect of 4 x 400 mg/kg (i.p. on 4 consecutive days) trans-stilbene oxide (TSO) was investigated on the clearance of intravenously administered 14C-heptachlor (2 mg/kg) in male Sprague Dawley rats. TSO or control treatment was started 4 days before (group I), immediately following (group II), and 4 days after (group III) heptachlor dosage. Rats were sacrificed 6 days after the last TSO or vehicle dose. Radioactivity in excreta was determined daily, in serum and tissues at the time of sacrifice. TSO increased fecal excretion of 14C-activity by 73, 48 and 21% in group I, II and III, respectively. Tissue radioactivity was lowered several fold in fat, kidney, brain, skin, heart, and muscle, not in serum, erythrocytes, liver and spleen. The table illustrates the effect on selected tissues of group I. Total body burden calculated from the sum of tissue radioactivity was reduced about 4-fold. It is assumed that TSO exerts its effect by phase II enzyme induction.

<table>
<thead>
<tr>
<th>Control</th>
<th>TSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>227 ± 29 a, 65 ± 9 b</td>
</tr>
<tr>
<td>Brain</td>
<td>8.8 ± 2</td>
</tr>
<tr>
<td>Kidney</td>
<td>266 ± 15</td>
</tr>
<tr>
<td>Serum</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>Liver</td>
<td>34 ± 6</td>
</tr>
</tbody>
</table>

a, mean ± S.E. (x 10^3 dpm); b, c, p < 0.01
ENHANCED ELIMINATION OF HEXACHLOROBENZENE AND PENTACHLOROPHENOL FROM CHICKENS BY FEEDING MINERAL OIL, CHELSTIPOL, OR LESS DIET. D. Polin, E. Lehning, B. Olson, and S. Burslan. Department of Animal Science, Michigan State University, East Lansing, MI.

Egg-type (White Leghorn) chickens at 21 days of age were fed diets with 10 ppm of hexachlorobenzene (HCB) or pentachlorophenol (PCP) for 14 days. Then cholestipol (CO) which is a bile acid binding resin, or mineral oil (MO), each at 5% of the diet, were fed during a 21-day withdrawal period. Some chickens were restricted in feed intake to 50% of ad lib (50AL) or treated with 50AL + CO or 50AL + MO. CO, MO, or 50AL reduced body burdens of HCB to 56, 58, and 59%, respectively, of chickens not treated during 21 days of withdrawal. Those given 50AL + CO or 50AL + MO had body burdens of HCB only 30% of non-treated birds formerly fed HCB and allowed 21 days for withdrawal. These latter values were 19% of body burdens detected on day zero (means + S.D., 573 ± 56). Body burdens of PCP were 362 µg at day zero, and 255 at day 21 of withdrawal. The birds on 50AL had 90 µg of PCP. Those treated with MO, CO, or 50AL + MO or 50AL + CO had no detectable PCP (< 1.0 ppb) by day 21 after withdrawal.


This study has examined the in vitro metabolism of MEHP in cultured rat hepatocytes, isolated and cultured by standard techniques for 4 days. The culture medium was replenished daily and 50 or 500µM MEHP added. Spent medium was analysed for MEHP metabolites by capillary column gas chromatography. At 50µM only minor time-dependent changes occurred in the profile of MEHP metabolism. However, at 500µM a time-dependent increase in total MEHP metabolism was seen. The production of mono-(3-carboxy-2-ethylpropyl)phthalate (I) increased by up to 4.5-fold during the experiment. Concomitantly, the formation of mono-(2-ethyl-5-hydroxyhexyl) phthalate (IX) and mono-(2-ethyl-5-oxohexyl) phthalate (VII) decreased by about 3-fold. Increases in peroxisomal CN⁻ insensitive palmitoyl CoA oxidation paralleled the increase in formation of I. This suggests that I is formed via peroxisomal β-oxidation of another MEHP metabolite; probably mono-(3-carboxy-2-ethylpentyl)phthalate (V). These data are similar to those obtained in vivo and indicate that hepatocyte cultures are suitable as in vitro models for the study of phthalate ester metabolism.


This study has examined the in vitro metabolism of MEHP and DEHP in male Alderley Park (Wistar) rats. DEHP and MEHP (both at 50 and 500 mg/kg) were administered by gavage for three consecutive days and urinary metabolites quantified by capillary column gas chromatography. At the low dose levels only minor changes were seen in urinary metabolite profiles over the 3 day period. However at the higher doses of DEHP or MEHP up to 6-fold increases in the quantity of mono-(3-carboxy-2-ethylpropyl) phthalate (I) were observed over the 3 day period. Increases in the amounts of mono-(5-carboxy-2-ethylpentyl)phthalate (V) were also seen. Small decreases in mono-(2-ethyl-5-hydroxyhexyl)phthalate (IX) and mono-(2-ethyl-5-oxohexyl)phthalate (VII) were apparent. Dose-dependent increases in hepatic peroxisomal CN⁻ insensitive palmitoyl CoA oxidation appeared to parallel the increases in metabolites I and V. These data indicate that DEHP and MEHP are: 1) metabolised via peroxisomal β-oxidation, and 2) they induce their own metabolism in rats, presumably by inducing α,ω-oxidase (P-450?) and peroxisomal β-oxidation.

REGULATION OF BENZO(a)PYRENE (BP) METABOLISM IN LUNG BY CYCLIC AMP. V.H. Schaeffer and R.J. Rubin. (Dept. Env. Hlth. Sci., Johns Hopkins Univ., Baltimore, MD 21205)

Male Sprague-Dawley rats (175-250g) were given dibutyryl cAMP (DCAMP) i.p. Lungs were removed at periods up to 48 hr. cAMP levels were maximally elevated (38E) by 1-2 hr. and at control levels by 4 hr. Lung slices were incubated for 90 min. with 4µM 3H-BP. Incubation media was extracted with ethyl acetate-acetone and 3H was assayed in the aqueous phase. A maximum increase (66%) in aqueous metabolites (AM) was found at 12 hrs. with DCAMP ≥ 50 mg/kg. Concomitantly, a 30% decrease in DNA binding by BP metabolites was seen. A phosphodiesterase inhibitor, theophylline (80 mg/kg) and adenylate cyclase activator, forskolin (0.75 mg/kg), increased AM by 54% and 80% respectively. The effect of DCAMP on induction of lung BP metabolism by 3-methylcholanthrene (NC) as measured by rate of formation of AM was also investigated. DCAMP (50 mg/kg) was given 45 min. prior to an i.p. injection of NC, followed by incubation of lung slices 24 hr. later. DCAMP had no effect on an optimal dose of MC (25 mg/kg), however it enhanced induction by a suboptimal dose of MC (0.1 mg/kg) by 21%. Experiments with isolated perfused lungs 20 hr. after in vivo administration of DCAMP confirmed the elevated rate of metabolism of BP to AM as well as to organic soluble metabolites. These results indicate that cAMP might serve to regulate the metabolic activation and inactivation of BP. (Supported by ES 07067 and 08454).
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METABOLIC AND MUTAGENIC ACTIVATION OF ACETYL-
AMINOFLUORENE (AAF) AND AMINOFUORENE (AF) BY TWO
3-METHYLCYLANTHRENE (MC) INDUCIBLE FORMS OF CYTO-
CHROME P-450 IN RAT LIVER. J. A. Goldstein,
I. G. C. Robertson, and D. W. Sundheimer. NIEHS,
Research Triangle Park, NC 27709

This study examines the metabolism of AAF by two
major MC inducible forms of cytochrome P-450,
P-448-52 and P-448-55 (P-450c). In a purified
reconstituted system, P-448-55 metabolized AAF at
a 10-fold greater rate than P-448-52 (Vmax for
total metabolites: 5 versus 5 nmol/min/mmol
P450). The primary metabolites for P-448-55 were
3-OH-AAF (30%), 5-OH(22%), 7-OH(22%), 9-OH(10%),
N-OH(3-4%). Although the turnover number of
P-448-52 for AAF was lower than that of P-448-55,
a greater proportion of N-OH-AAF was formed:
7-OH(30%), 5-OH(25%), 9-OH(12%), 3-OH(7%), N-OH
(15%). Antibody to P-448-55 inhibited total AAF
metabolism 43% in MC-induced microsomes and 30%
in 3,3',4,4',5'-hexachlorobiphenyl (HCBB)
-induced microsomes. In contrast, anti-P-448-52
inhibited metabolism 22 and 38% in these micro-
somes. However, anti-P-448-52 inhibited N-
hydroxylation by 60% in both MC- and HCBB-induced
microsomes while anti-P-448-55 (anti-P450c) did
not inhibit N-hydroxylation. N-Hydroxylation is
an obligatory step in the activation of aromatic
amines such as AAF and AF to mutagens and carcino-
gens. In a reconstituted system, P-448-52 was
also more active than P-448-55 or P-450-PB (the
phenobarbital-induced form) in activating AF to
mutagenic products using Salmonella typhimurium
TA98.

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2,6-DICHLORO-p-PHENYLENEDIAMINE: COMPARATIVE
DISPOSITION IN MALE AND FEMALE RATS AND MICE.
Y. M. Ioannou and B. B. Matthews. NIEHS,
Research Triangle Park, NC 27709

2,6-Dichloro-p-phenylene diamine (DPA) was recent-
ly reported to induce hepatocellular adenomas and
carcinoma in male and female B6C3F1 mice, but not in
F344 rats. The present investigation of comparative
disposition in both sexes of each species was designed to
detect species related variations in DPA disposition which
might explain variations in toxicity. In both species the
highest concentrations of DPA-derived radio-
activity were present in muscle followed by skin,
adipose and liver, respectively. Tissues of male
rats and mice contained similar concentrations of
radioactivity whereas tissues of female rats
contained significantly less and those of female
mice contained significantly more. Clearance by
each species was primarily in urine (60-70%) and
secondarily in feces (30-35%) primarily in the
form of metabolites though some parent compound
was excreted by mice. Metabolism was qualita-
tively similar but varied quantitatively with
species and with sex in mice. Rats excreted 3
major and 8 minor metabolites in urine while mice
excreted 1 major and 9 minor metabolites. Efforts to
detect covalent binding of DPA and/or metabo-
lites with hepatic DNA indicated no detectable
binding in either species. The present study
indicates that quantitative variations in dis-
position and metabolism exist between the two
species.

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STRAIN, SEX AND AGE DEPENDENT VARIATION IN THE
METABOLISM OF 2-ACETYLAMINOFLUORENE (2-AAF) IN
CULTURED RAT HEPATOCYTES. C.A. McQueen, M.J.
Miller, and C.H. Williams. American Health
Foundation, Valhalla, NY.

In vivo metabolism of xenobiotics are determined by
the strain, sex and age of the animal. To
investigate the influence of these variables, the
metabolism of a model aromatic amine, 2-AAF, was
studied in liver cultures. Hepatocytes were
isolated from adult male and female F344, adult
male Sprague Dawley (SD), and young and adult
male Long Evans (LE) rats. Monolayer cultures
were incubated with [14C]2-AAF for 20 hrs.
Metabolites were determined by reverse-phase
HPLC. In all cases, the deacetylated metabolite,
2-acetylfluorene, and ring and N-hydroxylated
metabolites of 2-AAF were observed and a linear
rate of disappearance of 2-AAF from the medium
was seen. The half-life (t-1/2) of 10^7 M 2-AAF
in the medium was lower for female F344 rats
(11.6 hr) than for male F344 (24.8 hr). No other
significant differences were seen in the t-1/2
for 2-AAF. Sex dependent differences were also
observed for the conjugated metabolites. The
ratios of sulfates (S) to glucuronides (G) was at
least 5:1 for male F344 rat hepatocytes but less
than 1 for cells from female F344. At 20 hours,
the S:G ratio for the male rat hepatocytes was
F344:adult LE>young LE>SD. These results show
the importance of age, sex and strain on meta-
bolic rate and conjugation. Additionally, the
usefulness of hepatocytes as an in vitro model of
metabolism is further demonstrated.

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POTENTIATION OF HEMATIN-MEDIATED INCREASES IN
PULMONARY MONOOXYGENASE ACTIVITY BY CYTOSOLIC
PROTEIN P.A. Dean, M. J. Nankung and M.R. Juchau,
Department of Pharmacology, School of Medicine,
University of Washington, Seattle, WA 98195.

Micromolar additions of hemat in reaction
mixtures containing 15-day pregnant rat pulmonary
S-9 fraction produced dramatic increases in aryl
A final hemat concentration of 18 µM produced a
15.4 ± 1.2 (mean ± S.O.) fold increase in specific
activity (picomoles product/mg protein/2 hr) with
benz[a]pyrene as substrate. The major metabolite
was 9-hydroxy-benz[a]pyrene. Time course
kinetics indicated initial product activation with
product inhibition at longer incubation times.
Superoxide dismutase, catalase, and peroxidas
did not significantly alter the hematin effect.
Washed microsomes exhibited lower increases in
monooxygenase activity after hematin addition
(6.6 ± 0.8 fold), but much greater increases were
observed (22.8 ± 3.8 fold) when small quantities of
the cytosolic fraction (104,000 g supernatant)
were added. The cytosolic component involved was
inactivated by heating (100°, 10 min) and protease
but was retained after dialysis. Albumin
exhibited no effect on microsomal hematin
activation. We conclude that a cytosolic protein
component is involved in hemat-in mediated
increases in pulmonary P-450-dependent
monooxygenase activity. Supported by N I H
Grants OH1231 and HL04839.

1-Nitropyrene (1-NP) originating from such sources as diesel exhaust emissions and coal combustion fly ash has been detected in the environment. The purpose of this study was to investigate the mutagenic potential of 1-NP in Salmonella typhimurium using rat liver, lung, and nasal tissue as the enzyme activating systems and to measure the rates of 1-NP metabolism in these same tissues. In both strains, TA-98 and TA-100, about 1.0 mg/ml liver and nasal tissue 5-9 was the optimal concentration that resulted in the largest mutagenic response to 1-NP (300 to 700 revertants/µg), whereas 2.0 mg/ml of lung S-9 was necessary to yield optimal responses (200 to 600 revertants/µg). When 1-NP was incubated with liver, lung or nasal tissue S-9 and strain TA-98NR, mutagenic responses were significantly decreased, compared to the response seen in TA-98. 1-NP was metabolized to several oxidized metabolites in all tissues examined. Total rates of formation of 1-NP metabolites for nasal, liver and lung tissue S-9 were 650, 390, and 60 pmol/mg protein/min, respectively, which represented 35%, 10% and 4% of the total amount of NP metabolized. These results suggest that the respiratory tract may be an important site for in vivo bioactivation of inhaled 1-NP. (Research was supported under the U.S. Department of Energy Contract No. DE-AC04-76EV01013.)
INHIBITION OF UDPGA GLUCURONYL TRANSFERASE (UDPGT) by BENZODIAZEPINES (BZD): SPECIES AND ISOMYO DIFERENCES.


(Sponsor R. M. McCann)

Previous studies have shown that the BZD can inhibit UDPGT. The inhibitory potential of diazepam (DZ), N-desmethyl-DZ (NDZ), and oxazepam (OXP) were investigated, in vitro with p-hydroxybiphenyl (PhBP), and p-nitrophenol (PNP) as aglycones. Hepatic microsomes from dogs, rats, and squirrel monkeys were incubated with BDZP in TrisCl buffer with 0.05% Brij 58 or 0.025% Triton X-100, and UDPGA (10M). A DZ concentration of 250M reduced the Vmax of dog and rat PNP-UDP GT by 33% and 12%, but 10M produced a similar reduction in Vmax with the BHPB isozyme from dog. Inhibition by BDZ also increased Km with both isozymes. The inhibitory effects of DZ and NDZ were greater than OXP. Dixon plots of the inhibition of PhBP-UDP GT yielded KI's for DZ, NDZ, and OXP, in the dog of 24, 22, and 47M, respectively. The K's for the monkey were 23 and 51M for DZ and NDZ, but 62 and 124M in the rat. Generally, the sensitivity of the species to the inhibition was dog > monkey > rat. Del Villar et al (Res Comm Chem Path Pharm 33, 433) reported that DZ was an inhibitor of morphine UDP GT. The present studies demonstrate that the inhibition of UDPGT by BDZ is the mixed type. The PNP-UDP GT was less sensitive BDZ than the PhBP (morphine) isozyme. The inhibition produced by NDZ indicates the N-methyl group is not required for the inhibition as previously proposed.

CHANGES IN GLUCURONIDATION AND DEGLUCURONIDATION

IN HEPATIC AND EXTRAHEPATIC TISSUES OF AGING RATS. S. J. Borghoff, E. S. Birnbaun. NIH, Research Triangle Park, North Carolina 27709

Uridine diphosphoglucuronol transferase (UDPGT) and α-glucuronidase (BG) were assayed in hepatic and extrahepatic tissues of 2 to 30 month old Fischer rats in order to evaluate changes in glucuronidation and degradomeratation with age. Tissue extracts were prepared from liver, lung, small intestine and kidney of male rats 2, 3, 6, 12, 18, 24 and 30 months of age. UDPGT activity was determined colorimetrically, using p-nitrophenol (PNP) and phenolphthalein (PT) as substrates, while p-nitrophenyl-α-D-glucuronide was used to measure BG activity. No age-related changes in the activity of either of these enzymes was detected in lung or small intestine. In liver, UDPGT-PNP activity exhibited a maturational decrease up to 15 weeks and then remained constant. UDPGT-PT, which was only detected in liver microsomes, increased after 72 weeks. Liver BG activity showed an increasing trend after 24 weeks in the S9 fraction. Kidney UDPGT-PNP activity decreased gradually with age, while BG increased. The data implicates that any compound normally glucuronidated in kidney would more likely be hydrolyzed in older animals or not conjugated at all while age-related changes in liver, UDPGT activity varies with the substrate. Thus, the changes seen in glucuronidation may not only depend on age or tissue, but also on various compounds.

BUTYLATED HYDROXYANISOLE INCREASES GLUCURONOSYL-

TRANSFERENCE ACTIVITY AND UDP-GLUCURONIC ACID

CONCENTRATION IN MOUSE SMALL INTESTINE. J.J. Hjelle, G.A. Hazleton and C.D. Klaassen, Dept. of Pharmacol., Toxicol. & Therap., Univ. of Kansas Medical Center, Kansas City, KS.

Buthylated hydroxyanisole (BHA) protects against the toxicity and carcinogenicity of various chemicals. In addition to its effects on glutathione conjugation, BHA also increases hepatic glucuronidation capacity. The present study was performed to determine if ingestion of BHA increases UDP-glucuronic acid concentration and/or glucuronosyltransferase activities in the small intestine. Female Swiss Webster mice received BHA in the diet (1% w/w) for 10 days (600-800 mg/kg/day). The proximal portion of the small intestine (approximately 12 cm) beyond the pylorus was used for enzymic and nucleotide analyses. BHA ingestion (1) increased the intestinal concentration of UDP-glucose (97% of control values), (2) enhanced UDP-glucose dehydrogenase specific activity to 234% of control, and (3) elevated intestinal UDP-glucuronic acid concentration (172% of control). Further, BHA ingestion produced approximately a two-fold increase in intestinal microsomal acetylcholinesterase activity in vitro but did not alter the rate of diethylstilbestrol conjugation. These findings show that oral BHA administration increases glucuronosyltransferase activity and elevates UDP-glucuronic acid concentration in small intestine of mice. (Supported by USPHS Grants ES 07079 and ES 03192).
PAPS plays a critical role in vivo as the sulfate donor for sulfation reactions. Information regarding hepatic PAPS levels is limited due to lack of a rapid and sensitive assay. Thus, a radiometric method was developed that measures the formation of $^{14}C$-1-naphthyl sulfate from 1$^4$C-1-naphthol and PAPS (limiting substrate) via a sulfotransferase catalyzed reaction. Conjugated and unconjugated $^{14}C$-1-naphthol were separated by a single chloroform extraction. The method was validated with regard to reaction components, time, tissue preparation technique and amount of tissue. Further, when PAPS was added to rat and mouse liver (30-120 nmol/g liver) prior to tissue preparation, recoveries of 87-101% were obtained. The assay can detect 150 pmoles of PAPS and consequently the sensitivity to measure PAPS in mg quantities of liver was obtained. Chromatographic separation (TLC) of reaction products showed that over 96% of the radioactivity in the aqueous phase was 1-naphthyl sulfate when corrected for blanks. PAPS levels in rat and mouse liver were determined to be 59 ± 5 and 20 ± 3 nmol/g liver, respectively. The ease and sensitivity of this method makes it suitable for routine use in toxicology and other disciplines. (Supported by USPHS Grants ES 07079 and ES 03192).

Both a glutathione-cytosol and a microsomal mechanism has been proposed for the covalent binding of 1,2-dichloroethane to DNA. DCE-$^{14}C$ was incubated either with $^{3}H$-glutathione in the presence of a cytosol fraction or with a microsomal suspension in the presence of polyadenosine (PA), polycytidine (PC), or polyguanine (PG) for 3 hrs. at 37°C. The polyribonucleotides (PN) were hydrolyzed with nuclease P1, acid phosphatase and alkaline phosphatase and the hydrolyzate was applied to an HPLC equipped with either an ODS or a SCX column. The ODS eluate of the glutathione-cytosol incubations of the different PN exhibited the same peak containing both $^{14}C$ and $^{3}H$ irrespective of adding the PA at the beginning or end of the incubation. Upon SCX chromatography, the same two peaks containing both $^{3}H$ and $^{14}C$, were eluted irrespective of whether the incubation contained a PN. The microsomal mediated binding to PA resulted in l, N$^\alpha$-ethenoadenosine, to PC in 3, N$^\alpha$-ethenocytodine and to PG in two major peaks. Thus, the binding of DCE to PN mediated by microsomes resulted in the formation of adducts while no evidence was obtained for a glutathione-cytosol mediated binding even though the formation of a glutathione containing metabolite was demonstrated. (This abstract does not necessarily reflect EPA policy).

The effect of varying microsomal vitamin E content on glutathione (GSH) inhibition of in vitro lipid peroxidation was determined with rat liver microsomes containing normal (60 ppm), depleted (0 ppm), or 10 times normal (1,000 ppm) amounts of vitamin E obtained by dietary manipulation. Peroxidation of normal microsomes was inhibited by 5 nM GSH over a 20-40 min time period, but reached an extent of peroxidation after 60 min equal to that obtained in the absence of GSH in 20 min. Microsomes containing a 10-fold higher vitamin E level showed protection by GSH which extended over a 3-5 hr time period, reaching an extent of peroxidation in 5 hr equal to that obtained in 3 hr in the absence of GSH. Integration of the GSH protection over the time required for complete peroxidation showed a 5-fold greater GSH inhibition in microsomes containing 10-fold higher amounts of vitamin E. No effect of GSH on peroxidation was seen with microsomes that were depleted of vitamin E or heat-denatured. These results demonstrate a heat-labile protective interaction between vitamin E and GSH, with enzymatic reduction of the chromanoxyl radical as a possible mechanism. (Supported by U.S. DOE Contract No. DE-AC02-76EV03490 and NIH Training Grant No. ES07026.)
Incubation of microsomes with NADPH and methimazole (MI; N-methyl-2-mercaptoimidazole), a high affinity substrate for flavin-containing monoxygenase (P450), has been reported to result in a loss of cytochrome P-450. Because we have previously shown that the P450-mediated metabolism of MI produces an inhibitor of P-450-catalyzed N-hydroxylation reactions, we have investigated the role of P450 in the loss of P-450 during MI metabolism. Liver microsomes from untreated rat were incubated with NADPH and MI for 15 min, resuspended by calcium precipitation, and assayed for protein and P-450. A 25% loss of P-450 detectable as its ferrous-CO complex was observed with 1 or 20 μM MI. When microsomal P450 was heat-inactivated prior to incubation with NADPH and MI, there was no loss of P-450. These results indicate that the P450-catalyzed metabolism of MI was necessary for the loss of microsomal P-450. Further studies showed that sulfoxide and N-methylimidazole, the ultimate products of MI metabolism by P450, did not cause P-450 loss. When glutathione was included in the incubation with MI, there was no loss of P-450. These results suggest that the loss of microsomal P-450 is due to an interaction with oxygenated metabolites of MI formed by P450.

The FAD-containing monoxygenase has been studied extensively only with the solubilized, purified pig liver enzyme. Little is known about the microsomal enzyme of common experimental animals. Antibodies against NADPH-cytochrome P-450 reductase have been used to determine the activity of the FAD enzyme towards 25 different sulfur-, nitrogen-, and phosphorus-containing substrates in the absence of cytochrome P-450-dependent oxidation. Maximal velocities (nmole/min/mg hepatic microsomal protein) include: mouse, 18; rat, 17; rabbit, 8; pig, 16. Lung Vmax values include mouse, 10; rat, 3; rabbit, 10. Although Vmax for this enzyme is said to be the same for all substrates, compounds metabolized at slower rates reflect either high Km values or solubility limits. N-Octylamine increases Vmax for the pig liver enzyme two-fold, has little effect on Vmax values with rodent liver microsomes, but always increases apparent K values. Km values from NADPH plots and KI values of thiacetamide oxidation indicate the rodent FAD-containing monoxygenase in microsomes has a lower affinity for methimazole, dimethylamine and secondary amines than that of the pig. The pulmonary monoxygenase of mouse and rabbit lung have unusual pH optima (c. 9.8). High activities in lung and kidney microsomes indicate this enzyme may be important, relative to cytochrome P-450, in these tissues.

Oxidative deactivation of most organophosphate pesticides is not catalyzed by pig liver microsomal FAD-containing monoxygenase. Exceptions are fonofos (0-ethyl-5-phenyl-ethylyphosphonodithioate) and other organophosphates containing at least one P-C bond. Fonofos is converted mainly to fonofos oxon by this enzyme. Other phosphorus-containing analogs of fonofos including, S-phenylidithiophosphonodithioate, C-ethyl-0-phenylethylphosphonothionate, and diethylphosphorylphosphate sulfide are also substrates for purified pig liver microsomal FAD-containing monoxygenase. The relationship between structure and activity of this series of compounds was determined. The action of the enzymes on trivalent organophosphorus compounds such as diethylphosphorylphosphate was also examined. The results of both kinetic and stoichiometric studies indicate that the microsomal FAD-containing monoxygenase is active as a P-oxidase in addition to its well characterized N- and S-oxidase activity.

The role of various enzymes and biological molecules on the activation and deactivation of the metabolites of benzene was investigated. Phenol, the major metabolite of benzene, is metabolized to hydroquinone and catechol. Activation of these and deactivation of their oxidized forms was assessed by the amount of covalent binding to microsomal protein in vitro. 14C-Phenol and NADPH were incubated with phenobarbital induced guinea pig microsomes. 2.25 Nmoles of hydroquinone and 0.07 Nmoles of catechol were formed/min/mg. The amount of covalent binding was 252 pmol/min/mg. Addition of superoxide diamutase decreased covalent binding 3% with induced microsomes, but had no effect with uninduced. Addition of S-adenosyl-L-methionine and catechol-O-methyltransferase decreased the binding 20%. H2O2 and horseradish peroxidase increased binding 4 fold if H2O2 was omitted the same increase was observed. Catalase had no effect on this increase. Purified hepatic DT-diaphorase decreased binding 45%, which could be reversed, with dicumarol. DT-diaphorase present in the 105g supernatant was also active in decreasing binding but only after glutathione was removed. These results indicate that hydroquinone, catechol and phenol and their oxidized forms can be activated and deactivated by the above systems which may play a role in benzene's toxicity to the bone marrow.
The pulmonary toxins, 3-methylfuran (3-MF), a
major constituent of atmospheric smoke, and 2-
methylfuran (2-MF), a natural product present in
cigarette smoke, coffee, and many foods, are ac-
tivated by microsomal monooxidases to reactive
electrophiles that bind to tissue macromolecules.
Using semicarbazide (SC) as a trapping reagent,
acetyl acrolein (AA) and methyl butenolide (MB)
were isolated as products of microsomal oxidation
of 2-MF and 3-MF respectively. A comparison of
the covalent binding of [3H]-3-MF and the amounts of
MB and semicarbazone produced in microsomal
incubation in the presence and absence of NADPH and
SC, revealed an inverse relationship be-
tween the two measures. NADPH dependent co-
valent binding of 3-MF was strongly inhibited by
SC, presumably by trapping the reactive diazide-
hyde intermediate (ME), before it could react
with tissue macromolecules. Although the ini-
tial formation of these metabolites was dependent
on NADPH, the binding of synthetic [14C]-AA to
microsomal protein was extremely rapid and was
not further enhanced by NADPH. Thus, the un-
satured aldehyde, AA and MB, appear to be
the principal reactive intermediates of 2-MF and
3-MF, that are bound covalently to tissue mac-
romolecules in these preparations.

Verapamil (V) is a calcium-entry antagonist
whose cardiovascular effects and toxicity correlate with its plasma levels. Since V
appears to be removed only by the liver, we have
determined the effects of various agents on its
metabolic elimination. The in vitro metabolism of
V by microsomes from pooled rat liver
homogenates was studied (37°C) at varying V
concentrations after 4 days of i.p.
phenoobarbital (PB) pretreatment, after single
i.p. doses of SKF-525A (SK), and using a 18-min preincubation with propranolol (P) (1.8
mg/ml). Lineweaver-Burk plots showed linear
slopes for studies with control liver
homogenates and for studies with PB, SK, and P.
In control studies, the Vmax (17.6
nmol/g/hr) and Km (9.6 nmol/1) values
were altered significantly by the interventions
used: SK decreased both Vmax (4.2 nmol/g/hr)
and Km (3.1 nmol/l); PB increased Vmax
(78.3 nmol/g/hr) as well as Km (35.3
nmol/1) (p<0.01, all values). P had no apparent effect. Conclusions: these data indicate
that V is metabolized by an hepatic microsomal
system which may be induced by PB and inhibited
by SK; thus, competing substrates may alter its
metabolic elimination.
The methyl-piperidinyl propylglycine derivative of the fluoroephenth aminodimethyl pyrazoles (FADP-1) has antispasmodic properties, induces mammary carcinoma in rats and hepatotoxicity in dogs. FADP-1 was evaluated for endoplasmic reticulum (ER) effects in a rodent hepatotoxicity model. Male rats received FADP-1 p.o. at 250 mg/kg/day for 4 days alone or with phenobarbital. Lower rats received a single FADP-1 dose of 250 mg/kg alone or after glutathione depletion by diethylmaleate (300 mg/kg, i.p.). Liver microsomes were isolated, assayed for cytochrome P450, glutathione-S-transferase (GSH), and antigenyze demethylase (APOM), and subfractionated to determine yields of smooth and rough ER membranes. FADP-1 reduced GSH and APOM activities and lowered P450 content and rough ER was increased with no change in the amount of smooth ER. The effects of ER enzyme activities were less marked with concurrent phenobarbital, and rough ER was unchanged. Rats pretreated with diethylmaleate exhibited effects similar to animals receiving FADP-1 alone. These observations indicate that this agent affects rough ER membranes, causing altered enzyme activity, apparent membrane proliferation, and potential hepatotoxicity.

Cultured hepatocytes are widely used to investigate the metabolism and toxicity of xenobiotic chemicals. Two methods for quantifying cytotoxicity were examined in these studies: (1) leakage of the cytosolic enzyme lactate dehydrogenase (LDH) into the culture medium, and (2) generation of malonaldehyde (MA), a product of polysaturated lipid peroxidation. Compounds tested included metal salts and chelated metal complexes. Hepatocytes were isolated from adult male Sprague-Dawley rats and cultured in Williams' medium E supplemented with 10% serum. Test chemicals were added in unsupplemented medium after 22 hr of culture. Aliquots of medium were removed at 2, 5, and 22 hr thereafter for MA and LDH analysis. The minimum concentration of CuCl₂ which was significantly toxic was approximately 10⁻⁴ M in both assays. However, Cu(II) was considerably more toxic when complexed with the chelator 1,10-phenanthroline: the maximal LDH release was produced by 5.10⁻⁶ M CuCl₂, while significant levels of MA were generated by a dose of 3.10⁻³ M. FeCl₂ did not cause significant LDH release at doses up to 5.10⁻³ M, but stimulated significant MA production at 10⁻³ M. These results suggest that, for chemicals which act by oxidative mechanisms, the MA assay may be a more sensitive measure of cytotoxicity than the LDH assay.

Because toxicity of cyanide (CN⁻) may involve cardiovascular actions, vascular effects of CN⁻ were determined in isolated aorta strips. Aortae were removed after sacrifice, placed in aerated Krebs-Henseleit solution (KHS) and helically cut into strips. Strips were suspended under tension in tissue baths containing KHS at pH 7.4, maintained at 38°C, and aerated with 95% O₂ - 5% CO₂ throughout the experiment. In the rabbit aorta, cumulatively added CN⁻ caused small contractions beginning at ≈ 10 nM CN⁻ and reaching a maximum at 10 μM. Concentrations between 10 μM and 1 mM caused relaxations. When CN⁻ was cumulatively added to norepinephrine (NE)-contracted rabbit aorta strips, no contractions were seen; CN⁻ concentrations above 10 μM caused relaxations. Sensitivity to NE varied among the species, with the ED₅₀ for contractions being dog and ferret, 400 μM and rabbit, 5 μM. Pretreatment with CN⁻ in concentrations up to 10 μM did not reduce contractions of dog aorta to NE, but 10 μM CN⁻ abolished contractions of rabbit aorta to NE, and reversed NE-contractions of ferret aorta to relaxation. Ten μM CN⁻ caused small contractions of aorta strips from each species. Thus CN⁻ may cause either contraction or relaxation of vascular smooth muscle, depending on the CN⁻ concentration and species.
CELL KINETICS IN MOUSE LUNG INITIATED WITH URETHAN AND PROMOTED BY BUTYLATED HYDROXYTOLUENE. H.P. Witschi and C.C. Morse. Biology Division and Univ. of Tennessee-Oak Ridge Graduate School of Biomedical Sciences, Oak Ridge National Laboratory, Oak Ridge, TN

A minimum of 4 intraperitoneal injections of butylated hydroxytoluene (BHT; 300 mg/kg) enhance lung tumor development in A/J mice pretreated with urethan (1000 mg/kg). It was originally thought that a mechanism for this was that each BHT injection would produce diffuse cell proliferation in mouse lung. When we measured cell prolif- eration by implanting 3H-thymidine filled minipumps in mice and by determining the cumulative 7-day labeling index (LI) of alveolar and bronchiolar cells, we found that only the first 2 BHT injections caused cell hyperplasia, whereas subsequent BHT injections were without effect. Piperonyl butoxide (800 mg/kg) given 60 min. before BHT produced a LI similar to the one found in controls. Whether given with or without piperonyl butoxide, 4 BHT injections significantly increased tumor development. It is concluded that lung tumors in A/J mice are promoted by BHT even in the absence of noticeable hyperplasia of the alveolar epithelium. Research sponsored by the Office of Health and Environ. Research, U.S. Dept. of Energy, under contract W-7405-eng-26 with Union Carbide and PHS Grant CA-33795 from NCI.

LACK OF GENOTOXICITY OF BUTYLATED HYDROXYANISOLE (BHA) AND BUTYLATED HYDROXYTOLUENE (BHT). G.W. Williams, T. Shimada, C. McQueen, C. Tong, and S. Ved Brat. Naylor Dana Institute for Disease Prevention, American Health Foundation, Dana Road, Valhalla, NY

BHA and BHT were examined in four in vitro systems for genotoxicity. They were tested in the Salmonella/microsome mutagenesis assay at four doses between 0.01 and 10 mg/plate. In tests in which a positive response was elicited by sodium azide, 9-aminoacridine, 2-nitrofluorene and 2-anthrone, neither BHA nor BHT was muta- genic in any of five tester strains without much toxicity either with or without an S9 fraction. Both chemicals were tested in the hepatocyte primary culture/DNA repair test at 10 doses ranging from 10^-5 to 1 mg/ml. BHA was toxic at 10^-3 mg/ml and BHT at 10^-2 mg/ml. Neither chemical was genotoxic in tests in which the positive control, 2-nitrofluorene, was active. Both chemicals were evaluated in the adult rat liver epithelial cell/hypoxanthine guanine phosphoribosyl transferase mutagenesis assay using adult rat liver line 18. Six doses ranging from 0.05 to 0.1 mg/ml were used. Both chemicals were toxic at 0.1 mg/ml, but neither was mutagenic at lower doses in a test in which the positive control benzo(a)pyrene was mutagenic. In the Chinese hamster ovary/sister chromatid exchange (SCE) assay, four doses from 1 to 10^-3 mg/ml were test- ed. BHT at 0.1 and BHA at 1 mg/ml were toxic; lower doses were negative whereas mitomycin C induced a six-fold increase in SCE.

MODIFICATION OF LUNG TUMOR DEVELOPMENT IN MICE BY BUTYLATED HYDROXYANISOLE. H.P. Witschi and D. G. Doherty. Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN

The food additive butylated hydroxyanisole (BHA) was recently found to produce forestomach cancer in rats. One possible explanation was that BHA would act as a promoting agent rather than as a complete carcinogen. The effects of BHA on lung tumor development in mice was examined. We found that a diet containing 0.75% BHA did not enhance the development of lung tumors in A/J mice if fed for 8 weeks after administration of urethan, benzo(a)pyrene (B(a)P) or dimethyl nitrosamine (DMN). Prefeeding animals with BHA partially protected animals against the tumorigenic effect of urethan and B(a)P. Partial protection was also seen in animals given B(a)P and then exposed to BHA. The two isomers of BHA (3-tert-butyl-4-hydroxyanisole and 2-tert-butyl-4-hydroxyanisole) were synthesized and injected ip. They had no effect on lung tumor development. Cell kinetic studies showed that BHA, unlike the closely related butylated hydroxytoluene (BHT) does not produce diffuse cell proliferation in mouse lung. It is concluded that BHA is not a promoting agent for lung tumors in mice. Research sponsored by the Office of Health and Environ. Res., US Dept. of Energy, under contract W-7405-eng-26 with Union Carbide and PHS Grant CA-33795 from NCI.

EFFECTS OF α AND β HEXACHLOROCYCLOHEXANE DURING TUMOR DEVELOPMENT IN MOUSE LIVER. F. Iverson, L. Tryphonas and E. Lok. Toxicology Research Division, Food Directorate, Health Protection Branch, Ottawa, Ontario, Canada, K1A 0L2

Cyclic chlorinated hydrocarbon compounds often produce a significant tumorigenic response in the mouse liver. It has been suggested that neoplasia results from promotion of spontaneously initiated hepatocytes and that the degree of response is related to either the enzyme induction, enhanced liver growth or cell proliferation produced by these compounds.

We monitored these parameters in mice fed α - Hexachlorocyclohexane at 500 ppm (α 500); 250 ppm (α 250); and 250 ppm β-hexachlorocyclohexane (β-250). Serial sacrifice revealed grossly visible nodules at 36 weeks with α 500 and at 50 weeks with α 250. No nodules were seen with β-250 at 80 weeks. Enzyme induction was approximately equal for all treatments and time periods. Liver growth increased an equal amount (2-fold) at all treatments after 4 weeks and appeared to increase above this level only when nodules were forming. Cell proliferation, measured as DNA synthesis activity, varied between treatments with no consistent relation- ship preceding neoplastic response. Thus while enzyme induction, liver growth or cell proliferation may be involved in the development of these benign tumors, there appear to be other factors that play a significant role in deter- mining the degree of response observed.

Methods have been developed to describe in quantitative terms the modification of ribosomal RNA gene sequences isolated from the total liver mitochondrial DNA (mtDNA) of male Fischer rats administered 1 mg/kg aflatoxin B1 dose. Liver mitochondrial DNA, enriched in ribosomal RNA gene sequences by one round of cesium salt density centrifugation, was incubated in alkaline buffer to stabilize AFB1-bound radioactivity and subsequently hybridized in formamide buffer with mitochondrial ribosomal RNA to form rDNA:RNA hybrids. These hybrids were purified by two successive CsCl centrifugations, and the level of AFB1 modification to ribosomal mtDNA was determined. As a function of time, the ribosomal DNA regions of mtDNA were found to be preferential targets within mtDNA for AFB1 modification; over a 12-hour period post-dosing, the ribosomal RNA gene regions contained 5-6 times more bound AFB1 moieties than unfractinated mtDNA. Comparison studies employing in vitro versus in vitro added mtDNA indicated that the observed preferential modification of the ribosomal gene regions is due to the relative accessibilities of these DNA sequences and not to the nucleotide content.

Supported by TAMU grant AH 6658, Center Grants 18820-11 and 18845, and DoI Contract DAAG29-83-88-88

POLYPOID ALTERATIONS IN PARTIALLY HEPATECTOMIZED RATS GIVEN MIRES OR DMN. C. Kruger-McDermott and R. Abraham, Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, NY.

Polyplodization was investigated in partially hepatectomized (PH) Sprague-Dawley rats following single or multiple exposures to a genotoxic carcinogen (dimethylnitrosamine, DMN) and an epigenetic carcinogen (mirex). A single exposure to mirex (4.5 mg/kg) or DMN (2 mg/kg) given 24 hours after PH failed to disturb ploidy. However, multiple exposures (7 daily doses of mirex or 3 i.p. injections of DMN) altered the pattern of polyplodization. Mirex increased the frequency of all polyploid populations, while DMN decreased tetraploids and increased the frequency of octaploids. After a 2-week period of recovery, rats given mirex displayed a normal pattern of ploidy, whereas the tetraploids were still decreased in the DMN-treated rats. Thus, multiple exposures of carcinogens in the model used yielded measurable changes in hepatic ploidy which may be useful as an early indicator in assessing the carcinogenic potential of chemicals.

Supported by New York State Health Research Council Fellowship.

DEVELOPMENT OF γ-GLUTAMYLTRANSPEPTIDASE POSITIVE (GGT+) FOCS IN HEPATOCYTES OF RATS ADMINISTERED AFLATOXIN B1 OVER A TEN-FOLD DOSE RANGE. G.E. Dunaf and T.C. Campbell, Field of Environmental Toxicology, Cornell University, Ithaca, NY.

GGT+ foci have been identified as an indicator of early preneoplastic liver lesions. This study was conducted in order to quantitate the response to administration of several doses of aflatoxin B1 (AFB1) on the emergence of GGT+ foci in the Fischer-344 rat. Male rats of 9-weeks of age, with a mean body weight of approximately 100 g were divided into 9 groups (n=12) and administered AFB1, p.o. dissolved in tricaprylin. The dose levels were 0, 40, 100, 150, 200, 250, 300, 350, and 400 μg/kg/day (10 doses over 12 days). Thirteen weeks post-dosing the rats were sacrificed. At the lowest dose no GGT+ foci were detected. However, over the remaining 5 doses a linear response was observed in terms of #foci/cm², #foci/cm², mean diameter of foci and % of section area occupied. The shapes of these 4 curves were similar and exhibited a threshold for emergence of GGT+ foci at the 100-150 dose range. These results are of particular interest when viewed in terms of the dose-response curve for the formation of various macromolecular adducts with AFB1, which has been shown to be linear over 5-orders of magnitude (10ng/kg-5mg/kg). Thus, the dose-response relationship between macromolecular adduct formation and GGT+ foci development in rat liver exposed to AFB1 may not be comparable at all dose levels. (Supported by NCI grant # PO1-CA26755 and NIH5 grant # ES0-0752.)


Hepatocarcinogenesis following limited feeding of FAA was studied in Fischer rats. The animals were fed a diet containing 0.02% FAA for 3 weeks and then a control diet for 1 week. One complete cycle (4 weeks) and two, three and four complete cycles were carried out. At the end of cycles one, two, three and four, 3 rats from each group were sacrificed. All surviving rats were sacrificed at 7 weeks of age when the experiment was terminated. Altered foci were observed in the liver of rats sacrificed at the end of the respective feeding cycles. Neoplastic nodules were present in all rats sacrificed at the end of two, three and four cycles of FAA feeding. At the end of the experiment, altered foci were observed only in rats fed FAA for two cycles; and no increase in the number of neoplastic nodules was noted in all groups. The feeding of FAA for two, three and four cycles was associated with a high incidence of hepatocellular carcinomas, which yielded a high rate of lung metastases only in the groups fed FAA for three and four cycles. These results show a dose-response relationship for the incidence of hepatocellular carcinomas following the limited feeding of FAA and partially confirm earlier work on regression and persistence of neoplastic nodules.
421 EFFECT OF BENZENE ON DNA SYNTHESIS IN MICE HEMOPOIETIC CELLS FOLLOWING EXPOSURE BY INHALATION. E. W. Lee, Biomedical Science Dept., GM Research Labs, Warren, MI 48080

Our previous study (Toxicologist, 3:112, 1983) revealed that a single dose of i.p. benzene (B) (440 to 880 mg/kg) to mice inhibited DNA synthesis in hemopoietic cells but it did not affect protein and hemoglobin synthesis. In this study, the effect of B on DNA synthesis in mice was evaluated following exposure to B by inhalation. Female ICR mice were exposed to B by "noise only" at 1000, 2000 and 3000 ppm. The effect of B on DNA synthesis was measured by a 30-min H-thymidine incorporation 4 or 16 hrs after exposure. The 2000 ppm B exposure for 4.5 hrs reduced DNA synthetic activity by 15% but was not significant statistically. At 3000 ppm, 53% of the DNA synthetic activity was reduced 4.5 hrs after exposure (15000 ppm-hr), indicating a dose-related response. However, when mice were exposed to 1000 ppm for 16.5 hrs (16,500 ppm-hr) DNA synthesis was not inhibited. These results suggest that the toxic response to B is related to the exposure concentration rather than the time-weighted dose at a "sub-threshold" level. At the inhibitory concentration (3000 ppm), an increased exposure period (16.5 hrs) resulted in increased inhibition of DNA synthesis (43% inhibition). Combined data from the inhalation and the i.p. injection studies showed that the relationship between bone marrow B concentration (x) and its early inhibitory effect on DNA synthesis (y) satisfied \( y = 63.8 - 3540 x \) with correlation coefficient of 0.99. This implies that an inhibitory effect of B on DNA synthesis in the in vivo situation is closely correlated with bone marrow B regardless of whether B is injected i.p. or given by inhalation.


Although certain organic solvents have been shown to be carcinogenic in laboratory animals, conflicting results have been obtained in short-term mutagenicity tests. This study describes a multiple end-point approach to the genotoxicity of dichloromethane, benzene, 1,2-dichloroethylene and trichloroethylene in L5178Y TK\(^+\)/ Mouse Lymphoma cells. Briefly, cells are exposed to the solvent for 4 hours with and without metabolic activation and then divided into two groups. One group is assessed for chromosome aberrations and the second for mutation at the TK locus. Results for dichloromethane indicate negative mutagenicity even at very toxic levels. Five doses from 4 \( \mu l/ml \) to 0.5 \( \mu l/ml \) were examined in three experiments. The toxicity ranged from 6% to 92% survival. None of the doses exhibited a significant increase in mutant frequency. However, chromo-

423 INHIBITION OF REPLICATION IN RAT LIVER MITOCHONDRIA BY BENZENE METABOLITES. C. Schwartz, R. Snyder, and G. Kalf, Dep. of Pharmacology and Toxicology, Rutgers Univ., Piscataway, NJ 08854 and Deps. of Biochemistry and Pathology, Thomas Jefferson Univ., Phila., PA 19107

Rat liver (RL) and bone marrow mitoplasts (MP) mediate the NADPF-dependent activation of benzene to metabolites which covalently bind to mitochondrial (mt) DNA and inhibit mt transcription. We report here that the metabolites p-benzazquinone (BQ) and 1,2,4-benzene triol also inhibit replication in RL mitoplasts. MP incubated with BQ showed a dose-dependent inhibition of \(^{(3)}H\)dUTP incorporation into mt DNA with an IC50 of 1 mM; a similar inhibition was seen with triol. Benzene and the metabolites phenol, catechol, and hydro-

424 N-NITROSO DIMETHYLAMINE-INDUCED FORESTOMACH TUMORS IN ZINC DEFICIENT RATS. P.M. Newberne, L. Fong, and W.L. Ng. Massachusetts Institute of Technology, Cambridge, MA

Groups of rats were fed either a zinc-deficient diet or a zinc supplemented diet pair-fed to the deficient group. Five weeks after initiation of the dietary treatment each animal was administered by gastric intubation dimethylnitrosamine (DMN) 2 mg/kg/B.W. 2 x weekly for 6 doses, then 4 mg/kg/B.W. 2 x weekly for 10 doses. All rats were sacrificed after 45 weeks. None of the control, pair-fed rats developed gastric lesions. Of the zinc-deficient rats 88% developed anacanthosis of the forestomach, 26% developed focal parakeratosis and dyskeratosis, 18% developed erosion and ulceration of the squamous epithelium and 63% developed papillomas. These data demonstrate that zinc deficiency permits DMN to induce tumors in a tissue not normally considered a target and indicates differences in metabolism of DMN to the proximate carcinogen. (Supported in part by ES 00597, Hoffman LaRoche and Hong Kong University).
425 ENHANCED ESOPHAGEAL CARCINOGENESIS BY RIBOFLAVIN DEFICIENCY. P.M. Newberne, Massachusetts Institute of Technology, Cambridge, MA

Rats were depleted of riboflavin and exposed to methylbenzylnitrosamine, 6 doses each of 2.5 mg/kg body weight. Results after 5 months were as follows:

<table>
<thead>
<tr>
<th>Neoplasms</th>
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<tbody>
<tr>
<td>control</td>
<td>0/10</td>
</tr>
<tr>
<td>control + MBN</td>
<td>8/20</td>
</tr>
<tr>
<td>riboflavin deficient</td>
<td>0/20</td>
</tr>
<tr>
<td>riboflavin deficient + MBN</td>
<td>23/26</td>
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</tbody>
</table>

The increased tumor induction in deficient animals was associated with decreased a-demethylase activity.

(Supported in part by grant ES00597).


Initial epidemiological studies of the Acquired Immune Deficiency Syndrome (AIDS) occurring in homosexual men identified the use of nitrite inhalants as possible risk factors contributing to the disease. Because no immunotoxicological data were available on these chemicals, we studied the effects of inhalation exposure to isobutyl nitrite (IBN) on the immune system. BALB/c mice were exposed to either 50 or 300 ppm of IBN (6.5 hr/day, 5 days/week) for up to 18 weeks. After 7, 13, or 18 weeks of exposure, mice were sacrificed and the following assays performed: antibody producing cells were enumerated by a slide plaque assay; lymphocyte proliferative response to mitogens was tested using several concentrations of each mitogen; mice were immunized with Freund's complete adjuvant 21 days prior to sacrifice, and tested for delayed hypersensitivity response; and the relative number of T-cells and T-cell subsets among splenic lymphocytes from exposed and control animals was determined. At the time periods tested, there were no discernible immunotoxic effects observed in the exposed animals in any of the assays performed.


It is not clear whether asbestos or other mineral fibers directly induce neoplasia of epithelial cells. In the present study we examined the effects of asbestos and fiberglass in a rat tracheal epithelial (RTE) cell transformation system. This system, which employs primary cultures of epithelial cells, has been well characterized and has been used to quantify neoplasia-related effects induced by a number of different physical and chemical carcinogens. Using this system, we found that chrysotile asbestos was both more cytotoxic and more transforming than crocidolite asbestos. The doses of chrysotile and crocidolite which resulted in 50% survival of cells were, respectively, 2.2 and 6.0 μg/cm². Chrysotile was also four-fold more potent in the induction of transformation than crocidolite. The maximum incidence of transformation (4%) was similar to that obtained after treatment of RTE cells with chemical carcinogens such as benzo(a)pyrene. The cytotoxic and transforming potencies of Code 100 fiberglass (Johns-Manville) were similar to those of chrysotile. Although asbestos and fiberglass may also have promoter or cocarcinogenic actions, we have clearly demonstrated their capacity to induce directly a preneoplastic change in epithelial cells. The progression of these preneoplastic cell variants to neoplastic variants is under study.


Although the concept of concentration-response is routinely used in many toxicologic tests, it has only recently been associated with induction of immunologic sensitization (Karol, Toxicol. Appl. Pharmacol., 1983, 88, 229-241). In the current study, the concentration-response relationship was explored for induction of pulmonary sensitization to bacterial subtilisin. Guinea pigs were exposed to 0.05% or 1.0% aerosols of subtilisin for 15 min per day on 5 consecutive days. The development of respiratory sensitivity was examined beginning on day 10 by bronchial provocation challenge. Animals were monitored during the 20 min challenge and for 24 hr to assess immediate-onset, delayed-onset or dual pulmonary reactions. Responses occurred only during or immediately following inhalation challenge. A concentration-response relationship was apparent between exposure concentration and (a) percent increase in respiratory rate (b) percent of animals responding. Comparison of severity of responses to subtilisin with that previously reported for TDI revealed more severe, at times fatal reactions were associated with subtilisin sensitivity. The concentration-response relationship shown here to govern respiratory sensitivity reactions should permit recommendation of a safe exposure level to protect workers from sensitization to this industrial material.

Psyllium, a relative of the lawn weed, English plantain, is commonly used in bulk laxatives. These non-prescription preparations are taken daily by millions of people. Recently, the allergenic potential of psyllium has received attention. In a current study, a patient was evaluated for psyllium-related workplace asthma and dermatitis. Using RAST, IgE antibodies were detected in serum samples. The antibodies were specific for psyllium. No cross-reactions were observed when RAST was performed with an extract of English plantain. Experiments were undertaken in guinea pigs to evaluate the sensitizing potency of psyllium. Using the bulk powder and the guinea pig maximization test, 4 of 12 animals were positive for sensitization. Bronchial provocation challenge of all animals, using an aerosol of aqueous psyllium extract, resulted in 4 immediate respiratory responses. Two of the animals were positive in both assays. These results suggest a mild but perhaps unrecognized allergenic potential of psyllium. In view of the widespread use of psyllium-containing laxatives, consideration should be given to appropriate statements in package inserts to warn of potential sensitization.


In earlier work we reported that a single intramuscular injection of MnCl₂ enhanced murine natural killer (NK) cell activity and inhibited the growth of B16-F10 melanoma lung tumors. Enhanced NK activity was found to be mediated by the induction of interferon. Increased NK cell activity following exposure of murine spleen cells to MnCl₂ in vitro was found to accompany interferon induction. We report here that other natural cell-mediated immune effector cell functions are also enhanced by MnCl₂. Antibody-dependent cell-mediated cytotoxicity (ADCC) against both chicken erythrocytes and P815 tumor cell targets was enhanced by MnCl₂. These results indicated that killer T lymphocytes and macrophages may also be affected by MnCl₂. Subsequent studies confirmed that macrophage function was also enhanced by MnCl₂. Resident peritoneal macrophages from mice injected intramuscularly with MnCl₂ displayed enhanced antibody-mediated phagocytic activity for chicken erythrocytes; enhanced cytolytic activity against P815 mastocytoma target cells; and enhanced cytostatic activity against MBL-2 lymphoma target cells. Studies are currently underway to determine if MnCl₂-enhancement of these effector cell functions is mediated by the induction of interferon as it is for NK activity.

IMMUNOSUPPRESSION BY ALKYLATING AGENTS: A PROPOSED BIOCHEMICAL MECHANISM. C.O. Joe and D.L. Busbee. Department of Physiology and Pharmacology, College of Veterinary Medicine, Texas A & M University, College Station, TX. Sponsor: T.D. Phillips.

The 7,8-dihydrodil-9,10-epoxide of benzo(a)pyrene is an alkylating agent which is known to produce adducts with DNA and to be mutagenic and carcinogenic. When human lymphocytes are treated with this compound at concentrations in excess of 5 x 10⁻¹⁷ g/mL both scheduled and unscheduled DNA synthesis are inhibited and DNA alpha polymerase is inactivated, but the cells are still viable (maintain an adenylate charge between 0.5 and 0.8). Mutagen inhibition studies of DNA alpha polymerase show Lineweaver-Burk plots indicating uncompetitive enzyme inhibition when DNA is varied, and noncompetitive enzyme inhibition when the mixture of nucleotides is varied. Formation of mutagen-DNA adducts is apparently not the key factor in inhibiting either scheduled or unscheduled DNA synthesis; rather, the data suggest that the alkylating agent binds to DNA alpha polymerase, noncompetitively inhibiting triphosphonucleotide interaction with the DNA-enzyme complex. Since scheduled DNA synthesis is an integral part of the lymphocyte mitotic process, inhibition of DNA alpha polymerase results in decreased blastogenesis.

SPERMICIDE-INDUCED ALTERATIONS IN THE IMMUNE AND HEMATOLOGICAL SYSTEMS IN MICE. L.D. Caren,1 J.A. Leveque,1 and A.D. Mandel,2 1Dept. of Biology, Univ. of Santa Clara, Santa Clara, CA, and 2NASA Ames Research Center, Moffett Field, CA. Sponsor: J. Wesley Clayton.

Spermicides are increasingly being studied for possible teratogenic, mutagenic, and toxicological effects. In the present immunotoxicology study, mice were injected daily or every other day for several weeks with 3% octoxynol-containing spermicide. A dose-dependent anemia occurred within 30 days. Leukocyte counts were usually unaffected but sometimes were decreased. In some but not all experiments, immunization with sheep erythrocytes resulted in decreased antibody titers and serum protein levels. Immediate and delayed hypersensitivity reactions, as measured by the footpad swelling test, and skin graft rejection were not affected. Seven to 20 weeks of treatment with spermicide did not affect body weight, but resulted in significant enlargement of one or more organs (kidneys, liver, lungs, spleen, or heart) and shrinkage of the thymus, depending on the experiment. In one of two experiments, spermicide cream base which lacked octoxynol also caused decreased hematocrits and enlargement of the spleen, lungs, and heart. These studies indicate that in mice, chronic ip exposure to 0.05 to 0.1 ml spermicide, four to seven times a week for several weeks, has some adverse effects on the immune and hematological systems.

Numerous industrial and environmental chemicals have been identified as causing contact sensitivity. A method is proposed to assess the sensitizing potency of such chemicals using dose-response data. Studies were undertaken in guinea pigs and mice to explore a dose-response relationship for contact sensitivity to three chemicals: dicyclohexylmethane 4-4, 1-diisocyanate (HMDI), formaldehyde (HCHO) and piryl chloride (PICl). Guinea pigs were sensitized by topical application using doses of 10-10,000 µg. Animals were challenged by patch testing and responses graded at 24 hr. Application of high doses resulted in extensive erythema and in a great proportion of animals becoming sensitized. Lower doses produced less sensitivity. Topical sensitization was also performed in BALB/cby mice. Sensitivity was assessed by extent of ear swelling upon challenge. A dose-response relationship was also apparent in mice between sensitization dose and a) severity of response b) number of animals responding. Comparison of the dose of each chemical required to sensitize 50% of the mice (SD50) yielded 7 µg for HMDI and 10 µg for PICl. Use of the SD50 should provide a method for assessing the ability of various chemicals to cause contact sensitivity. Supported in part by Contract NA-AF 26 from the International Isocyanate Institute.


The mouse has been suggested to provide an advantageous model for predictive allergic contact dermatitis to simple chemicals over existing guinea pig models. Evidence to support the utilization of the murine model for elucidation of allergenic potential has been determined by 1) a study of the variables influencing optimal conditions for induction of hypersensitivity 2) response to a number of compounds exhibiting concordance and discordance in humans and 3) application of inferential statistical procedures to the system i.e. power analysis. Experimentally, groups of BALB/c mice of either sex are induced by the split adjuvant technique i.e. 25 µl of a supra-irritating dose of the test compound in vehicle is administered epicutaneously for 4 consecutive days. On day 4, 20 µl of Freund's Complete Adjuvant (CFA) is injected intradermally adjacent to the test site. On day 7 animals are challenged with 20 µl of a 50% concentration of the test compound applied to the dorsal aspect of the right ear. Response to challenge is quantitated by the ear swelling test and the radiometric ear test.


Ear swelling, localization of in situ 125I-labelled immune cells, and localization of exogenously labelled 51Cr bone marrow cells (BMC) were used to evaluate the delayed response to dinitrofluorobenzene (DNFB) in mice. The abdomens of the mice were shaved and sensitized (sens.) with DNFB by 2 daily applications of 0.2, 0.5, 1.0, and 2.0 µg DNFB. Prior to challenge with 0.2% DNFB, the 125I-TDR animals were given FUDR and then 125I-TUDR. Similarly, the 51Cr animals received 51Cr-labelled BMC from naive donors. All animals were challenged on the left ear. Ear swelling was measured at 2, 10, 24, and 48 hrs post-challenge. Ear biopsies of the 51Cr and 125I-TUDR treated mice were removed at 24 hrs and gamma-counted for radioactivity. The ear swelling data demonstrated a delayed response with maximal swelling for all sens. doses at 24 hrs. The greatest response was observed at sens. doses of 0.5 and 1.0% DNFB (Late-Rear = 0.16mm, control = 0.44mm). The radioisotope localization studies confirmed a sensitizing dose-dependency with maximal stimulation indices of 4.80 (125I-TUDR) and 5.5 (51Cr-BMC) at sens. doses of 1.0% DNFB. The 3 methods of evaluating contact sensitivity correlated well with each other. Sensitizing dose dependency was best demonstrated with the radioisotope techniques. (Supp. by NIHS 1T52NE07087).


T-2 toxin is a potent inhibitor of eucaryotic protein and DNA synthesis. Immuno-toxic evaluations of T-2 toxin, both acute and subchronic, have implicated it as an immunosuppressive compound. The present study indicated T-2 toxin stimulates specific immunologic functions. Male CD-1 mice were gavaged with T-2 toxin (0.00, 0.02, 0.10, 0.50, 2.50 mg/kg body weight) every third day. The vehicle, 4% ethanol in corn oil, was employed in all groups including controls. Lymphocyte blastogenesis was assessed following two and four weeks of T-2 toxin exposure. Mitogenic stimulation at two weeks was enhanced in the lower dose groups. This phenomenon was not observed at four weeks. Production of IgM antibodies in splenic lymphocytes, evaluated by hemolytic plaque response to sheep erythrocytes (SRBC), was unaffected by low levels of T-2 toxin in SRBC sensitized animals. Delayed hypersensitivity, determined in SRBC sensitized animals following challenge in foot-pad, also was not affected by low level T-2 toxin treatment. Observations on food consumption, body weight gain, organ weights, hematology and serum chemistry indicated systemic toxicity in the high dose group only. It is suggested that T-2 toxin, at the low doses studied, has had a stimulatory affect on the murine immune system, followed by depression at higher doses.
ALTED IMMUNOCOMPETENCE AND HOST RESISTANCE TO
MURINE MALARIA IN TUMOR SUSCEPTIBLE OBESE YELLOW
(AVY) MICE. D. W. Roberts, R. L. Suber, W. L.
Campbell, R. W. Benson and G. L. Wolff. National
Center for Toxicological Research, Jefferson, AR.

Immunologic consequences of the obese and tumor
susceptible yellow AVY/a mouse phenotype as com-
pared to either genetically identical but lean
pseudogout AVY/a mice or congenic black a/a
mice included decreased antibody responses to
tetanus toxoid (p < 0.005), enhanced antibody re-
sponses to Type III pneumococcal polysaccharide
(p < 0.03), decreased (unadjusted) rates of car-
bon clearance (p < 0.01), and diminished host
resistance to the murine malaria Plasmodium
yoelii (Py). Yellow mice had lower parasitemias
and altered survival curves as compared to their
non-yellow littermates. Evaluation of complete
blood cell count parameters in uninfected
animals revealed that yellow mice had increased
(p < .001) osmotic erythrocyte fragility. Altered
immune function and erythrocyte fragility
seen in yellow mice are probably related to
diminished host resistance to Py as resolution of
infection involves multiple immunologic
factors including clearance of damaged erythro-
cytes by an immunologically activated reticulo-
endothelial system. The relationships of the
altered immune function and erythrocyte fra-
gility we observed in obese yellow AVY/a mice
to their enhanced susceptibility to tumors
remains to be clarified.

MEMBRANE-MEDIATED EFFECTS OF ESTROGEN
METABOLITES ON LYMHCYTE AND MACRAPHAGE
ACTIVATION AND EFFECTOR CELL FUNCTION. R. W.
Pfeifer and R. M. Patterson, STB, NIHS, P.O. Box 12233, Research
Triangle Park, NC. Sponsor: J. A. Goldstein

At pharmacological concentrations (5x10^-5-10^-6M),
estrogen metabolites modulated phytosynergic
metabolite (PHA)-induced lymphocyte blastogenesis in vitro.
Identical effects were observed on the agglutina-
tion response of activated cells occurring within
minutes. These results suggested a role for
estrogens in modulating lymphocyte activation at
the cell surface. Catechol estrogen metabolites
(2-hydroxyestrone, 20H) demonstrated a potent inhi-
bition which was sulfhydryl-dependent. Suggestive
of membrane partitioning, nonreactive metabolites
(estrene, E) demonstrated effects which were depen-
dent on lectin concentration, while 17beta estradiol
had negligible effects. Estrogens demonstrated
similar immunomodulatory effects on natural killer
cell-mediated tumor cell growth inhibition. How-
ever, although 20H inhibited macrophage activating
factor (MAF)-induced macrophage cytosis, all
the compounds, including 20H, enhanced growth
inhibition mediated by activated macrophages in vitro.
Therefore, unlike lymphocytes, alterations in
macrophage membrane properties accompanying activa-
tion and the expression of effector cell function
demonstrate different sensitivities to estrogen
modulation.

DELTAS-9-TETRAHYDROCANNABINOL DECREASES
HOST RESISTANCE TO HERPES SIMPLEX VIRUS TYPE 2 VAGINAL
INFECTION

Mishkin, E.*; Marciano-Cabral, F.; and Cabral,
G. A. Sponsored by: S. G. Bradley

Department of Microbiology and Immunology and
Department of Pharmacology, The Medical College
of Virginia/VCU, Richmond, Virginia

Delta-9-tetrahydrocannabinol (Delta-9-THC),
a major psychoactive component of marihuana,
elicits suppressive effects on the immune system.
This study was undertaken to determine whether
Delta-9-THC decreases resistance to herpes simplex
virus type 2 (HSV2) genital infection in the
guinea pig. The guinea pig was selected as the
host since HSV2 induces a spectrum of disease
similar to that in humans. Animals inoculated
intravaginally with 10^3.5 plaque-forming units
(PFU) of HSV2 and treated intraperitoneally with
4mg/kg and 10 mg/kg Delta-9-THC exhibited sig-
nificant increases in lesions and mortalities when
compared to non-drug treated controls. Of in-
fected vehicle-treated animals, 50% exhibited
lesions and 33% died. In contrast, 75% of in-
fected animals receiving 4mg/kg Delta-9-THC had
lesions and died. Of infected animals treated
with 10mg/kg Delta-9-THC, 80% had lesions and 66%
died. These results indicate that Delta-9-THC at
these doses decreases host resistance to HSV2
genital infection in the guinea pig.

Supported by a Center of Excellence Award
to MCV/VCU by the Commonwealth of Virginia.

CORRELATIONS BETWEEN POLYCHLORINATED BIPHENYL (PCB)
ISOMER IMMUNOTOXICITY, MICROSOmal ENZYME INDUCTION,
AND THE AROMATIC HYDROCARBON (Ah) RECEPTOR. J. B.
Slikworth, L. Antrim, and L. S. Kasinsky. Center for
Laboratories and Research, New York State Department of
Health, Albany, NY, 12201.

PCB immunotoxicity is dependent on isomer planarity and
the Ah gene complex in mice. To evaluate structural
requirements for Ah receptor binding and the
relationship between concomitant microsomal enzyme
induction and PCB immunotoxicity, pure PCB isomers (35
or 350 umoles/kg) which are (in rats) either
phenobarbital (PB)-type, 3-methylcholanthrene (MC)-type
or mixed-type inducers were given to either C57BL/6
(80, Ah') or DBA/2 (02, Ah) mice 2 days prior to
immunization with sheep erythrocytes. The splenic
direct antibody forming cell (PFC) response was
evaluated on day 5. Hepatic aminopyrine N-deethylase
(PB-inducible) and aryl hydrocarbon hydroxylase
(MC-inducible) activities were also measured.

2,2',4,4'-PCB was a PB-type inducer only in B6 mice and
had no immune effects. 2,2',5,5'-PCB was inactive.
3,3',4,4'-PCB was an MC-type inducer, caused a 90%
suppression (350 umoles/kg) in PFC/spleen, and a 43%
reduction in thymus weight in both in B6 mice.
2,3',3',4,4',5'-PCB was a PB-type inducer in D2 mice but
a mixed-type inducer in B6 mice and caused 78%
suppression (350 umoles/kg) of PFC/spleen but not thymic
atrophy in B6 mice. These results suggest that MC-type
induction is associated with immunosuppression but not
thymic atrophy, that the thymus may not be the critical
target tissue in PCB immunotoxicity, and that ortho
chlorination does not prevent Ah receptor binding and
subsequent immunotoxicity. (NIH grant ES06897)
Reduced binding of low density lipoprotein to its hepatic plasma membrane receptor of guinea pigs treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin. D.W. Bombick, B.V. Madhukar, and F. Matsumura. Pesticide Research Center, Michigan State University, East Lansing, MI.

Low density lipoprotein (LDL) binding was lower in the hepatic plasma membrane of male guinea pigs administered a single i.p. dose (1.0 mg/kg) of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). This reduction in LDL binding in treated animals was not caused by decreased food consumption since pair-fed control animals exhibited similar LDL binding to ad lib controls. Rat hepatic plasma membranes from treated animals also had lower LDL binding compared to controls. The relationship between reduced LDL binding, increased levels of LDL, and very low density lipoproteins (VLDL) was examined by comparing LDL internalization in isolated hepatocytes from control and treated animals. Hepatocytes from treated guinea pigs showed a reduced rate of LDL internalization compared to controls. The internalization process represents the essential step in metabolism of LDL. Therefore, these results indicate the cholesteryl ester-carrying lipoproteins (LDL) in the serum are not readily metabolized in TCDD-treated animals. In addition, animals possessing low LDL receptor activity (e.g., human familial hypercholesterolemic patients) also show elevated levels of VLDL production in the liver. The evidence obtained in the current investigation indicates a possible causal relationship between reduced LDL binding at the hepatic plasma membrane and the hypercholesterolemia observed in TCDD-treated guinea pigs. (Supported by research grant ES01953 from NIEHS.)

Inhalation pharmacokinetics: inhibitory interactions between N-hexane (HX), methyl-N-butylketone (MBK), and 2,5-hexanedione (HD). H.J. Clewell, M.E. Andersen, and M.G. MacNaughton. Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB, OH.

HX is metabolized to MBK which is in turn oxidized to HD. Both steps are \( \omega-1 \) microsomal oxidations. We have studied the metabolism of inhaled HX in male rats by gas uptake methods. The uptake of HX indicated both saturable and first order metabolic processes. Pre-treatment of rats with either MBK or HD significantly inhibited HX metabolism. Baker and Rickard (1981) found that increasing concentrations of inhaled HX led to a decrease in HD during exposure, suggesting inhibition of MBK oxidation by HX. We have developed a physiological kinetic model for inhaled HX, incorporating mutual inhibitory interactions and used optimization methods to estimate inhibitory constants. The successful model assumed (1) that the inhibition of MBK oxidation by HX was more effective than MBK or HD inhibition of HX metabolism, (2) that both pathways were low capacity, but high affinity reactions, and (3) there was a second, non-inhibitable pathway for MBK oxidation (presumably \( \omega-1 \)-oxidation). In HX inhalation exposures, inhibition of MBK to HD by HX ceases rapidly at the end of exposure due to rapid HD exhalation. This release of inhibition leads to complex kinetics for MBK and HD in the post-exposure period.

A physiological model of the intravenous and inhalation pharmacokinetics of three dihalomethanes - CH₂Cl₂, CH₂BrCl, and CH₂Br₂ - in the rat. M.J. Andersen, R.L. Archer, H.J. Clewell, and M.G. MacNaughton. Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB, OH.

A physiologically based pharmacokinetic (PB-PK) model has been developed to describe simultaneously the kinetic behavior of dihalomethanes and 2 metabolites - bromide ion and carbon monoxide (as CO) - in rats. Tissue partition coefficients were determined for the 3 chemicals by vial equilibration methods and metabolic constants estimated from steady-state inhalation exposures. CO kinetics were modeled by a Cabourn-Forster-Kane description in which blood CO partitioned between free CO and HbCO and was eliminated by the lung according to CO diffusivity and alveolar ventilation. Bromide kinetics were modeled by retention in extracellular water and first-order elimination by the kidney. CH₂Cl₂, CH₂BrCl, or CH₂Br₂ were injected iv in a saline vehicle into unanesthetized 200-300 g rats and time courses determined for parent chemical, Br⁻ and HbCO. Inhalation exposures were for 4 hr to various concentrations and the time course followed until HbCO levels were below 1%. A single PB-PK model with appropriate partition coefficients and metabolic constants successfully described the kinetic data for all 3 dihalomethanes. Scale-up simulations were run to predict human kinetics of dihalomethane and HbCO.


TE has been reported by NCI to increase the incidence of hepatocellular carcinoma (HC) in the mouse but not the rat following long-term exposure to high gavage doses. The fate of \( ^13 \)C-TE was followed for 72 hrs in male B6C3F1 mice and Osborne-Mendel rats after oral administration of 150 mg/kg for 6 hr exposure to 10 ppm of \( ^13 \)C-TE vapor. \( ^13 \)C-TE was extensively metabolized (> 90%) in both species, both hepatic and extrahepatic. In the mouse, \( ^13 \)C activity was associated with hepatic DNA. HPLC analysis, however, revealed this activity to be associated with parent DNA bases, presumably the result of \( ^13 \)C-1 incorporation, rather than due to DNA adduct formation. Following 4 daily oral doses of 150 or 300 mg/kg, the approximate NCI dose levels in the mouse study, hepatic DNA synthesis was enhanced 1.7- and 6-fold in the mouse and was associated with centrilobular swelling. In the rat, 150 mg/kg/day was associated with a 4.7-fold increase in hepatic DNA synthesis, which may have been influenced by apparent altered feeding patterns as frank hepatotoxicity was not observed. These data suggest that the hepatic tumors induced by TE in the B6C3F1 mouse result from recurrent cytotoxicity which enhances the normal spontaneous incidence of HC, and not from direct genotoxic events.
THE PHARMACOKINETICS AND MACROMOLECULAR INTERACTIONS OF INHALED [14C-METHYLENE CHLORIDE (CHCl₃)] IN HAMSTERS AND RATS. A. M. Schumann, T. R. Fox, K. D. Nitschké, and P. G. Watanebe, Dow Chemical HAEs, USA, Midland, MI.

The fate of inhaled [14C-CHCl₃] was evaluated in the liver and salivary tissue of male Sprague-Dawley rats vs Syrian Golden hamsters to determine whether differences observed after chronic inhalation exposure were related to differences in metabolism or macromolecular events. Similar to the dose-dependent metabolism reported in rats (TAP 66, 1-10, 1982), hamsters metabolized 89% (50 ppm) and 73% (1500 ppm) of their entire body burden after a 6 hr exposure to CHCl₃ vapor. On a body wt. basis, the hamster metabolized 1.1 (50 ppm) and 1.9-fold (1500 ppm) more CHCl₃ than the rat, resulting in a similarly greater extent of 14C activity irreversibly associated with hamster liver and submaxillary gland macromolecules. After a 3.5 hr exposure to 3500 ppm [14C-CHCl₃], a 1.5 and 3-fold greater extent of 14C activity was associated with purified liver and submaxillary gland DNA, respectively, in the hamster vs the rat. HPLC analysis, however, did not reveal DNA adduct formation but showed the 14C activity to be associated with normal DNA bases, presumably via 14C-1 incorporation. These results suggest the in vivo genotoxic potential of [14C-CHCl₃] to be nil and therefore not a significant factor in the toxicity of this material.

DERMAL ABSORPTION OF DIBROMOMETHANE VAPORS. J. N. McDougall, C. W. Jeppson, M. G. MacNaughton, and M. E. Andersen. Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB, OH.

Determination of the dynamics of dermal absorption of vapors is important to general hazard assessments in inhalation toxicology and to a basic understanding of the skin as a barrier. In this study we determined the absorption characteristics of dibromomethane (DBM), a representative halogenated hydrocarbon. Rats were exposed to DBM vapors at concentrations from 500 ppm to 10,000 ppm for 4 hours in a dermal absorption chamber, which prevented vapor inhalation. Serial blood samples were withdrawn through jugular canulas for determining blood DBM and percent carboxyhemoglobin (HbCO). After exposures rats were exsanguinated for analysis of plasma bromide. DBM appeared in the blood within 0.5 hour at all concentrations and blood concentrations ranged from 1 µg/ml at 500 ppm to 140 µg/ml at 10,000 ppm. Steady state was only achieved at the lower concentration. HbCO, an indicator of oxidative metabolism, were from 4% at 500 ppm to 15% at 10,000 ppm. End exposure serum bromide, an indicator of total metabolism, ranged from 0.6 mM at 500 ppm to 2.5 mM at 10,000 ppm. These data were successfully simulated by a physiological model with first-order dermal uptake and a two-subcompartment representation of the skin.


Male Hilltop-Wistar rats were exposed percutaneously to three concentrations of 14C-EDA (10%, 25%, or 50%) using water, acetone or DMSO as the vehicle. Four rats were dosed by applying 0.2 ml of each solution over a 7 x 7 cm area on the back with occlusion for 24 hours. A material balance study was conducted on each rat and a skin sample was taken from the dosing area for histologic evaluation and autoradiography. Mean recoveries (61-83%) were low due to losses during dosing and holding. Urine was the predominant route of radioactivity excretion. Based on 24-hour 14C-balance, the estimated absorbed doses were comparable for all vehicles at the 25% and 50% EDA concentrations. The absorbed dose of EDA by rats receiving 10% EDA in acetone, 25% EDA in water, and 50% EDA in DMSO was estimated to be about twice the amount obtained with 10% EDA in water or acetone. Pharmacokinetic studies were conducted only on rats receiving the 50% concentration of EDA. The absorption rate was fastest when DMSO was used as the vehicle. Histologic examination of skin sections revealed full thickness epidermal necrosis in rats receiving 25% or 50% EDA with all vehicles. At the 10% EDA concentration the epidermis remained normal and intact when water or acetone was used as the solvent but mild epidermal necrosis was evident with DMSO.

ANTHRALIN, A POTENT INHIBITOR OF MOUSE EPIDERMAL PROTEIN SYNTHESIS. C.J. Molloy, M.A. Gallo, and J.D. Laskin, Dept. of Environ. and Community Med. Rutgers Medical School/Rutgers Program in Toxicology, Piscataway, NJ.

Anthrallin is a widely used topical agent for the treatment of certain hyperproliferative skin disorders, eg. psoriasis. In the present study anthrallin was applied to the shaved dorsal skin of mice and 24 hours later effects on epidermal protein synthesis were monitored. Skin fragments from control and treated animals were pulse-labeled with [35S]-met. to assay total protein production. Anthrallin (1-80 µg /animal) caused a marked dose-dependent irritation, hyperemia with increased epidermal thickness, skin discoloration and desquamation. This was accompanied by a significant inhibition of protein synthesis. Thus, at 1.0 µg. epidermal protein synthesis was inhibited by 56% when compared to untreated animals. At doses of 20, 40, and 80 µg /animal, inhibition was 72%, 78%, and 85% respectively. Other skin irritants tested, including phorbol esters and benzoyl peroxide did not inhibit protein synthesis. Analysis of epidermal extracts by polyacrylamide gel electrophoresis revealed several changes in protein production induced by anthrallin in the 45-60 kd. range. Further studies characterizing these specific changes will be useful in understanding the precise mechanism of action of anthrallin. Supported in part by a grant from the Society of Cosmetic Chemists.
The pharmacokinetic profile of the herbicide Dicamba (3,6-dichloro-2-methoxybenzoic acid) and an isomer present in Dicamba formulation (3,5-dichloro-2-methoxybenzoic acid) was examined in rats given single dermal applications of 100 mg/kg Dicamba, of 20 mg/kg isomer and of their combination at those rates. The concentration of the isomer in the blood increased regularly up to 9 hours following dosing followed by a slow decline. At time periods later than the first hour, the isomer concentration in blood exceeded that of Dicamba. The clearance of Dicamba from the blood greatly exceeded that of the isomer and was apparently of first order. The rate constant for first order elimination (Ke) were 1.7 and 0.19 hr⁻¹ for Dicamba and the isomer, respectively. The rate of absorption of the two chemicals through the skin as determined by urinary excretion of Dicamba was 0.0029 hr⁻¹ and for the isomer was 0.0012 hr⁻¹. Urinary excretion was 16% and 7.5% for Dicamba and the isomer, respectively, with regard to the initial dose applied dermally to rats. Following their combined administration, the kinetics of uptake and clearance was similar to that determined for each chemical when administered alone. The low rate of absorption of the two isomers through the skin suggests removal of this herbicide acid from the skin by washing after exposure.

The major halogenated hydrocarbons identified as residues in human adipose tissue, blood and breast milk include DDT, DDD, DDE, isomeric hexachlorocyclohexanes, several cyclodiene-derived insecticides, polychlorobenzene and several PCB isomers and congeners. Based on average relative concentrations of these pollutants in human breast milk, a reconstituted breast milk halogenated hydrocarbon mixture was prepared. The lowest dose (0.7 mg/kg) was estimated to be the amount of halogenated hydrocarbons which would be absorbed by an infant nursing for 180 days. No effects were observed at the low dose, however, pretreatment of rats with 1.4 mg/kg of the mixture induced hepatic microsomal monooxygenases. Pretreatment with higher doses (14 and 140 mg/kg) induced several hepatic drug-metabolizing enzymes, caused mild alterations in thyroid architecture, changes in hepatocellular nuclei, and changes in hepatocellular cytoplasm organization.

Contaminated soil is a significant source of environmental exposure to TCDD. Previous studies have demonstrated that TCDD is an inducer of hepatic microsomal aryl hydrocarbon hydroxylase (AHH) and cytochrome P-450 but not ethylmorphine N-demethylase (ED). However, no information is available about TCDD’s effectiveness when encountered as a contaminant in soil. In the present study, female rats were treated with TCDD in either corn oil or contaminated soil from the Minker site in Missouri. Doses of 0.3, 1, 0.2, 0.04 and 0 μg TCDD per kg body weight were used. A third group received equal amounts of soil uncontaminated with dioxin. The results showed that at any dose, TCDD in corn oil and soil are equally effective inducers of hepatic AHH. The induction ranged from about 80-fold at the highest dose to 6-fold at the lowest dose studied. TCDD also caused an increase in cytochrome P-450 concentration and a shift in spectral peak from 450 to 448 nm. There was no effect of TCDD on ED, consistent with previous reports. Our results suggest the bioavailability of TCDD in soil in rats is at least 50%. Therefore, intestinal exposure to TCDD contaminated soil may constitute a significant health hazard in view of its extremely high toxicity and relatively high bioavailability. Further studies will be required to establish the degree of availability in humans.
EFFECT OF PARASITISM ON SEVERAL INSECTICIDE DETOXIFYING ENZYME SYSTEMS IN THE TOBACCO BUDWORM. L.A. Fix. Toxicology Program, Entomology Dept., North Carolina State University, Raleigh, NC. Sponsor: W.C. Dauterman.

Tobacco budworm larvae, Heliothis virescens (Fab.), parasitized by the braconid Cardioglossa nigripennis Viereck and comparable control larvae were assayed for the activity of several enzyme systems important in insecticide metabolism. For most of the enzyme systems tested, there was little difference in activity between control and parasitized larvae. The activity differences in gut preparations were the reduction of carboxyesterase activity in parasitized as compared with control larvae. And the activity differences in the fat body preparations were an increase in parasitized larval microsomal monooxygenases relative to the controls. These differences in the ability to detoxify insecticides offer an explanation for the previously observed greater susceptibility of parasitized larvae to insecticides, such as methyl parathion and permethrin.

THE EFFECTS OF THE QUINONE TYPE DRUGS ON THE HYDROXYL (OH-) PRODUCTION BY RAT LIVER MICROSONES. A. J. Tobia, D. Couri, and A. Sagone, Departments of Pharmacology and Medicine, Ohio State University, Columbus, OH 43210.

The quinone drugs are known to be metabolized to the semiquinone free radical intermediate and to enhance NADPH oxidation in microsomal system. We therefore, studied the ability of these drugs and related structural drugs to enhance the decarboxylation of 14C-benzoic acid via OH* production in the microsomal system. The drugs tested were: adriamycin (ADRIA), mitomycin C (MITO. C), 5-fluorouracil (5-FU), cytoxan (CTX), and methotrexate (MTX) and the concentration of each drug was 100 µg/ml. We found ADRIA and MITO. C stimulated decarboxylation of benzoic acid above the control by 100 and 49%, respectively. Conversely, 5-FU, CTX, and MTX did not stimulate decarboxylation. We also found that superoxide dismutase increased decarboxylation of benzoic acid in the reaction with and without the drugs present, as opposed to catalase which inhibited both circumstances. This provides evidence that the quinone drugs enhanced OH* production by liver microsomes in our system. Suggesting a possible mechanism for damage to cellular components by these agents.

ALTERNED NEUROMUSCULAR TRANSMISSION FOLLOWING ACUTE THALLIUM ADMINISTRATION. W.D. Atchison and M. Rosner. Dept. of Pharmacology and Toxicology, Center for Environmental Toxicology, Michigan State University, East Lansing, MI 48824

Poisoning due to accidental ingestion of thallium pesticides is still a problem in human and veterinary medicine. Muscle weakness and paralysis are among the symptoms associated with thallium poisoning however the mechanism underlying these effects is unknown. A potential paralytic site of action of thallium is the neuromuscular junction, where a number of heavy metals are known to disrupt transmission. The goal of the present study was to examine effects of bath applied thallous sulfate on neuromuscular transmission in the rat hemidiaphragm preparation using conventional intracellular microelectrode recording methods. Under conditions of reduced quantal content (Ca**+ = 1 mM, Mg**+ = 8 mM) 100 µM thallium produced a marked increase in nerve-evoked acetylcholine (ACh) release as measured by its effects on end-plate potential (EPP) amplitude, within 1-2 min. With continued application, thallium decreased EPP amplitude. This later effect may be due to depolarization of the presynaptic nerve terminal because end-plate membrane potential was depolarized with continued thallium application. Spontaneous ACh release, measured by miniature end-plate potential frequency, was increased by thallium. This increase could be reversed by washing with thallium-free solutions. Thus, acute administration of thallium alters synaptic transmission perhaps by depolarization of the presynaptic nerve terminal. (Supported by NIH grant ES-1361 and a BRSG grant to the MSU College of Veterinary Medicine.)

VISUAL CORTICAL AND HIPPOCAMPAL LOSS OF GABAERGIC RECEPTORS IN LEAD EXPOSED RATS: ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL CORRELATES. D.A. Fox and L.B. Costa. College of Optometry, Univ. Houston, Houston, TX and Dept. Env. Hith., Seattle, WA.

Children poisoned by Pb have decreased spatial vision and recurrent seizures. Adult Long-Evans hooded rats developmentally exposed to low Pb have decreased visual acuity (Fox et al., Toxicologist 3: 69,1983) and altered seizure responsiveness (Fox et al., Neurotoxicologist 1: 149,1979). To determine possible GABAergic contributions to the spatial deficit, pattern-reversal evoked potentials were recorded in awake rats following biccuculline (5-50 µg/kg, ip). To examine the pharmacological basis of the seizure alterations, the iv D50 of biccuculline was determined. These findings were correlated with 3H-muscimol (GABA ligand) binding data in retina, superior colliculus, lateral geniculate nucleus, visual cortex (VvX), frontal cortex, caudate, hippocampus (HIPP) and cerebellum. Biccuculline produced dose-response decreases in visual acuity in both groups, however, the decreases were greater in the Pb group. The CD50 was decreased approx. 20% in the Pb group. Decreases in 3H-muscimol binding were only found in VvX (~35%) and HIPP (~25%). These electrophysiological and pharmacological results demonstrate biccuculline supersensitivity. They suggest that the longterm effects of developmental Pb exposure may be on intrinsic GABAergic receptors in the VvX and HIPP. Supported by ES 01831 (LGC), and OH 07085 and ES 03183 (DAF).
457 EFFECTS OF TRIETHYL AND TRIMETHYL TIN AND LEAD ON REGIONAL CONTENT OF AMINO ACIDS IN THE RAT BRAIN. T.J. Walsh, M.E. Wilson, P.N. Hudson, T. Kanamatsu, J.S. Hong and H.A. Tilson. National Institute of Environmental Health Sciences, Research Triangle Park, NC.

To better understand the neurochemical substrate of organometal toxicity, the effects of triethyl and trimethyl lead and tin (TEL, TML, TET and TMT) on amino acid levels in the brain were studied. Adult, male Fischer-344 rats were injected s.c. (3/4 LD50) with one of the metals and sacrificed 7, 14 or 28 days later for neurochemical analysis. Levels of taurine (TAU), aspartate (ASP), glutamic acid (GLN), glutamate (GLU), glycine (GLY) and gamma-aminobutyric acid (GABA) were determined in the hippocampus (HIPP) and frontal cortex by HPLC. Levels in the corpus striatum (CS) of rats exposed to TMT were also examined. TML, TET and TEL had no effect on any of the amino acids in any of the regions studied. TMT decreased TAU in the HIPP and FC up to 20 and 7 days postdosing, respectively; GLN was increased in these areas up to 14 and 7 days after dosing, respectively. TMT also decreased GABA in the FC and HIPP and decreased GLU in the HIPP 7 days postdosing. GLN in the CS was increased 7 days after TMT. These data indicate that TMT, but not TET, TML or TEL, affects the putative inhibitory transmitters GLU, TAU and GABA in the HIPP and FC. Regionwise increases in GLN suggest that TMT alters the disposition of GLN and/or GLU or that it increases ammonia content in the brain.


Itoi–Ito disease in Japan, characterized by extensive skeletal demineralization and attributed in part to cadmium (Cd) ingestion, occurred primarily in postmenopausal women with a history of multiple childbearings. To investigate effects of Cd exposure, diet, and reproductive history on the endocrinology of this disease, 3 groups of female mice (260–340 mice/group) were given purified diets containing 0.25%, 5%, or 50 ppm Cd. At each Cd level, mice received either a sufficient diet (+) or an "Itoi–Ito disease" diet (−) (deficient in certain vitamins, minerals, and fat). One-half of the females (PL) were bred for 6 consecutive pregnancy/lactation cycles; remaining females were nonpregnant controls (NP). Cd-dependent decreases in Ca content, dry weight, Ca/dry weight ratio, and ash/dry weight ratio of femurs were not significant (NS) on either diet at 5 ppm Cd (compared to 0.25 ppm). For mice on the + diet at 50 ppm Cd (compared to 0.25 ppm) the parameters listed above decreased significantly: by 21%, 18%, 12% and 8%, respectively, for PL females and by 9%, 8%, 7%, and 8%, respectively, for NP females. Decreases on the diet at 50 ppm Cd were smaller than on the + diet. In conclusion, Cd-dependent bone mineral loss occurred in multiparous mice at 50 ppm Cd; this loss was not extensive. (Work supported by the U.S. Department of Energy under contract No. W-31-109-ENG-38.)

460 ENHANCED ACCUMULATION OF MANGANESE IN RAT LUNG: A POSSIBLE EXPLANATION FOR THE INCREASED TOXICITY IN MMT IN THE RAT. P.A. McGinley, J.B. Morris, G. Glantzos. Section of Pharmacology and Toxicology, School of Pharmacy, University of Connecticut, Storrs, CT.

MMT (methylcyclpentadienyl manganese tricarbonyl) can cause death resulting from pulmonary edema and hemorrhage independent of the route of administration. We attempted to produce an accelerated neurotoxicity in the rat with MMT as was previously demonstrated in the mouse (Glantzos and Murray, Neurotox. 3:75, 1982), but were unsuccessful due to enhanced MMT pulmonary toxicity in the rat. In investigating this phenomena further, we noted the increased ability of the rat lung to accumulate manganese. Twenty-four hours after injection of MMT 10 mg/kg s.c. manganese concentrations in the blood, brain and lung of rats and mice were determined by carbon furnace atomic absorption spectroscopy. The levels of manganese in the blood and brain were similar in both species. However, the lung to blood ratio of manganese in the rat and mouse were 19 and 6, respectively. We assume the manganese we measured is in the form of MMT or a closely related metabolite, since another manganese compound, manganese oxide (MnO2) when given by diet or injection did not accumulate in the lungs of either rats or mice. This demonstrated enhanced ability of rat lung to accumulate MMT could be a possible mechanism for the enhanced toxicity of MMT observed in the rat. (Supported by NIH Grant ES02471.)

458 THE MOBILIZATION OF INTRACELLULAR CADMIUM DEPOSITS UNDER THE INFLUENCE OF DITHIOCARBAMATES. Mark M. Jones, Shirley G. Jones and Leslie A. Shinobu. Department of Chemistry and Center in Environmental Toxicology, Vanderbilt University, Nashville, Tennessee 37235

Dithiocarbamates are effective antidotes for both acute and chronic cadmium intoxication in mice. Unlike other chelating agents, with dithiocarbamates the antitotal efficacy does not drop off rapidly as the time interval increases between the administration of the cadmium and that of the antidote. In chronic cadmium intoxication, dithiocarbamates can mobilize cadmium from intracellular deposits in the kidney and liver which are two or more weeks old. Structure-activity relationships indicate that the most effective dithiocarbamates are those in which groups of modest polarity are attached to the nitrogen atom.

Trimethyl tin-Cl (TMT) and triethyl tin-SO₄ (TET) have been shown to be potent neurotoxins in many species. Rats were trained to respond on a schedule of reinforcement in which only those responses separated by a 10 to 14-sec period of no responding produced a food pellet (DRL 10-14 sec). Each rat received a single dose of TMT (5.6, 7.5 or 10 mg/kg) or TET (1, 3 or 5.6 mg/kg). The lowest dose of TMT (5.6 mg/kg) and the lowest dose of TET (1 mg/kg) were without significant effect. At 5.6 mg/kg and 10 mg/kg TMT, the percentage of the total responses spaced 10-14 sec apart decreased over the first 7-9 days after TMT. Those rats receiving 5.6 mg/kg TMT gradually returned to control values over the next 2-3 weeks while those rats receiving 10 mg/kg never recovered. Rats receiving 3 and 5.6 mg/kg TET showed a decrease in the percentage of reinforced responses immediately after receiving TMT. The behavior of those rats receiving 3 mg/kg returned to control levels in 24 hrs while the health of the rats receiving 5.6 mg/kg failed rapidly, and they were sacrificed 6 days after receiving TET. These results led to several criteria, including amino acid content (Pharmacol 25:248, 1963). Despite this apparent lack of TMT, the Cd-hemoglobin assay for MT detects high basal levels in tests. Initial experiments indicated that the Cd-hemoglobin method detected primarily TMBP-2 in gel-filtered G-75 testicular cytosol, suggesting polymerized forms of MT. Like MT, TMBP-2 separated into two forms by anion exchange chromatography. However, amino acid analysis showed marked differences between TMBP-2 and MT, particularly in cysteine content. TMBP-2 was similar to TMBP-3 in amino acid content. Freezing/thawing or heat treatment of tests resulted in disappearance of TMBP-2 with a concomitant increase in TMBP-3. Furthermore, addition of a reducing agent (dithiothreitol) or protease inhibitor (N-ethylmaleimide) inhibited the appearance of TMBP-3. Results suggest that the low MW Cd-binding protein of rat testes is a breakdown product of a higher MW sequence. (Supported by USPHS Grants ES 01142 and ES 07079).

463 TRIMETHYLtin EXPOSURE RETARDS SERIAL PATTERN ANTICIPATION LEARNING IN RATS. S.B. Fountain and Z. Annau. Dept. of Environmental Health Sciences Johns Hopkins Univ., Baltimore, MD 21205.

Trimethyltin (TMT) is a neurotoxic organometal which produces a variety of learning and memory impairments in laboratory animals and humans including impairments of avoidance learning, maze learning, and problem solving. The present study investigated the effect of TMT exposure on serial pattern anticipation learning in rats. Rats were intubated once with either 0 or 7.0 mg/kg TMT one week prior to the experiment. Rats learned serial patterns composed of various quantities of brain stimulus reward (BSR) pulses; they received BSR quantities in a predetermined order for leverpresses in a discrete-trial operant task. All rats received two serial patterns (18-1-0 versus 18-1-0 pulses of BSR) that alternated within each daily session. Each day rats received 50 repetitions of each pattern. The results indicate that TMT-exposed rats required significantly more repetitions of their patterns than controls to learn to respond fast for large BSR quantities and slowly in anticipation of small BSR quantities. However, TMT exposure did not affect either the reinforcing properties of BSR or rats' asymptotic performance. These and other results support the notion that TMT exposure impairs the cognitive abilities involved in serial pattern anticipation learning in rats. (Supported by NIH Research Service Award MH 08759, ES 07094, and ES 02277.)

462 RAT TESTICULAR Cd-BENDING PROTEINS: APPARENT SOURCE OF THE LOW-MOLECULAR WEIGHT (MW) PROTEIN. M.P. Wakelee, B.B. Sarnoff and C.D. Klaassen, Dept. of Pharmacol., Toxicol. & Therap., Univ. of Kansas Medical Center, Kansas City, KS.

Three major groups of testicular metal binding proteins (TMBP), with approximate MW of > 80,000 (TMBP-1), 25,000 (TMBP-2), and 8,000 (TMBP-3) were separated by gel-filtration. TMBP-3 has been previously classified as metallothionein (MT), although we recently demonstrated that TMBP-3 is distinct from MT by several criteria, including amino acid content (Pharmacol 25:248, 1963). Despite this apparent lack of MT, the Cd-hemoglobin assay for MT detects high basal levels in tests. Initial experiments indicated that the Cd-hemoglobin method detected primarily TMBP-2 in gel-filtered G-75 testicular cytosol, suggesting polymerized forms of MT. Like MT, TMBP-2 separated into two forms by anion exchange chromatography. However, amino acid analysis showed marked differences between TMBP-2 and MT, particularly in cysteine content. TMBP-2 was similar to TMBP-3 in amino acid content. Freezing/thawing or heat treatment of tests resulted in disappearance of TMBP-2 with a concomitant increase in TMBP-3. Furthermore, addition of a reducing agent (dithiothreitol) or protease inhibitor (N-ethylmaleimide) inhibited the appearance of TMBP-3. Results suggest that the low MW Cd-binding protein of rat testes is a breakdown product of a higher MW sequence. (Supported by USPHS Grants ES 01142 and ES 07079).


Cigarette smoking has been associated with an increased risk of cardiovascular disease as well as lung cancer. Dietary Se levels have also been shown to correlate inversely with the incidence of cancer. VIT E is an antioxidant and prevents formation of free radical lipid peroxides which may be involved in cancer as well as atherosclerosis. This study determined the impact of dietary Se and VIT E on prostaglandin generation to prostacyclin (PGI2) or thromboxane (TXB2) as a function of smoke exposure. Rats were placed on basal diets supplemented with 0 to 3 ppm Se as well as 0 or 20 ppm VIT E for 41-43 wks. Daily exposures to fresh smoke from one UK ZR1 cigarette was given for 31-32 wks. Percent COHb values were 0.75±0.12 and 4.73±0.12 in sham and smoke-exposed groups, resp. Rats were sacrificed 24 hrs. after the last smoke exposure and platelets, aortic rings and lung homogenates were prepared. Lung AHG activity was increased nearly 2 fold in smoked rats over shams. 0 ppm Se caused a 28% decrease in aortic PG12. Smoke exposure produced a 20-50% decrease in aortic PG12 generation compared to shams in the 3 ppm Se, 0 ppm VIT E, and 0.03 ppm Se groups resp. Smoking increased platelet TXA2 2.8 and 2 fold in 3 ppm Se and 0 ppm VIT E groups, resp. It is concluded that smoke and dietary Se alter PG12-TXA2 generation. Supported by KTRF 48006 and 4A015.

Fifty-four Holstein bull calves were maintained on an all milk diet and given daily oral doses of Pb acetate (1 mg Pb/kg of body weight) for 8 weeks or (5 mg Pb/kg b.w.) for 1 week. BbPd rose dramatically after the first 6 hours following either a single dose of 1 mg Pb/kg or 5 mg/kg (3 \& 12 - 0 time, 4 and 5 \&/dl - 1 hr., 15 and 34 \&/dl - 3 hrs., 37 and 86 \&/dl - 6 hrs., respectively). Each following dose of Pb further increased BbPd so that the mean concentration reached 110 \&/dl after 1 week at the higher dose and after 5 weeks at the lower dose. ALAD activity decreased to <20% of pretreatment values in both groups within 6 hours after the 1st dose of Pb. The activity remained at this level in both groups throughout the experiment, and could not be used to differentiate intake levels of Pb. ZPP did not change significantly until 1 week following the initial dose of Pb.

Base levels in both groups was 2-3 \&; ZPP/Sm of Hb, while following 1 week of Pb the mean levels were 6-7 \&; ZPP/Sm of Hb in both groups. ZPP continued to rise in the calves dosed with 1 mg Pb/ Kg and reached a mean of 100 \&; ZPP/Sm Hb at the end of 8 weeks. These data clearly indicate that BbPb is still the best available diagnostic parameter for Pb absorption in cattle, but that ALAD and ZPP are helpful in evaluating the type and length of exposure. (Supp. by USDA Grant #79-CSR5-2-0485)


The kidney is considered as the critical organ in excessive exposure to inorganic mercury (Hg). In animal models, a significant amount of Hg is also deposited in the kidney even after organic Hg exposure. Dimercapto propionate sulfonate (DMPS), a hydrophilic analogue of BAL was found to mobilize Hg actively from the kidney. In order to evaluate the pattern of Hg chelation by DMPS and its usefulness in the estimation of renal burden of Hg, rats were exposed to 130 HgCl (0.1 to 2 mg/kg i.p.) or Hg vapor (0.5 to 2 mg Hg/m$.^3$). At different periods (7 to 38 days) after Hg exposure, DMPS (1 mmol/kg) was injected. Urine and feces were collected for 24 hrs. before and after DMPS treatment. Whole body burden, tissue levels and excretion of Hg were determined by measurement of radioactivity. Analysis of the data showed a significant decrease in the whole body burden of Hg and increase in urinary excretion after DMPS treatment. There was a direct correlation (r=0.94) between renal levels and urinary excretion of Hg. No such relationship was observed for other organs or fecal excretion of Hg. These results suggest that DMPS can chelate a certain fraction of renal Hg (61 to 67%) and the measurement of DMPS induced increase in urinary excretion can be used to estimate the renal burden of Hg in excessive exposures. (Supported by Grant ES01247).

468 CADMIUM AND LEAD CONCENTRATIONS IN THE HUMAN AMNIOTIC FLUIDS OF SMOKERS AND NON-SMOKERS. C.-P. Siegers, J. Jungblut, F. Klink, and F. Oberheuser. Departments of Toxicology, Gynecology and Obstetrics, Medical School of Lübeck (FGD).

Several reports have linked cadmium to both cigarette smoking and hypertension. We therefore investigated the amniotic fluids of 155 women, non-smokers (n=128) and smokers (n=27), with respect to their cadmium and lead concentrations. Both cadmium and lead were measured by atomic absorption spectrophotometry with carbon rod atomization after wet digestion of the samples. The mean ± s range of cadmium in the amniotic fluid of non-smokers amounted to 2.58 ± 1.36 \&/l, that of smokers to 7.29 ± 2.39 \&/l. Also there was a correlation between the extent of daily cigarette consumption and cadmium concentration. No correlations existed between the age of the patients, week of pregnancy, blood pressure, disturbances of pregnancy and the cadmium levels in the amniotic fluid. For lead higher concentrations were found as compared to cadmium, the mean ± s range for non-smokers amounted to 23.98 ± 4.92 \&/l, for smokers to 21.53 ± 7.16 \&/l. No correlations were found between smoking, disorders during pregnancy and the amniotic lead concentrations. The maternal and fetal risks of higher cadmium levels in the amniotic fluids of smokers remain to be clarified.
THE EFFECTS OF TRIMETHYL Tin CHLORIDE (TMTC) ON ACETYLCHOLINESTERASE (AChE) IN THE HIPPOCAMPUS OF RAT BRAIN.


The effects of TMTC on cholinergic systems in rat brain were investigated using AChE histochemistry in the hippocampus. Adult male Wistar rats were dosed with 12.25 mg/kg TMTC. Control and treated rats were dosed ip with 1.8 mg/kg di-isopropyl-fluorophosphate to inhibit AChE. Five hours after injection the animals were killed and processed for AChE in brain. Sections preincubated with paraoxon served as reaction controls. AChE histochemical staining revealed sharply decreased staining intensity around the CA2 and CA1 hippocampal areas of TMTC treated animals. Control animals showed uniform staining densities throughout the entire pyramidal cell tract (CA1-CA4). No differences were found in septal staining patterns between control and treated rats. Preincubation with paraoxon blocked the reaction. These results revealed the development of specific cholinergic lesions in the rat brain hippocampus related to TMTC, thus contributing to the elucidation of the pathogenesis of lesions by this toxicant.

CHELATE ANTIDOTES FOR GOLD (SODIUM BIS(THIO-SULFATE) GOLD (I) INTOXICATION IN MICE. Mark A. Basinger, Mark M. Jones, William M. Mitchell, Robert L. Forti and Stephen J. Gibbo. Department of Chemistry and Pathology and Center in Environmental Toxicology, Vanderbilt University, Nashville, Tennessee 37235.

A study of the relative efficacy of various chelate antidotes for acute gold intoxication in mice (200 mg/kg Na[Au(S₂O₃)₂]·2H₂O i.p.) showed 2,3-dimercaptopropanionic acid (DMSA) to be the most effective in terms of survival rate enhancement for the compounds tested. D-penicillamine as an antidote appeared to hasten the onset of death in animals when compared to control animals receiving gold alone. The effectiveness of DMSA in reducing gold concentrations of liver and kidney tissue was examined in animals receiving 140 mg/kg Na[Au(S₂O₃)₂]·2H₂O. Gold levels in control animals receiving gold alone were found to be 886 ± 113 ppm in kidney tissue and 109 ± 27 ppm for liver. In animals treated with DMSA gold levels were reduced to 163 ± 22 ppm and 60 ± 4 ppm for kidney and liver respectively. A pathological assessment of liver and kidneys of these animals showed involvement of proximal and distal tubules in the kidney. No difference was found between control groups and groups receiving DMSA for the dose and time sequence examined, both sustaining extensive renal tubular necrosis at 48 hours, even though DMSA treated animals showed a marked reduction in renal gold concentrations.


Both lead sulfate (PbSO₄) and lead dioxide (PbO₂) are used in the production of automotive and industrial batteries. A two-week endotracheal comparative toxicity study of PbSO₄ and PbO₂ was conducted. Because the toxicity expressed by any lead compound is the result of absorbed lead ions, any differences in absorbance of the two compounds should reflect the corresponding differences in systemic toxicity between the two lead compounds. Adult Sprague Dawley rats were dosed endotracheally with 15, 60 or 125 mg/kg of either the sulfate or the oxide for two consecutive weeks followed by a two-week recovery period. Blood lead, zinc protoporphyrin (ZP), erythrocyte protoporphyrin (EPP), aminolevulinic acid dehydrase (ALAD), urine and serum chemistries, a complete blood count, kidney and femur lead and a neurobehavioral screen were performed. The study was designed to observe signs of toxicity by monitoring those parameters that lead "classically" alters, i.e. heme synthesis, the neurobehavioral system and the renal system. No signs of frank toxicity were observed. No effects on either the neurobehavioral or renal system were found. The early stages of an effect on heme synthesis was seen in an alteration of the EP value, but not with ZP or ALAD. In this study blood lead values and tissue lead (kidney and femur) values were the best biological indicators of low level lead exposure. The results also indicate that the sulfate compound was more efficiently absorbed systemically than the oxide.
473 EFFECT OF LEAD TREATMENT DURING PREGNANCY AND LACTATION ON VITAMIN D METABOLISM. M.E. Shackelford and M.H. Bhattacharyya. Argonne National Laboratory, Argonne, IL. Sponsor: R.A. Fowler

This study was conducted to determine if lead ingestion during pregnancy and lactation will affect normal plasma calcium levels and the increase in 1,25-dihydroxyvitamin D levels normally observed during pregnancy and lactation. Rats (9-10/group) were given American Institute of Nutrition 76A diet containing either 250 or 500 ppm lead from day 1 of pregnancy to day 14 of lactation. Blood lead and plasma calcium content were measured by atomic absorption spectroscopy and 1,25-dihydroxyvitamin D levels by procedures of Shepard et al. (Biochem. J. 1979;182:55-69). Mean blood lead was elevated to 409 μg/dL and 479 μg/dL and mean plasma calcium was decreased 1.5% and 2.6% in the 250 and 500 ppm lead treated groups, respectively. The mean 1,25-dihydroxyvitamin D levels were decreased 20% and 52% in the 250 and 500 ppm lead treated groups, respectively. Evaluation of the toxic effects of lead on 1,25-dihydroxyvitamin D concentrations is limited in the rat because pre-treatment and post-treatment values cannot be determined in the same animal, and animal variability is large.

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474 URINARY CALCIUM AND ZINC EXCRETION IN LEAD-EXPOSED RATS DURING CALCIUM, DISODIUM EDTA TREATMENT. W. Victor, C.R. Miller, and R.A. Goyer. National Institute of Environmental Health Sciences, Research Triangle Park, NC

Essential trace metal excretion was evaluated in rats prior to, during and subsequent to chelation with Ca(Na)₂EDTA. Two groups of rats were exposed to either 10,000 ppm Pb (as acetate) or equimolar Na acetate in drinking water for 6 wks. On two baseline days prior to chelation, daily Ca excretion was 4.7 fold higher and Zn excretion 1.8 fold higher in Pb-treated rats. Urinary Na and K excretion were not different in the two groups. On 3 subsequent days, half of the rats in each group received i.p. injections of EDTA (40 mg/100 gm body wt.) while the other animals received saline. Urinary Ca excretion increased in both Pb-treated and control animals to an amount nearly equal to Ca administered in the EDTA. Urinary Zn increased 10 and 30 fold over baseline values in Pb-treated and control animals respectively. There was no change in Na or K excretion. During 3 recovery days, urine losses of Ca fell rapidly in EDTA-treated animals but Pb-treated animals still excreted levels significantly higher than controls. Urinary Zn levels decreased gradually in EDTA-treated rats. This study demonstrates exposure to lead produces hypercalciuria and hyperzincuria which is further exacerbated by chelation therapy. The magnitude of essential metal loss emphasizes the need for trace metal supplementation during chelation.


The sensitivity, magnitude, and persistence of urinary enzyme activities were studied in male and female rats gavaged with mercuric chloride. Changes in hematology, serum enzymes, bilirubin, and creatinine did not occur except at frank nephrotoxic doses. After 12 days treatment with 5.0 mg/kg mercury the most sensitive urinary enzymes were ALK-P and GGT which doubled in activity. There was a trace of tubular nephrosis in 3/5 males and 1/5 females at this dose. Higher mercury doses (10 and 20 mg/kg) caused progressive increases in tubular nephrosis correlated with 3 to 9-fold increases in ALK-P, GGT, and GGT, and up to 11-fold increases in urinary LDH enzyme activity. Renal mercury was 45-65 ppm. All of these urinary enzymes were elevated at 120 days in rats given 5.0 mg/kg doses. After 180 days dosing ALK-P and GGT remained higher than control urinary enzyme activity, when renal mercury levels were 87-123 ppm. There were only mild changes in kidneys of some of the males and none in female rats, characterized as cytoplasmic vacuolation of renal tubular cortical cells. Measurement of urinary enzyme activity is a sensitive, non-invasive method for the evaluation of chronic as well as acute nephrotoxicity.


Trimethyltin (TMT) increases latencies and decreases amplitudes of the acoustic startle response and produces substantial destruction of the organ of Corti. This experiment assessed the effect of TMT on auditory thresholds of rats by recording BAERs at three suprathreshold levels and extrapolation of amplitudes to electroencephalographic (EEG) noise levels. Adult male Long-Evans hooded rats (N=40) were divided into 4 groups: 0 (saline), 4, 5, and 6 mg/kg body weight TMT, administered by gavage. Responses to 512 rarefaction clicks were averaged to produce each BAER waveform. Three BAERs were recorded from each animal in response to clicks at 95, 85, and 75 dB peak spl. EEG noise level was determined by averaging 312 samples in the absence of an acoustic stimulus. The latency of the action potential of the auditory nerve was delayed, with little evidence of central auditory damage. Click thresholds were raised in a dose-related manner. Amplitudes were significantly reduced in treated animals. All of these findings are indicative of damage to the auditory periphery.
**Effects of Chronic Lead Exposure on Behavioral Reaction to a Complex, Novel Environment in Adult Rhesus Monkeys.** N.K. Laughlin and R.E. Bowman, Psychology Department, University of Wisconsin, Madison, WI. Sponsor: R.E. Peterson

Adult, female rhesus monkeys were studied to assess potential effects of lead exposure on behavior in the absence of other signs of toxicity. Lead-exposed monkeys (n=10) had received a total of about 4 years of daily lead exposure during the past 5 years. Lead was administered as lead acetate in daily drinking water. At the time of testing, group mean (+SEM) lead intake was 6.3 (+0.57) mg/kg/day and blood lead level was 95.7 (+10.4) µg/dl. Mean blood lead level for control monkeys (n=8) was 4.8 (+0.3) µg/dl. All animals appeared normal. Each animal was given access to a large, novel, complex environment for 15 minutes a day on 7 consecutive days. A variety of behaviors was scored. In general, compared to controls, lead-exposed monkeys were slower to enter the test room and once in the test room fear-induced more often, were less active, spent less time in visual exploration of the environment, and were less likely to engage in certain self-directed behaviors. The chronic lead levels employed in this study produced behavioral effects suggestive of increased timidity or fearfulness and decreased activity levels in a novel environment.

**Comparison of Metal Neurotoxicity: biochemical changes in brain regions and analysis of patterns of behavior.** D.R. Brown, M. Cleva, R. Schatz, and B. Callahan. Northeastern U., Boston, MA

Biochemical and behavioral changes have been reported with both adult and neonatal exposure to metals. Few general approaches are available to identify both site of injury to the brain and to detect subtle behavioral changes. Lipid peroxidation (LP) and beta-galactosidase (BG) were used to assess cellular toxicity in brain regions. Changes in activity patterns were used to quantitate behavior. Rats were dosed (i.p.) with thallium, lead or triethyl lead (TEL). (Tl dose was 4 or 8 mg/kg for 7 day-old (Adults). Pb dose was 3.75 or 7.5 mg/kg and TEL was 1.5 and 3.0 mg/kg in 3-11 day old rats). After dosing rats were videotaped during exploration of a novel cage and patterns of behavior were assessed by computer analysis. Brain regions were removed for LP and BG analysis. Dose-related changes in LP and BG were found in selected regions. For Tl the cerebellum, brain stem, midbrain, cortex and hippocampus had elevated levels of BG. LP changes were similar, but not identical to BG. For Pb the brain stem and cerebellum showed elevated LP. Behavioral links between exploratory and grooming patterns were altered in a dose responsive manner. The Chi² statistic provided a quantitative measure of the change. This study demonstrates dose dependent comparisons between behavioral and regional biochemical effects. The approach appears to be useful in determining no effect levels for neurotoxins.

**Four Essential Trace Metals and Three Specific Organ Weights in Rats With Restricted Dietary Food Intake - B.C. Lee, K.L. Schmieder, and H.C. Petering. Kettering Lab (#56), Univ. Cincinnati Medical Center, Cincinnati, OH 45267.**

Reduced food intake is a typical response in animals during toxicological investigations, especially after exposure to cancer chemotherapy agents. Although pair-feeding is assumed to cancel the differences in food consumption, effects of reduced food consumption alone cannot be examined with a diet restriction design. A 40% restriction of lab chow diet was imposed on 140 male Sprague-Dawley rats for 8 days. An ad lib fed group was the control. Manganese, copper, iron, and zinc were determined in heart, liver, and kidney. Organ and body weights were recorded. Elevated concentrations of Cu (12%) in the liver and Mn (12-26%) and Fe (17-69%) in heart, liver, and kidney were found in the diet restricted group. In remained constant in the three organs. Specific heart weight was unchanged, specific liver weight decreased 13% and specific kidney weight increased 13%, despite 40% body weight difference between the groups. Reduced dietary food intake alters essential trace metal levels and specific organ weights. Since these trace metals are involved in mechanisms which modify toxicity, the response to a toxicant can be modified.

(Research supported by NIEHS Toxicology Training Grant ES-07073)

**Effect of Pulmonary Instillation of Gallium Arsenide on the Biochemical and Histological Composition of the Rat Lung.** D.R. Webb, S. Wilson and D.E. Carter. Dept. of Pharmacology & Toxicology, Univ. of Arizona, Tucson, AZ 85721.

Male, Fischer-344 rats were intratracheally dosed with suspensions of gallium arsenide (GaAs) at 100 mg/kg. Particle size analysis determined the mean count and mean volume diameters to be 1.63 and 5.92 µm, respectively. GaAs administration resulted in significant increases in lung wet weight, dry weight, protein and DNA content two weeks after dosing. The lung content of 4-hydroxyproline (4-OH), an indicator of fibrosis, was also elevated but not significantly. The lack of a fibrogenic response was confirmed by an observed decrease in the 4-OH/lung wet weight and 4-OH/protein ratio. Approximately 36% of the dose as arsenic and 49% of the dose as gallium was retained after 14 days. Dissolution of GaAs was indicated by significant blood arsenic levels (116 ppm) while gallium was not detected. Alveolar deposits, believed to be the purported dissolution product Ga(OH)₃, were also observed microscopically. A decrease in the liver/B.W. ratio and an increase in the kidney/B.W. ratio accompanied systemic arsenic intoxication. The primary, histopathological observations in lungs receiving GaAs were pneumocyte hyperplasia, alveolar proteinosis, and interstitial pneumonia.

(Supported by NIEHS T32-ES07091.)
There is evidence that suckling rats have a greater capacity for intestinal transport of metals, both essential and non-essential, than do mature animals. It has been suggested that the iron transport mechanism provides a way for non-essential metals like Cd to be absorbed. The effect of iron on intestinal uptake of Cd (109Cd) was investigated in suckling (14 day old) adolescent (28 day old) and young adult rats (42 day old) using an in situ incubation technique. In the presence of 0.4 and 2.0 mM FeSO4, intestinal uptake of Cd from a 20 μM CdCl2 solution was significantly decreased in 14 day old pups. When dams and pups were placed on an iron-deficient diet (TD) tissue iron levels decreased in 28 and 42 day old rats but not in 14 day old rats. Intestinal uptake of Cd was significantly greater in iron deficient 28 and 42 day old rats but did not change in 14 day old pups compared to controls of the same age. Suckling rats injected with iron-dextran (SC) had a significant increase in tissue iron levels and showed a significant decrease in Cd uptake compared to controls. The results of the present studies suggest that intestinal Cd transport in the suckling, adolescent, and young adult rat is mediated, at least in part, by iron transport pathways. (Supported by Universidad de Oriente - Caracas, Venezuela and by NIH grant ES-02416)

Because of association of lead intoxication with gastrointestinal symptoms, the effect of 7-week ingestion of lead in rats on contractility of g.I. smooth muscle was determined under in vitro conditions. Male Wistar rats were fed 1/2 lead acetate in their diet (NIN-07); controls were pair-fed. After 7 weeks lead concentration was 389 ± 85μg/dl in blood and 4.51 ± 1.0μg/g in the colon. The in vitro isometric contractile responses of the fore-stomach, ileum and colon were evaluated with methacholine and KCl as agonists. Tissues were suspended in a physiologic saline which for lead-exposed rats contained 1.2x10^-5M lead acetate. The dose-effect curves for methacholine were analyzed by logistic function for ED50, slope and Emax/dry wt. Lead exposure significantly decreased the ED50 in the forestomach to 59.7% of control without changing the slope or Emax/dry wt. No changes were observed in ileum or colon. Maximum phasic and tonic responses to KCl (80mM) were not affected by lead. However, the initial rates of response to KCl were significantly less in forestomach and ileum from lead-exposed rats 54.6% and 48.6% of control, respectively. These data indicate that substantial accumulation of lead in g.I. smooth muscle does not impair and may potentiate response to a cholinergic agonist, but may slow the response to membrane depolarization. (Supported by NIEHS grant #02665)

Lead poisoning alters smooth muscle function in humans and causes crop dysfunction in pigeons. Most probably this effect is the result of toxicity at sites on the smooth muscle structure or the associated nerve plexus. We have devised a bathing medium for working with muscle strips in vitro which supports the activity of the crop tissue and accommodates the solubility characteristics of lead. Strips of crop muscle in this solution relax in response to the addition of lead chloride. Tetrodotoxin, propranolol and other neurotransmitter antagonists failed to alter lead-induced relaxation in our preparations, which indicates that the effect is not neurally mediated. Cumulative calcium concentration-response curves were shifted to the right by lead, by the calcium influx blocker verapamil, and by the phosphodiesterase inhibitor theophylline. Also, contractions produced by potassium-rich medium or betahistine chloride in the presence of 2.5 mM calcium were inhibited by verapamil, but not by lead or theophylline. These results suggest that lead acts at some step beyond the influx of calcium, possibly by inhibiting phosphodiesterase activity or the activity of contractile proteins. (Supported by NIEHS)

Our previous pure-tone auditory brainstem evoked potential (ABR) results in triethyltin-Br (TET) intoxicated rats (Evans et al., Toxicologist 3: 169,1983) demonstrated selective decreases to high frequency (HF) stimuli. Although TET is a known CNS toxicant, these HF deficits are characteristic of known cochlear (PNS) ototoxicants. To differentiate the possible PNS and CNS interactions in TET rats (1.5 mg/kg/day x 3 days, ip) we utilized cochlear microphonic (CM) and 5th (auditory) nerve compound action potential (CAP) recording techniques at the round window of the cochlea. Semi-thin cochlear sections were examined to correlate with the electrophysiological findings. At 24 hr following the final dose, the CM was decreased over 90% and the CAP was absent. Cochlear histology revealed deterioration of the tectum vasculosa in the basal turn (HF region) and less damage in the apical turn (low-mid frequency region). Patchy demyelination was observed in the spiral ganglion axons. The electrophysiological and histological findings describe previously unreported toxic effects of TET on peripheral sensory receptors. The results demonstrate a clear PNS tonotopic site of action for TET that is similar to known ototoxicants. In combination with our ABR data, these studies reveal that TET has both PNS and CNS sites of action. Supported by UTGSBS and ES-03183 (DAP)
Fetal uptake of mercury in pregnant rats exposed to elemental mercury vapor. C.S. Rao, Y.S. Tong, and V. Radchenko, Research Institute, American Dental Association Health Foundation, Chicago, IL 60611.

Industrial and occupational exposures to elemental mercury (Hg) vapor are well documented. In the dental profession, it continues to be of concern since the Hg-based alloys are currently in wide use. As a part of our studies on the teratogenic effects of Hg in pregnant Wistar rats, we studied the fetal uptake of Hg. The Hg levels in fetuses from dams continuously (24 h/day) exposed to Hg vapor at concentrations of 0.1 to 2.0 mg/m³ during days 1 to 21 of gestation ranged from 23.1 ± 4.7 to 106.0 ± 18.0 ng Hg/g of fetal tissue, while the corresponding maternal blood Hg levels were 100 ± 20 to 538 ± 166 ng/ml. The results indicate that: 1) fetal uptake of Hg occurs during maternal inhalation exposure to elemental Hg vapor, 2) Hg readily crosses the placental barrier, 3) fetal Hg levels parallel maternal blood levels, the former being about one-fourth lower, and 4) fetal Hg levels depend on the maternal Hg exposure concentrations. (Supported in part by a grant from the American Fund for Dental Health)

Effect of lead intoxication on Ca metabolism in cultured bovine brain capillary endothelial (BBE) cells. A.C. Nye and J.G. Pounds. National Center for Toxicological Research, Jefferson, AR and Division of Interdisciplinary Toxicology, Univ. of Arkansas for Medical Sciences, Little Rock, AR, and P.D. Bowman and C.W. Goldstein, University of Michigan, Ann Arbor, MI.

The brain capillary endothelium has been proposed as a possible site of action of Pb-induced brain edema. Experiments were performed to examine concentration dependent effects of Pb on the steady state metabolism of 45Ca in cultured BBE cells. Isolation was accomplished by subjecting bovine brain homogenates to a series of centrifugations. The cellular toxicity of Pb was measured by LDH release and 3H-leucine incorporation into protein. BBE cells were incubated for 20 hr in 0, 10, 50, or 100 µM Pb in all experiments. No leakage of LDH was observed at tested Pb concentrations. 3H-leucine incorporation into protein was depressed 8% by 100 µM Pb. Pb effect on 45Ca metabolism was examined by labeling BBE cells with 45Ca (1.8 mCi) for 20 hr to achieve steady state (SS) concentrations of 45Ca. The SS efflux curve was kinetically modeled by 3 intracellular mathematical compartments. Concentration dependent increases in each compartment were observed with 50 and 100 µM Pb treatments. Total cell 45Ca was increased to 400 and 800% of control in 50 and 100 µM Pb treatments, respectively. These findings support the conclusion that Pb alters 45Ca metabolism in BBE cells at concentrations which produce no measurable cellular toxicity.


To understand potential Cd-fetotoxicity, we investigated MT levels in human fetal liver (n=13), preterm- (n=13), and term-placenta (n=6). Samples from 13-21 week abortions were weighed and homogenized in buffered sucrose. Quantitation of MT was performed by the Cd193/Mb affinity assay of Eaton & Toal (TAF, 66:134). The relative MT content in preterm-, term-placenta, and fetal liver was 1:30:36 resp. while Zn content (µg/g wet wt.) in the crude cytosol was 2.8, 2.2, and 85.4 resp. Qualitative characterization of MT involved gel filtration on Sephadex G-75, followed by DEAE Sephadex A-25 column chromatography and identification of MT by zine quantitation using atomic absorption spectrophotometry. DEAE A-25 profiles of liver preparations revealed 3 peaks (A, B, C) of which the middle peak-B exhibited the highest affinity for Cd193, while A and C showed low but significant isotope binding. Peak B expressed bimodality and amino acid analysis is pending. In conclusion, we have resolved that: (a) as compared to fetal liver, preterm placental MT levels are very low, (b) term placenta exhibits high levels of Cd binding capacity, (c) available data suggest the presence of multiple forms of MT in human fetal liver. It is most probable that the human placenta affords little protection against cadmium insult during the early periods of organogenesis.


Recent studies suggested that the cellular metabolism of lead is modulated by physiological stimuli which regulates Ca homeostasis. These experiments were conducted to compare the effects of a) a calmodulin inhibitor on the cellular homeostasis of Ca-45 and Pb-210, and b) a calmodulin inhibitor and lead intoxication on the cellular homeostasis of Ca. Cells were isolated from adult Sprague-Dawley rats by collagenase perfusion in situ, and placed into culture in Williams' medium E. Cells were labeled for 20 hr with 25 µCi/ml Ca-45 (1.8 mCi) or 1 µCi/ml Pb-210 (3 µCi), with or without 10 µM TFP. The steady state metabolism of Ca and Pb was determined by kinetic analysis of washout curves. TFP increased total cellular Ca (120%) and Pb (190%) compared to controls. These increases were largely due to an increase in the fraction of the kinetic pool associated with mitochondrial Ca (140%) and Pb (190%). The kinetic pool S1 was increased only with lead (150%) while S2 was increased only with Ca (130%). No kinetic pool of Ca or Pb was decreased by TFP treatment. The data indicate that a) the effect of TFP on Ca and Pb homeostasis is similar, but not identical, and b) lead intoxication and TFP have very similar effects on cellular calcium homeostasis.
The effect of cadmium (Cd\(^{2+}\)), a component of cigarette smoke, on the pathogenesis of atherosclerosis has been examined in a number of studies and remains controversial. Determining the uptake of Cd\(^{2+}\) by platelets is a step toward examining the effects of Cd\(^{2+}\) on platelet function, which in turn affects the development of atherosclerotic plaques. Platelets obtained from adult male Sprague-Dawley rats were incubated with Cd-109 for 1 hr. Platelet Cd\(^{2+}\) uptake was calculated following measurement of supernatant Cd-109 concentration by liquid scintillation spectrometry. Platelet Cd\(^{2+}\) uptake increased with increasing Cd\(^{2+}\) concentration (0-1000 \(\mu M\)). At low Cd\(^{2+}\) concentrations (<109 \(\mu M\)), platelet-poor-plasma (PPP; 33-67%) increased Cd\(^{2+}\) uptake while at higher Cd\(^{2+}\) concentrations (182-344 \(\mu M\)) PPP decreased Cd\(^{2+}\) uptake. Bovine serum albumin decreased Cd\(^{2+}\) uptake, suggesting that nonspecific binding of Cd\(^{2+}\) to albumin might cause the decreased Cd\(^{2+}\) uptake observed with PPP at higher Cd\(^{2+}\) concentrations. The platelet uptake of Cd\(^{2+}\) in whole blood was determined to be less than the platelet Cd\(^{2+}\) uptake in washed platelets and platelet-rich-plasma. It is of note that little Cd\(^{2+}\) was taken up by erythrocytes during the 1 hr incubation. These data suggest that platelets absorb Cd\(^{2+}\) and that further studies will be necessary to determine the characteristics and consequences of platelet-Cd\(^{2+}\) interactions. Supported by U. of Ky. THRI Project No. KTRB 58516.

**EFFECTS OF METHYLMERCUY AND SODIUM SELENIUM ON TISSUE LEVELS OF CALCIUM, COPPER, IRON AND ZINC IN GUINEA PIGS.**


Female guinea pigs were given (per os) 10 doses of methylmercuric chloride with or without concomitant equimolar doses of sodium selenite. Tissue levels of calcium, copper, iron and zinc were determined 24 hours after the final dose. Selenium decreased the level of mercury in all analyzed organs. Mercury given alone was associated with increases in calcium levels in several organs but a marked decrease in cerebellum. Also, significant differences were found in brain, kidney and pancreas (copper), blood, pancreas and brain (iron), and spleen, pancreas, kidney and liver (zinc). Increased kidney copper may be involved with heavy metal induced renal toxicity, while the sharp decrease in calcium in cerebellum may be involved with neurotransmitter release inhibition and neural lesions ultimately produced by methylmercury, against which selenium appears to be protective. Histopathological studies are consistent with this hypothesis.

**INTESTINAL ABSORPTION OF Pb AND ITS INFLUENCE ON Zn ABSORPTION.**

L.B. Sassar, R.J. Chertok, D.K. Burbank, and F.H. Cross. Pacific Northwest Laboratory, Richland, WA.

The body burden of Pb is determined by intestinal absorption which can be influenced by age and dietary factors. Everted gut sacs were used to characterize the movement of Pb across the rat intestine at Pb concentrations of 10\(^{-6}\), 10\(^{-3}\), and 10\(^{-4}\)M. Tissue Pb concentrations approached a steady state (0.7-3x10\(^{-7}\)moles/g tissue) within 30 min. of incubation. There was a pronounced increase in the rate of Pb accumulation in the serosal fluid after 15 min. of incubation. Results suggest that Pb diffuses into intestinal tissue where part is bound and the remainder passes into the serosal fluid. As tissue Pb concentration approached a steady state, efflux of Pb into serosal fluid was equal to influx into the tissue. In another study the influence of Pb on intestinal Zn absorption was determined. Intestinal uptake of Zn was increased by increasing concentrations of Pb. Transport of Zn into serosal fluid was inhibited by 10\(^{-3}\) and 10\(^{-4}\)M Pb. Thus, Pb appeared to alter the permeability of the luminal membrane to increase the uptake of Zn but did not alter the transport of Zn across the membrane.

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**METHYLIFICATION NEUROTOXICITY IN THE RAT. I. TISSUE CONTENT OF ORGANIC AND INORGANIC MERCURY.**


A technique for separating organic and inorganic mercury (Copper & Smith 1978, Bull. Env. Contam. Toxicol. 19, 560) was used to investigate the nature of the mercury (Hg) distributed in tissues during development of methylmercuric chloride (MeHgCl) intoxication. Male Sprague-Dawley rats (300 g) were given p.o. 8 mg MeHgCl kg\(^{-1}\) containing Me\(^{203}\)HgCl on 6 consecutive days. Tissue distribution and conversion to inorganic form (Hg\(^{2+}\)) were determined during the latent phase (24 hr), and early (7 days) and late (30 days) symptomatic phase of intoxication. Total Hg detected in liver, kidney, whole blood and plasma reached a peak at 24 hr (36±8,110±3,1 and 1.6 mg g\(^{-1}\) wet wt., respectively) and declined thereafter by 28,40,37 and 50% at 30 days. At all time points, the Hg\(^{2+}\) in whole blood was less than 0.5% of total Hg and in the plasma it accounted for 11 to 13%. In the kidney, Hg\(^{2+}\) increased from 3.7% to 19.2% between 24 hr and 30 days. By contrast, in the CNS, less than 1% of the Hg was inorganic at 24 hr and 7 days, rising to 2% after 30 days. Total Hg in various CNS regions increased on average by 20% between 24 hr and 7 days, and values at 30 days suggested tissue clearance to be slow (e.g. at these times in the cerebellum 12.6,15.1 and 12.2 μg g\(^{-1}\) wet wt., respectively). The delay before concentrations of total Hg reached a maximum in the CNS may have been a factor contributing to the occurrence of a latent period. The low levels of inorganic Hg indicate that this form, unless much more toxic than organic Hg, plays a relatively small role in the development of MeHg-induced neurotoxicity. (Supported by Food and Drug Industries Council and Wellcome Trust).

The relationship between the cellular distribution of mercury (Hg) within the CNS and the development of methylmercury-induced neurotoxicity was studied in an animal model which displayed latent and symptomatic phases. Sprague-Dawley rats (200 g) were given 6 consecutive daily doses of 8 mg methylmercuric chloride kg\(^{-1}\) po. Sections of brain regions and spinal cord were examined by light microscopy after staining with hematoxylin and eosin, or a silver precipitation method to visualize Hg (Danusser & Schroder, 1979. Histochemistry, 60, 1). During the latent phase no histopathological changes were evident and Hg was detected predominantly within glial cells. Soon after development of neurotoxic signs degeneration of the cuneate and gracile tracts was observed and Hg deposition extended to the ependymal cells, Purkinje cells of the cerebellum and neurons of the medulla and spinal cord. In the chronic stage of symptomatic intoxication, these lesions were accompanied by extensive pyknosis and loss of granular layer neurons in the cerebellum. Few glial cells then contained Hg which was localized within all neuronal types except the granular layer. This study suggests that methylmercury follows an intracerebral pathway across the blood-brain barrier from capillary to glial cell, with neurotoxic signs coinciding with accumulation of Hg in the neurons.

(Supported by Food and Drink Industries Council and Wellcome Trust.)


Cerebral blood flow and two parameters of blood-brain barrier (BBB) function were investigated during the pathogenesis of methylmercury intoxication in an animal model which displayed latent and symptomatic phases. Treatment comprised 6 consecutive daily doses of 8 mg methylmercuric chloride kg\(^{-1}\) po to male Sprague-Dawley rats (300g). During both phases of neurotoxicity groups of animals were anaesthetized with pentobarbitone (40mg kg\(^{-1}\) ip) and estimations made of regional cerebral blood flow (rCBF) using \(^{125}\) iododeoxytryptamine. BBB integrity and transport function were assessed by measuring \(^{14}C\) mannitol penetration and the unidirectional flux of \(^{14}C\) D-glucose into regions of the CNS using the programmed infusion technique of Daniel et al. (1975, Med. Biol. Engng. 13, 214). In the latent phase a reduction in rCBF to all brain regions was observed, but the pattern of distribution was unchanged. During the symptomatic phase rCBF was similar to controls except in hind-brain regions where subsequently histopathological changes were most evident. Although the permeability of the BBB to mannitol was unchanged, glucose influx to the CNS was greater than control at equivalent plasma glucose levels but only in the latent phase. The reduction in rCBF at a time of increased uptake of glucose by the CNS suggests an uncoupling of the normal relationship between glucose influx, cerebral metabolism and blood supply. These findings suggest an early pathophysiological change occurring in the CNS before outward signs of toxicity appear. (Supported by Food and Drink Industries Council and Wellcome Trust.)

LEAD INDUCED MODIFICATION OF BEHAVIOR IS DEPENDENT ON THE AGE AT WHICH RATS ARE EXPOSED. D.H. Taylor, K.E. LaGory, C. Barnes, D.J. Zaccaro, R.J. Pfohl and R.J. Bull. Department of Zoology, Miami University, Oxford, OH 45056 and Toxicology and Microbiology Division, AFFL, USAFA, Cincinnati, OH 45268

Two different behavioral tests were used to evaluate the effects of lead on locomotor and exploratory behavior of rats under three exposure regimes. Rats that were exposed to Pb (200 mg/l in drinking water) during gestation exhibited no differences in exploratory behavior when tested in a novel test arena at either 28 or 60 days of age. Rats exposed to similar levels of lead, but for a longer time (through lactation) exhibited significant increases in exploration at 28 days, although not at 60 days of age. In a residential cage, both the gestationally exposed and those exposed through lactation exhibited depressed running-wheel activity from 50 to 60 days of age. In the third regimen, rats were exposed to the same concentration of Pb as young adults. These animals exhibited no significant differences in locomotor, feeding or drinking activity. These data suggest that the behavioral changes induced by lead are dependent on the age at which rats are exposed. (This abstract does not necessarily reflect EPA policy.)
EFFECT OF ANIMAL CAGING ON RESPONSE OF RATS TO URANIUM TOXICITY. E.G. Damon, A.F. Edson and T.C. Marshall. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM

F-344 rats, reared in polycarbonate (poly) cages, then housed in metabolism (metab) cages immediately after subcutaneous implantation of yellowcake (composed of ~82% ammonium diuranate and ~18% U3O8; 10 mg/kg body weight), to simulate a contaminated wound, died from uranium toxicity. Identically exposed rats maintained in poly cages, showed no overt toxic effects. Therefore, studies were conducted to assess effects of these two types of cages on nephrotoxic response of rats to implanted yellowcake. The LD50/21 days was 6 mg/kg (95% C.L. = 3-8 mg/kg) for rats (Naive) housed in metab cages beginning on day of implantation. However, acclimated rats (housed in metab cages for 21 days prior to implantation) had an LD50 of 360 mg/kg (95% C.L. = 220-650 mg/kg), which was the same value obtained for rats housed continuously in poly cages. This highly significant difference (P < 0.05) in response of "Naive" rats in metab cages compared to response of "acclimated" rats in metab cages or rats in poly cages can be related to significantly lower water consumption by "Naive" rats. The effects of changing cage types on water and food consumption of rats are pertinent to proper design and interpretation of toxicity studies. (Research performed for U.S. Nuclear Regulatory Commission via an Interagency Agreement under U.S. Department of Energy Contract DE-AC04-76EV01013.)


In two previous investigations, 50 ppm lead (Pb) acetate given to rats in drinking water was associated with increased rates of responding on a fixed interval (FI) schedule of food reinforcement. To determine if lower Pb concentrations would result in FI rate increases, chronic exposures to 0 and 25 ppm Pb acetate were initiated in 21 day old male weanling rats. Median response rates of the treated group increased to 200% of control over the first 40 experimental sessions. Differences were less apparent over the final 50 sessions. As in previous studies, individual differences in susceptibility to Pb-induced behavioral changes were apparent. Blood Pb and ZPP determinations were carried out after 30, 60 and 90 sessions. In previous studies, 50 ppm exposure produced blood Pb levels averaging 20-50 ug/dl. Exposure to 25 ppm produced blood Pb values of about 20 ug/dl. ZPP values did not differ between groups, remaining at approximately 23 ug/dl throughout the study. Brain Pb values averaged 0.028 and 0.07 ug/g for control and treated animals, respectively. Even lower Pb levels must be investigated to determine a no-effect level for behavioral impairment. NICHS Grants ES-01247, ES-0248, ES-03504 and ES-03079.


Sub-lethal doses of Cd had been shown to protect mice from a subsequent lethal dose. The effect was possibly due to the induction of metallothionein (MT). This study is to investigate the effect of cytosolic Zn-MT on Cd toxicity in isolated rat hepatocytes. Viability of hepatocyte preparations was assessed by trypan blue exclusion test and leakage of cytosolic enzyme. Groups of cells were exposed to 0, 4.5, 9.0, 18.0 and 36.0 μM of Cd respectively for 15 mins. Cells were then washed, and resuspended in fresh media. No significant difference was observed between any of the treatments groups and control in exclusion of trypan blue and leakage of cytosolic enzyme 6 hrs. after exposure. A dose-dependent effect was observed in the incorporation of 3H-Leu into cellular proteins upon Cd treatment. Exposure to 9.0, 18.0 and 36.0 μM of Cd significantly reduced the incorporation of 3H-Leu into proteins to 73%, 61% and 53% of the control at 6 hrs. after exposure. In hepatocytes, isolated from rats that were pre-induced with Zn for 24 hrs., significant protection against the reduction was observed. In the two highest dose groups, the values were 89% and 87% of the control. In conclusion, presence of Zn-MT in the cytosol protects isolated rat hepatocytes from Cd toxicity. The responses of the two groups could be correlated to the amount of non-MT bound Cd.

ALTERATIONS IN FIXED RATIO PERFORMANCE AND ZINC PROTOPORPHRYIN LEVELS PRODUCED BY CHRONIC LEAD EXPOSURE. D.A. Cory-Slechta. Div. of Toxicol., Dept. of Rad. Biol. & Biophy., Univ. of Rochester School of Med. & Dent., Rochester, NY 14642

Behavioral changes on a fixed interval (FI) schedule are reliably produced in the rat by chronic postweaning lead (Pb) exposure. To determine whether such effects were specific to the FI schedule and to further characterize the behavioral toxicity of Pb, performance on another reinforcement schedule was studied. On the fixed ratio (FR), behavior is rewarded on the basis of responses, while the FI rewards the first response after a specified time interval elapses. Animals were tested on FR values ranging from 1 to 100. Chronic exposure of rats to Pb in drinking water began at 21 days of age. Unlike the FI, the FR schedule was not disrupted by a 50 ppm Pb exposure. At 500 ppm, overall response rates decreased to 50-80% of control at the lower FR values (5 and 25) during the initial 15-20 sessions. Later, rates increased to control levels. Rate decreases did not reappear at the higher FR values. Blood Pb values at 500 ppm generally increased with exposure duration to levels of 80-100 μg/dl. ZPP levels of 50 ppm animals diverged from controls after 1 month of exposure (28.8 vs. 43.5 μg/dl), and continued to increase to 71.6 μg/dl after 8 months. Pb affects performance at a lower concentration than that at which FR behavior is disturbed; these data indicate that Pb has selective effects. NICHS Grants ES-01247, ES-01248, ES-03054, and ES-03079.

Chronic postweaning lead (Pb) exposure in the rat increases response rates on a fixed interval (FI) reinforcement schedule. To determine the species generality of the behavioral impairment, and the correlated biological exposure indices, the same exposure protocol was used in studies with the mouse. Chronic exposure to Pb acetate in drinking water began at 21 days of age. Fifty ppm produced performance changes similar to those seen in the rat: median response rates increased to 275% above controls over the first 35 sessions, then dropped to 175-200% over SS subsequent sessions. At 250 and 500 ppm, treated animals did not differ from controls; the delayed response rate increases characteristic of the rat were not observed in the mouse. During the first 30 days of treatment, 500 ppm retarded weight gain; this effect was not seen in the rat. The mean bloodbrain ratio for the mouse (1.05) was comparable to values reported both for the rat and human. Absolute tissue Pb levels (see below), however, were substantially lower than those produced in the rat with the same exposure protocol. The differences in performance may reflect species differences in the disposition of lead.

Pb Exposure (ppm) | 0 | 50 | 250 | 500
Blood Pb (ug/dl) | 1.0 | 5.7 | 36.1 | 76.4
Brain Pb (ug/g) | 0.016 | 0.15 | 0.57 | 0.82

503 Combined Unit for Housing and Nose-only Exposures in Conduct of Inhalation Carcinogenesis Studies. R.K. Wolff, J.A. Lopez, I. Wolf, W.F. Beierman, M.D. Hoover and R.O. McClellan. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM

A compact modular system has been implemented for the conduct of chronic inhalation carcinogenesis studies in a nose-only mode while minimizing contamination. A data logging isolation unit (CIU) is a glove box enclosure consisting of individual wire mesh caging for 80 rats and an 80-port nose-only exposure chamber. Nose-only exposures reduce pelt contamination, and consequent ingestion from grooming; also, less aerosol generation material is required. The rats are housed in the CIU for 24 hrs/day for up to 2.5 years. Pass boxes are used to introduce food, clean cages and cageboard, and to remove excreta and other contaminated materials. For nose-only exposures, rats are removed from their cages and placed in polycarbonate exposure tubes, which are plugged into the exposure chamber for 3-5 hrs/day. The rats are then removed from the tubes and returned to their cages. Computer monitoring of environmental conditions and exposure atmospheres is carried out with alarms for adverse conditions. Sham exposures have shown that this system works, but is labor intensive. Rats adapt well to the exposure regime, with body weight gains similar to those of controls. (Research supported under U.S. Department of Energy Contract No. DE-AC04-76EV01013.)


Conventional inhalation exposure systems suffer from a number of deficiencies. One particular problem is the inability to follow complex concentration-time profiles mimicking urban air pollution profiles. To provide an inhalation system capable of this type of concentration-time profile, we have developed a microcomputer control system. The exposure system consists of a Tylan mass flow controller, a Vinnis exposure chamber, a gas analyzer, a three-way valve and a Commodore Vic 20 microcomputer to coordinate the components. The computer, using a PID (proportional, integral and derivative) control loop, adjusts concentration levels within the chamber by modifying the flow of concentrated pollutant gas and comparing the chamber concentrations with the preset concentration levels. A three-way valve intermittently samples room air to ensure that toxic gases are not leaking into the room from the gas lines or the chamber. An alarm system sounds and shuts off the exposure under malfunction. A data logging system is incorporated to provide quality assurance and exposure data. The system has been found effective in controlling O₃, NO₂ and SO₂ exposures at minimal costs. (Supported by an EPA Cooperative Agreement and NIH Grant ES02916.)


Inhalation toxicology studies typically represent complex experimental designs with a wealth of data for processing. To address these challenges, Northrop Services, Inc. (NSI), through the auspices of a research program with NIHES, has developed and installed a computer-assisted inhalation exposure system that will significantly expand the capabilities of existing manually controlled systems. This program automatically addresses exposure control, data collection, and data processing requirements. The automation package handles not only steady-state exposure control but also preprogrammed dynamic (time-varying) exposure profiles. A unique calibration system, jointly developed at NIHES, allows the user to quantify all known sources of error and automatically run checks on analyses as needed. The system provides real-time display of all operating parameters including statistics on minute averages. Data validation is accomplished interactively for all data. Data presentation, including graphics, are available to the investigator on daily, weekly or any time basis desired. Except for calibration and maintenance all operation of the system including real-time data display are accomplished through computer terminals, located in the facility and at remote sites.

A system for simultaneously measuring cardiopulmonary function in 4 awake animals (200-800g) using a 4-channel plethysmograph has been developed. One day prior to testing, intrapleural and optional intravascular and arterial catheters are implanted. A unique restraint system reduces movement artifacts and keeps animals away from implanted catheters without imposing constraints on functional measurements. During testing, tidal volume, intrapleural pressure and electrocardiogram are measured. All primary signals are recorded on a polygraph, converted to digital form and stored on a computer. Six sec. breathing epochs are recorded, analyzed and the resulting cardiopulmonary measurements displayed on-line. Each of the 4 plethysmographs is mounted on the door of a 32 m³ standard inhalation chamber. Optionally, for small volume exposures, each animal's head is covered by a 500 ml funnel that is connected to a manifold inside the chamber. With this arrangement, all 4 animals can be exposed in the chamber while measuring cardiopulmonary function. After pollutant exposure, aerosolized bronchoconstricting agents are administered via the funnel to detect airway reactivity. So far, several sizes of rats and guinea pigs have been tested after exposure to air pollutants and bronchoconstricting agents. (This abstract does not necessarily reflect EPA policy).


Primary rat hepatocyte cultures and washed erythrocytes (RBCs) were used alone and in combination to study the effect of KCN on cellular ATP, urea synthesis and/or lactate dehydrogenase (LDH) release. Hepatocytes isolated by collagenase perfusion and plated at 2.5 x 10⁶ cells per 60 mm dish in hormone-supplemented culture medium were held for 24 h at 37°C under air-CO₂ (95:5). With the hepatocytes alone the three parameters studied were time and concentration dependent on KCN. ATP was the most sensitive, being depressed the earliest and at the lowest concentrations (0.1 mM). RBC LDH and ATP were unaffected up to 2.5 mM KCN. At this concentration hepatocyte ATP was barely detectable. In the combined system, RBCs in suspension over the hepatocyte cultures were aspirated just prior to analysis of hepatocyte ATP. Hepatocytes from the combined system responded similarly to hepatocytes alone; KCN-depressed ATP was readily reversed by replacing the medium with medium containing no KCN. After 10 min exposure to KCN, therapeutically used antides, sodium thiosulfate and sodium nitrite, were also effective in restoring ATP to control levels and in preventing further LDH release. The combined system may offer a convenient model for investigating the mechanism of action of KCN and its antidotes and evaluating antidote efficacy. Supported by DAMD17-82-C-2211.


The capability of subchronic (90-day) studies in laboratory animals to detect the cataractogenic activity of chemicals has been examined. A comprehensive survey of the literature reveals that cataracts were induced, after systemic administration, by 49 chemicals in the rat; 18, dog; 11, guinea pig; and 11, rabbit (sugars and chemicals that are cataractogenic only in neonate animals were excluded). Major classes include alkylating agents, organic solvents, naphthalene derivatives, and enzyme inhibitors. In continuous administration studies in which time of cataract development could be determined, cataracts were observed within 90 days for 26 of 39 (74%) chemicals that induced cataracts in the rat; 8 of 8 (100%) in the dog. Chemicals requiring longer than 90 days for cataract development include sulfonyl urea hypoglycemics and dilauroylglycerol (cataractogenic in the rat), synthetic progestational or estrogenic chemicals and diguat (rat and dog), and sodium cyanate (dog). Based on studies of all durations of exposure (acute through continuous) in which time of cataract development could be determined, cataracts were observed within 90 days of start of treatment for 31 of 41 (76%) chemicals in the rat; 10 of 14 (71%), dog; 7 of 7 (100%), guinea pig; and 8 of 8 (100%), rabbit. (Supported by EPA, Contract No. 68-01-6651. D. Sellers, Project Officer. Contents do not reflect agency policy.)

HEMOROGRAF AND COAGULATION DATA: A REAL TIME DATA COLLECTION AND REPORTING SYSTEM. R. Ruhren, V. Sciot, T. Scarneczias, R.A. Schroer, M. Brodeek Medical Research Division/Information Services Division, American Cyanamid Co., Pearl River, N.Y. Sponsor: L. Sarnano

The LEOFOX Hematogy system represents the latest development of the integrated on-line computer systems which have been developed for the collection and reporting of data from preclinical safety evaluation studies. The Hematology system was designed to facilitate the acquisition of Hematology data from automated instrumentation and manual work stations. The system features enhanced user interaction with the utilization of color CRTs, and improved data manipulation capabilities by the use of the VAX 2110 BNS system.

The hematogran data is captured as it is being generated from the instruments (Coulter S Plus, Hematrak), via manual work stations (manual WBC differentials, reticulocyte counts, cell morphologies) and CTR terminals. Various color displays are used throughout these processes for appropriate operator information and data flagging. Extensive error checking for data integrity, completeness, and protocol compliance is performed during data collection and reporting. System outputs include laboratory archive reports, edit and audit trail reports, data summary reports by individual animal, by single bleeding intervals per study and across time periods, as well as statistical summaries.
509 TOXICOLOGICAL USE OF THREE MICROASSAYS FOR IODINE AND IODIDE. H.G. Shertzer and M.W. Tabor, Kettering Lab, University of Cincinnati Medical Center, Cincinnati, OH 45267.

Accurate determinations of I₂ and I⁻ levels in tissues, diet and water are often of toxicological importance. Three useful procedures for determining I₂ and I⁻ were scaled to micro methods and evaluated. The Leuco Crystal Violet (LCV) dye binding procedure, the Cerium Catalytic Reduction (CCR) method, and the Iodide Specific Electrode (ISE) method had comparable precision and accuracy. However, the sensitivity ranges for the 3 assays were very different: LCV, 5-50nmol I₂; CCR, 1-10nmol I⁻; ISE, 0.25nmol-0.25nmol I⁻. Each method was useful for both I₂ and I⁻, by assaying before and after conversion of the redox forms: I₂ to I⁻ oxidation with ammonium persulfate; I₂ to I⁻ reduction with sodium thiosulfate. Dilute samples were concentrated by converting I₂ to I⁻ followed by treatment with anion exchange resin. Resin treatment and ethylacetate extraction eliminated most interfering substances. Tissue extracts could not be analyzed by LCV due to nonspecific bleaching of the dye. At 0.25, 5 and 10nmol I⁻, the ISE required 50, 4 and 2 min, respectively, to equilibrate. These results suggest that at more than 10nmol I⁻ the ISE has the range and response time to make it useful for small numbers of samples, while for large numbers of samples with more than 1nmol I⁻, the CCR method is recommended. The CCR method is applicable to inorganic I₂ and I⁻ analyses of water samples, diet and biological tissues.

510 QUANTITATIVE DIGESTION OF DIMETHYLARSENIC ACID TO INORGANIC ARSENIC AND ANALYSIS USING DIRECT HYDRIDE AAS. D.R. Webb and D.E. Carter, Dept. of Pharmacology & Toxicology, Univ. of Arizona, Tucson, AZ 85721.

The presence of dimethylarsinic acid (DMAA) in biological samples can cause an underestimation of total arsenic content when analyzed relative to an inorganic arsenic standard by direct-hydride flame atomic absorption spectrophotometry. An acid digestion procedure is described that quantitatively recovers DMAA as well as monomethylarsonic, inorganic arsenic(III), and arsenic(V) from aqueous and biological samples. Methylated arsenicals are converted to inorganic arsenic by wet digestion with HNO₃, H₂SO₄, and eq Cr₂O₇ and subsequently reduced to arsenic(III) with NaI. Arsines are generated with NaBH₄ and converted to atomic arsenic following immediate introduction into a nitrogen-entrained air-hydrogen flame. This method produced a linear relationship between absorbance and a concentration range of 0-300 ng arsenic/arsine reaction. A sensitivity of 2 ng arsenic and a detection limit of 7 ng arsenic/arsine reaction was also obtained. Recovery of DMAA from water, urine, feces, or whole blood ranged from 93-100% with a coefficient of variation of 5-10%.

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511 A MICRO-COLUMN METHOD FOR AFLATOXIN DETECTION. E.C. Shepherd and T.D. Phillips, Department of Veterinary Public Health, College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843.

An improved mini-column method for the rapid detection of aflatoxins has been scaled-down to a micro-column method. The micro-column uses less packing material, requires a smaller sample volume and is equal in sensitivity to larger mini-columns. Improved mini- and micro-columns were compared to established mini-column methods in terms of readability and sensitivity. The new columns, employing a bentonite-type processed clay as the primary adsorbent layer, overlayed with a thin layer of silica gel, were less subject to the band-spreadening sometimes seen with other mini-columns yet were equal in sensitivity and more economical. The column sensitivity for extracted peanuts and extracted peanut oil was determined to be 5 ppb. It was possible to analyze aflatoxin-contaminated peanut oil directly thus avoiding an elaborate extraction procedure, however, the detection limit was raised to 40 ppb. The new columns were tested for their reliability in screening peanuts and other commodities for aflatoxins. The strong affinity of aflatoxin for bentonite suggests that this sorbent may be used not only for detection but also in decontamination and/or detoxification of aflatoxin contaminated commodities.

(Supported by Title XII AID PROJECT 50305-2.)

512 QUANTITATION OF TRITIUM EXCHANGE IN IN VITRO AND IN VIVO EXPERIMENTS USING TRITIUM-LABELED AFLATOXIN B₁. H.E. Olsen, Y. Shao and D.D.H. Haich, Department of Environmental Toxicology, University of California, Davis, CA 95616.

Tritiated aflatoxin B₁ (3H-APF₁) has been widely used in experiments designed for elucidation of the mechanism of action of this potent carcinogen. However, the stability of the 3H label has posed a problem in the interpretation of experimental results. To evaluate the extent of this problem, the present study was undertaken to quantify 3H exchange in experiments involving 3H-APF₁. The 3H-APF₁ was first dissolved in ethanol, followed by evaporation of the solvent to remove readily exchangeable tritium. The ethanol-treated compound was then compared with ring labeled 14C-APF₁, for radiochemical stability in the subsequent in vitro and in vivo experiments. The in vitro experiments included incubation of 3H- and 14C-APF₁ with liver microsomes and storage of the labeled compounds in physiological saline at 37°C and -80°C. The in vivo studies consisted of i.p. injection of Fisher 344 rats followed by sacrifice at 6, 48 and 72 hours to determine covalent binding to DNA, RNA, and protein. In an aqueous environment 3H-APF₁ undergoes a slow, temperature dependent rate of 3H exchange. After 24 hours at 37°C this slow rate of exchange resulted in a 6 percent loss of the 3H label to water. At -80°C, 3H exchange was not significant. (Supported by NIH 5 Training Grant PBS PS07059.)
ANALYTICAL PROCEDURES FOR DETERMINATION OF POLYCHLORINATED DIBENZO-P-DIOXINS AND RELATED COMPOUNDS IN HUMAN AND ANIMAL TISSUES. M.L. Taylor, T.O. Tietman, J.H. Garrett, G.F. Van Ness, N.J. Fendler, D.J. Wagel, C.E. Eversen, S.R. Bultman, G.L. Ferguson, J.C. Bolch, Wright State University, Dayton, Ohio 45435. Analytical methodology has been developed and applied for the determination of polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) in human and animal tissues. These procedures entail digestion of the tissues, extraction of the compounds of interest into an organic phase, a sequence of liquid chromatographic fractionations, and detection and quantitation by coupled gas chromatography-mass spectrometry (GC-MS). Various WOT capillary GC columns are utilized to achieve complete isomer-specific detection for certain chlorinated classes of the PCDDs, (for example, the 22-TCDD isomers) for which adequate numbers of isomer standards are available. Data obtained by utilizing these analytical methods for determining PCDDs, PCDFs and PCBs in various tissues of animals collected at sites to which chlorophenoxyl herbicides have been applied will also be presented. These analytical procedures have also been employed to measure the concentrations of these compounds in human adipose and blood taken from persons who were exposed to sources of PCDDs and related compounds. Results of studies of this type will be described, along with similar data for unexposed control and/or general population subjects.

TRYPAN BLUE UPTAKE AS A NEW METHOD FOR STUDYING HEPATOTOXICITY IN PERFUSED LIVER. S.A. Belinsky, J.A. Popp, F.C. Kaufman, and R.G. Thurman, Depts. of Pharmacology, U. of N.C., Chapel Hill, NC; U. of Maryland, Baltimore, MD, and CITT, RTP, NC. Many hepatotoxins damage specific zones of hepatocytes within the liver lobule by mechanisms which require elucidation. A good method to study the events leading to selective toxicity should be simple, nearly physiological, and maintain the hepatic architecture. A new method which has these characteristics was developed employing the perfused rat liver. The uptake of trypan blue (5% 5 mM for 10 min) into livers following treatment with hepatotoxins was studied. N,N-bis (2-aminoethyl)diethylether, a hepatotoxin which causes necrosis of periporal (PP) regions, caused TB staining of nuclei of PP but not midzonal or pericentral (PC) cells when infused (350 μM) into livers from phenobarbital-treated rats for 60 min. Studies of TB uptake facilitate examination of the temporal relationship between alteration of key biochemical processes and the onset of damage. For example, following 30 min of AA infusion, bile flow decreased concomitant with the onset of lactate dehydrogenase release. 02 and AA uptake, glycogenolysis, and glycogenolysis were also monitored continuously in this model. When used in conjunction with biochemical studies, minute 02 electrodes and fiber optic light-guides, TB staining in the perfused liver will provide a better understanding of the development of tissue-specific hepatotoxicity. (ES-02759; ES-07126).

ASSESSMENT OF ISOLATED HEPATOCYTES IN SUSPENSION FOR TOXICOLOGICAL PRESCREENING. C. Haberk and J. Brodeur, Dép. méd. trav. et hyg. mill, Univ. de Montréal, Montréal, Canada. H3C 3J7. The toxicity of bromobenzene (BB), styrene (ST) and o-dichlorobenzene (DCB) was studied in vivo and in vitro to assess the use of isolated hepatocytes in suspension for the toxicological prescreening of aromatic hydrocarbons. In vivo hepatotoxicity was estimated by measuring plasmatic aminotransferases (ALT) 24 hr after i.p. injection of the compound to male rats. In vitro cytotoxicity was evaluated by measuring ATP release and glutathione (GSH) depletion in solvent-treated hepatocytes, during a 2 hr incubation. In vivo, maximal tolerated non-lethal doses were 12 mmol ST, 5 mmol BB and 4 mmol DCB per kg. In vitro, pronounced cytotoxic effects for the 3 solvents were observed at a similar final concentration of 5-6 mM. In vivo, ST was observed to be least hepatotoxic, followed by BB and DCB. Interpretation of the in vitro cytotoxic potential of the 3 compounds was much less distinct, relative toxicity being concentration- and time-dependent. The optimal predictive value of different biochemical parameters for toxicity evaluation varies with substrate concentration and incubation time, rendering it difficult to predict relative toxicity in vivo without having done detailed concentration-effect studies in vitro. Evidence of hepatocyte toxicity is not an indication that in vivo the same solvent will be selectively hepatotoxic. (Supported by IRSST, Québec).

MEASUREMENT OF IN VIVO LIPID PEROXIDATION USING THE THIOBARBITURIC ACID ASSAY FOR LIPID HYDROPEROXIDES. D.T. Kirkpatrick and R.D. Mavis, Division of Toxicology, University of Rochester Medical Center, Rochester, NY 14642. Conditions for the production of malondialdehyde chromophore (MDAC) from lipid hydroperoxides and thiobarbituric acid (TBA) were examined in order to assess the utility of the TBA assay for measuring lipid hydroperoxides following in vivo peroxidation. MDAC equivalent to 0.086 μmol TBA per mole of hydroperoxide was obtained when pure linoleic acid or arachidonic acid hydroperoxide, 50 μM Fe3+, and TBA at pH 3.7 were heated at 95°C under an N2 atmosphere. Chromophore production was reduced 50 percent when no iron was added, 70 percent with no added iron and under air, and 40 percent by 0.2 mM butylated hydroxytoluene. Production of MDAC from hydroperoxides is optimum in the absence of O2 and appears to be stimulated by Fe3+. When liver microsomes from CCl4 exposed and control rats were heated in this assay, MDAC from exposed animal microsomes was increased above control with color development in air, but not in N2. These results are not consistent with the existence of hydroperoxide formation in vivo, since the product would be expected to yield optimal MDAC in N2. Since TBA reagents can apparently form in vitro from exposed microsomes, such oxidation must be blocked by N2 to allow valid detection of in vivo peroxidation products by this TBA assay. (Supported by U.S. DOE Contract No. DE-AC02-76EV03940 and NIH Training Grant No. ES 07026.)
517 USE OF A MODIFIED 109-CADMIUM-HEMOGLOBIN ASSAY FOR QUANTITATION OF METALLOTHIONEIN IN BIOLOGICAL TISSUES. G.J. Chellman and G.L. Diamond, Division of Toxicology, University of Rochester, Rochester, NY 14642. Sponsor: P.A. Smith

This study reports on the development of a modified version of the 109-Cd-hemoglobin assay for determination of metallothionein (MT). Tissue 18,000 x g supernatant (SN) from rat liver, kidney, testes and pancreas was incubated with 109-Cd at a final concentration of 250 µg Cd/ml. Most of the excess free Cd was removed by 3 successive additions of 2% hemoglobin followed by heat denaturation and centrifugation. The final assay SN was fractionated on Sephadex G-75 Superfine to determine the distribution of Cd. Recovery of a purified Cd,Zn-thionein standard (MT II from rat liver) was 98.8 ± 2.6% under these conditions. Incubation with less than 250 µg Cd/ml resulted in significant losses in recovery due to isotope dilution. G-75 analysis of assay SN from all tissues examined revealed that significant amounts of Cd were not bound to MT. This was most pronounced in testes, in which 45.5% of the protein-bound Cd in the SN was associated with MT, but with a 90,000 Mw protein. Therefore, measurement of Cd in the final assay SN was not adequate for accurate quantitation of MT. From measurement of 109-Cd-MT in SN, the following MT concentrations (µg MT/g wet weight tissue) were obtained: liver = 20.1 ± 1.0, kidney = 88.0 ± 1.8, testes = 53.3 ± 1.4, pancreas = 29.4 ± 0.8. (Supported by NIH Grant ES 01247-Pilot Project, and NIH Training Grant ES 07026).


Disobutamide induces cytoplasmic vacuoles in a variety of cells in dog and rat, and has recently been shown to induce similar vacuoles in cultured dog coronary artery muscle cells (CM). Various cultured primary, cloned, or transformed cells were exposed to media containing 10^-4 to 10^-2 M disobutamide for 24 hours and examined in situ by light microscopy. Vacuoles were induced in all cell types exposed to drug. Chinese hamster ovary tumor cells were least sensitive (vacuoles in only 10% at 6x10^-4 M) and cloned bovine aorta endothelial cells were most sensitive (vacuoles at 10^-4 M). Human skin fibroblasts (Detroit 351) and mouse L-929 (transformed fibroblasts) were similar, with cell death at a drug concentration only slightly higher than that required for vacuole induction. Rat bladder carcinoma (BCR) and transformed rabbit aorta muscle cells (AMR) were more similar to CM with a threshold for vacuole induction of 2x10^-4 M, whereas cell death in BCR and AMR cells was observed only at 10^-3 M. Drug-induced vacuolation appeared not to be related to the cell doubling time or initial seeding density. Since culturing of BCR or AMR is reproducible, and since their response to the drug was most similar to that of CM, we concluded that BCR or AMR are most suitable in screening assays for drug-induced cytoplasmic vacuoles and for experiments on the mechanism of vacuole formation.


The response of the pulmonary alveolar type II cell to injury was investigated by evaluating the plasma and mitochondrial membrane electrical potentials as determined by the use of 86Rb" and [3H]TPMP". These cationic probes were shown to accumulate to steady state within the type II cell, with a resting value for the trans-plasma and trans-mitochondrial membrane potential of -61.9±4 Mv and -147.8±10 Mv respectively. The steady state concentration was found to respond in an expected fashion to the action of known depolarizing agents. In toxic exposure studies, a direct dose dependent depolarization of the cell was observed within 15 minutes after in vitro exposure to low levels of ionizing radiation (less than 20 Gy), or Phorbol myristate acetate (PMA). Radiation or PMA exposure which resulted in membrane depolarization was also found to be associated with the release of lamellar body phospholipid by the type II cell. These observations indicate the mitochondrial and plasma membranes of the type II cell are directly affected during lung injury. The results suggest a role for ion movements in the initiation of surfactant release. Supported by NIH Grant CA 27791.


A diffusion cell has been developed for automatic collection of samples for measurement of the absorption rate of chemicals through skin. Its accuracy was determined by comparing absorption data with results from the standard static diffusion cell (using rat skin) and in vivo experiments with rats. The required flow rate of saline solution through the cell was determined to be at least 5 ml/hr. For water, benzoic acid, and cortisol, absorption values and profiles were nearly identical in the 2 cells. Steady-state absorption rates of tritiated water were 4.3 and 4.4 µg/cm²/hr in the flow cell and static cell respectively. Absorption of cortisol (petroleum vehicle) after a 24-hr application was determined in the 2 types of cells and in vivo in rats. The results were similar: in vivo, 19.6%; flow cell, 20.1%; static cell, 22.8%. The value of a flow-through cell in the in vitro measurement of the absorption of hydrophobic compounds was examined. With normal saline receptor fluid, absorption of cinnamyl anthranilate was increased 1.7-fold by the flow-through cell. However, absorption was less than in vivo and less than that obtained in diffusion cells with a nonionic surfactant as the receptor fluid. The flow-through cell gives results equivalent to those of the static diffusion cell, and may be superior for measuring skin permeation of hydrophobic compounds.

Chinese hamster cells (V79) seeded in 25 cm² flasks at a density of 6 x 10⁵ were treated with the alkylating agents ethyl nitrosourea (ENU) and methyl nitrosourea (MNU) alone and in combination. At the completion of a 4 hour drug exposure, cells were rinsed (2x) and complete medium returned to the flasks. Following exposure, cell count and viability were determined by counting duplicate, trypsinized cell samples (trypan blue) using a hemocytometer. Over the concentration range of 2 x 10⁻⁴–10⁻³M, ENU caused 25 to 40% cytotoxicity compared to that of untreated controls. MNU produced 20 to 60% cytotoxicity over the concentration range of 10⁻⁴–10⁻³M, respectively. The combination experiment employed a factorial design in which cells were treated with both agents simultaneously in various concentration combinations. Analysis of the resulting concentration-response surface indicates that as the level of ENU increases in the combination, the toxic effect of MNU falls, i.e., antagonism. These experiments demonstrate the use of a powerful statistical procedure for analyzing the biological effects resulting from exposure to multiple cytotoxic agents. The analysis can be extended to other endpoints and is not limited by the number of treatment agents. (Supported by NIH ES02992).


Quantitative risk assessments require the estimation of the parameters in mathematically defined relations between dose and response. These relations may then be used to predict the increased risk above background levels corresponding to a given dose, the excess risk (ER), or to predict the dose corresponding to an acceptable increase in risk, the virtually safe dose (VSD). In most situations the model parameters, as well as ER, VSD, 1/VSD, and log(VSD), have distributions which are asymptotically normal. Results from simulated animal bioassays suggest that even for studies with relatively large numbers of animals, these quantities are not normally distributed. The use of confidence bounds based upon normality is therefore not supported empirically and alternative methods must be considered. For a situation where the exact distribution may be calculated, lower 95% confidence bounds on the VSD using normality are compared with the bounds obtained using 1) a likelihood ratio statistic, 2) sets of 1000 simulations, and 3) the exact distribution. Use of the normal approximation for for VSD or log(VSD) is very conservative, whereas use of the normal approximation for 1/VSD is usually anticonservative. The likelihood ratio and simulated bounds are however generally close to the exact bounds.

523 A CIGARETTE SMOKE EXPOSURE MODEL FOR ASSESSING EFFECTS ON CARDIOVASCULAR TISSUE CALCIUM RECEPTORS IN RATS. S. D. Harrison, Jr. and J. L. Cox. Grad. Ctr. for Toxicology, U. of Kentucky, Lexington, KY.

A well-standardized protocol for chronic exposure of rats or mice to mainstream or sidestream smoke from reference cigarettes has been developed at the U. of Ky. Tobacco and Health Res. Inst., permitting studies of various aspects of the toxicology of cigarette smoke inhalation in vivo. Male Sprague-Dawley rats received nose-only exposure to mainstream smoke once/day, 7 days/wk for 20 wks in a Griffith-Hancock MS-55 exposure system. Controls consisted of sham smoked and untreated rats. Weekly body wts, daily total particulate measurements, and daily COHgb data were recorded. Rats (at least 8/group) were killed 24 hrs after the last smoke exposure. Wt gain was reduced by sham and smoke exposure. Blood COHgb was typically 10 times higher in smoked than in sham rats immediately after exposure. Smoke exposure had no effect on terminal hematocrit or platelet counts. 

524 EFFECTS OF BUTYLATED HYDROXYANISOLE (BHA) ON HEPATIC GLUCURONOSYLTRANSFERASE ACTIVITIES AND UDP-GLUCURONIC ACID METABOLISM IN MICE. G.A. Hazelton, J.J. Hjelle and C.D. Klasing, Dept. of PharmacoL., Toxicol. and Therap., Univ. of Kansas Medical Center, Kansas City, KS.

The present study has examined possible biochemical mechanism(s) by which BHA increases the glucuronidation of xenobiotics. The process of glucuronidation (in vitro) was studied in male and female Swiss Webster mice that received BHA in the diet (1% w/w) for 10 days (600-800 mg/kg/day). UDP-glucuronosyltransferase activities were measured in native and detergent activated microsomes using 7 aglycones. In general, transferase activities toward acetaminophen, 1-naphthol, 4-nitrophenol, estrone and diethylstilbestrol were increased (136–241% of control) whereas activities with chloramphenicol and digitoxigenin monodigitoxoside were unchanged. In all cases (i.e. female/male or native/activated) transferase activity with acetaminophen exhibited the largest increase (approx. 230% of control). Further, BHA elevated hepatic UDP-glucuronosyl transferase concentration (1.9-fold) by increasing UDP-glucose concentration (1.4 fold) and enhancing UDP-glucose dehydrogenase activity (1.6 fold). The findings indicate that BHA feeding increases the capacity for glucuronidation in mouse liver by elevating UDP-glucuronosyltransferase activity towards specific substrates and increasing levels of UDP-glucuronic acid. (Supported by USPHS Grants ES 07079 and ES 03192).
THE EFFECTS OF GLUCURONIDE CONJUGATION ON BENZO(a)PYRENE-INDUCED CYTOTOXICITY AND MUTAGENICITY IN THE CHO/HGPRT ASSAY. L. Recio, A. W. Hsie. Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40536 and Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831.

Conjugation of lipid soluble xenobiotics with glucuronic acid is considered to be a major pathway of detoxification and elimination. Benzo(a)pyrene [B(a)P], a model carcinogenic mutagen, is biotransformed by the mixed function oxidase system to numerous phenols, quinones, dihydrodiols and epoxides. Using a mammalian cell gene mutational assay, Chinese hamster ovary cell/hypoxanthine guanine phosphoribosyl transferase (CHO/HGPRT), we found that the addition of uridine diphosphate α-D-glucuronic acid to a rat liver homogenate (S9) reaction mixture reduces B(a)P (0.5-6 μg/ml)-induced cytotoxicity but does not affect mutagenicity. Since the toxic nonmutagenic phenols, but not the proximate mutagen dihydrodiol, were shown to be the preferred substrates for glucuronide conjugation, the enhanced cell survival is most likely due to the reduction of phenols and quinones.

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Inhibition of uroporphyrinogen decarboxylase (UROD) by halogenated biphenyls such as 3,4,3',4'-tetrachlorobiphenyl (TCB) in chick embryo hepatocyte cultures is most easily detected by accumulation of uroporphyrin (URO), rather than protoporphyrin (PROTO) when cells are subsequently exposed to 0.15mM d-amino levulinate (ALA). Addition of 30μM piperonyl butoxide or 10μM ellipticine stopped the URO production and PROTO now accumulated at the same rate as in control cells. Ellipticine and piperonyl butoxide are known inhibitors of cyt P450's, ellipticine being a specific inhibitor of cyt P446. In cells preinduced for cyt P448 with 3-MC, TCB caused 50-60% inhibition of heme synthesis from 14C-ALA. Piperonyl butoxide restored heme synthesis to control values. Enzymatic assay of homogenates of cells previously treated with TCB (thus accumulating URO) failed to detect any UROD inhibition. UROD values using pentagone III as substrate were (pmol/min/mg protein) control: 15.4 ± 1.8 (4); TCB (3μM): 15.2 ± 2.2 (4); TCB + piperonyl butoxide: 14.0 ± 4.5 (3).

CONCLUSION: The inhibition of UROD by TCB in intact cultured liver cells is transient, reversible and dependent upon cyt P448. The results argue against postulated mechanisms for covalent binding of inhibitory metabolites to the enzyme.

Auranofin (AF) is an orally active gold-containing compound which is used in the treatment of rheumatoid arthritis. Since transition metals have been demonstrated to cause reductions in hepatic cytochrome P-450 (P-450), it is important to determine if gold alters hepatic drug metabolism. These studies were designed to investigate the effects of AF treatment on hepatic drug metabolism in the rat. AF was administered to male Sprague-Dawley rats (p.o.) as a single dose of 17, 34, or 68 mg/kg, or as daily doses of 0.2, 0.6, or 2.0 mg/kg/day for 3 or 14 days. Single doses (s.c.) of CoCl2 (60 mg/kg) and Co-protoporphyrin IX (33 mg/kg) were used as positive controls. Single dose AF treatment did not alter hepatic microsomal P-450 content, benzphetamine- N-deethylase (BPD) and ethoxyxycoumarin-0-deethylase (ECOD) activities. Three daily AF treatments caused no significant alterations in drug-metabolizing enzymes. Administration of AF for 14 days at 0.2 or 0.6 mg/kg did not alter drug-metabolizing enzymes; however at 2.0 mg/kg a significant reduction (41%) in P-450 content was observed. This decrease was not associated with reductions in BPD or ECOD activities. Treatment with cobalt compounds reduced P-450 content (19-33%), BPD and ECOD activities (42-75%). The results suggest that repeated high dose (20x therapeutic dose) AF administration causes isozyme specific P-450 content decreases.

THE EFFECT OF DIETARY EXPOSURE TO A MIREX AND CHLORODECOE COMBINATION ON CC14 INDUCED HEPATOTOXICITY. Andrea N. Bell and Hariharan M. Mehenale, Dept. Pharmacol. & Toxicol., Univ. MS Med. Ctr., Jackson, MS 39216.

Previous work has shown that dietary exposure to chlodeco (CD) at 10 ppm for 15 days potentiates CC14 hepatotoxicity and lethality while an identical exposure to its structural analog mirex (M), does not. While the effect of dietary exposure to M and CD alone have been studied, the effect of the two combined has not. The purpose of this study was to investigate the effect of M plus CD combined on CC14 hepatotoxicity. Male Sprague-Dawley rats were maintained on control diets or on diets containing 10 ppm Cd, 10 ppm M or M + CD (10 ppm each; MCD) for 15 days. On day 15, the rats received a single ip injection of CC14 (100 μl/kg) and hepatotoxicity was assessed 24 hr later. Significant increases in liver-to-body weight ratios and in serum enzymes (SGPT, SGOT and ICD) occurred in all 3 pretreatment groups following CC14 challenge. MCD and CD pretreatments led to significant cholestasis and decreases in biliary excretion of phenolphthalein glucuronide, while M did not. The combination treatment, MCD, did not potentiate hepatotoxicity above that seen with CD alone. These results provide additional evidence that CD pretreatment results in unique sensitization of animals to CCl4 toxicity in ways independent of the actions of M. (Supported by ES-07045.)
Previous studies from this laboratory reported an excessive accumulation of Ca$$^{2+}$$ in the liver tissue of rats upon receiving CCl$$\text{4}$$ after chlorocone pretreatment, both at individually nontoxic doses. A large accumulation of Ca$$^{2+}$$ in hepatic mitochondria was evident. Present study deals with the uptake of 45Ca in the mitochondria and microsomes from the livers of rats treated with chlorocone and CCl$$\text{4}$$. Male S-D rats were maintained on a diet containing 10 ppm chlorocone and on day 15 of dietary pretreatment, they received a single ip injection of 100 µl CCl$$\text{4}$$/kg in corn oil vehicle (1 ml/kg). The animals were sacrificed at 0, 6, 3, 6, 12, 24 and 36 hr after CCl$$\text{4}$$ administration and the 45Ca uptake in liver mitochondria and microsomes was measured. The uptake was studied at 4 Ca$$^{2+}$$ concentrations of 0.1, 1, 10, and 40 µM. Mitochondrial Ca$$^{2+}$$ uptake remained suppressed until 12 hr after CCl$$\text{4}$$ administration to normal rats, whereas microsomal Ca$$^{2+}$$ uptake remained suppressed until 24 hr after CCl$$\text{4}$$ administration to rats maintained on normal diet. Chlorocone by itself inhibited the mitochondrial Ca$$^{2+}$$ uptake but did not affect the microsomal Ca$$^{2+}$$ uptake. CCl$$\text{4}$$ administration to chlorocone pretreated rats significantly inhibited the mitochondrial as well as microsomal Ca$$^{2+}$$ uptake at all time points. (Supported by ES-01365.)

We have developed a system of primary cultures of postnatal rat hepatocytes to investigate the role of extracellular Ca$$^{2+}$$ in carbon tetrachloride (CT)-induced cell injury. The cultured hepatocytes were exposed to initial concentrations of 2.3 and 4 mM CT for durations of 15, 24, 45, and 60 min, in medium containing 1.88M Ca$$^{2+}$$. A dose-dependent decrease in microsomal glucose-6-phosphatase activity was observed ranging from 85% of control for the 2mm dose to 60% of control for the high dose. Leakage of lactate dehydrogenase (LDH) showed both time and dose-dependent changes caused by CT. The 2mM dose produced a significant increase in LDH leakage only at 60 min, while significant changes occurred at all time points for the 3 and 4mM doses, reaching 100% of control. Measurement of the loss of intracellular K$$^+$$ as a marker of membrane integrity compared favorably with the LDH leakage data. Interestingly, significant changes in total cellular ATP content paralleled the damage seen by LDH leakage and loss of K$$^+$$, with ATP levels dropping to 70% of control for the 4mM dose. When the hepatocytes were exposed to a Ca$$^{2+}$$ concentration of 3.6mM, a slight increase in CT-induced LDH leakage versus 1.86M Ca$$^{2+}$$ was noticed. But when the cells were treated without Ca$$^{2+}$$, a two-fold increase in LDH leakage occurred in 4mM CT treated cells. This study suggests that toxic injury of the liver may not necessarily be dependent upon extracellular Ca$$^{2+}$$. The major tobacco-specific carcinogen in tobacco smoke is nicotine-derived NNN. This cyclic nitrosamine induces lung adenomas in mice, tracheal tumors in hamsters and esophageal and nasal cavity tumors in rats. Chen et al (Cancer Res. 38:3639, 1978) showed NNN to be activated by α-hydroxylation at the 2'$^\text{nd}$ and/or 3'$^\text{rd}$ C of the pyrrolinium ring. The metabolism of NNN to reactive intermediates which bind covalently was assessed using male Sprague-Dawley rat liver microsomes. Incubations (37°C) contained 5mM NAD$$\text{P}$$, 1mM EDTA, an NAD$$\text{P}$$-generating system, 1mM [14C]-NNN and protein in 0.1N Tris pH 7.5. Blanks (−NAD$$\text{P}$$) were determined for each time and protein or substrate concentration. Protein was precipitated with 10% TCA and extracted six times with hot methanol to assess binding. Covalent binding of 14C was linear with protein to 3 mg/ml and with time to 90 min and was 168±64 pmol bound/mg protein/hr (N=5). EDTA extended the time course of linearity of binding. V$$\text{max}$ and Km of binding were determined from initial velocities (10, 30, 45, 60 min) at 0.125, 0.25, 0.5 and 1mM NNN. V$$\text{max}$ was 4.7 mmol bound/min/mg protein and Km was 0.91 mM. Thus, the covalent binding to microsomes in vitro indicates that NNN is activated to a reactive metabolite which binds tissue nucleophiles. (Supported by Kentucky Tobacco and Health Research Institute).

THE ROLE OF EXTRACELLULAR CALCIUM IN CCL$\text{4}$ INJURY OF CULTURED RAT HEPATOCYTES. K.S. Santone and D. Acosta, College of Pharmacy, The University of Texas, Austin, TX 78712.

The ability of two 5-Fluorouracil analogs to inhibit [14C]-incorporation into L1210 DNA and RNA was evaluated to clarify mechanisms of enhanced cytotoxicity observed after prior analog activation by hepatic mixed function oxidases (MFO). L1210 cells (4x10$$^5$/ml) were exposed to 100 µM of either 5-Fluorouracil (FU), 5-Fluoro-2'-deoxyuridine (FUdU), or 5-(2'-deoxy-2'-fluorooxyethyl)-5-fluorouracil (FUS) for 3 hours with or without MFO (0.25% rat S-9). After incubation, aliquots were measured for cell viability. One ml aliquots were further incubated with 0.05 µC [14C]-uridine. [14C]-incorporation into RNA did not differ between FU and the RNA analogs and was not affected by MFO activation. MFO activation did not affect FU cytotoxicity or FU inhibition of 5-FU, either in DNA or protein synthesis. FT-172, FUS-383% and [14C]-incorporation into DNA (FT-140X, FUS-772) of the analogs to values greater than measured for FU. Data suggest that FT and FUS induce L1210 cytotoxicity by additional mechanisms than only FU inhibition of thymidylate synthetase.

1983 Silas M. Burroughs - APPE Fellow in Pharmacology/Toxicology.
533 Intermediacy of 1-Naphthol in the Covalent Binding and Pulmonary Toxicity of Naphthalene. A. R. Buckpitt, L. S. Bahnsen and R. B. Franklin. Community and Environmental Medicine, University of California, Irvine, CA, and *Lilly Research Labs, Indianapolis, IN.

The covalent binding (CB) of reactive metabolites from aromatic hydrocarbons such as benzene, bromobenzene and naphthalene (NA) has been postulated to require intermediate formation of phenols. This study has further examined the involvement of 1-naphthol (1-NOL) in (a) the in vitro and in vivo metabolism of NA to covalently bound metabolites (CBM) and (b) the bronchiolar necrosis caused by NA. The ratio of 1-NOL formed in mouse pulmonary vs hepatic microsomes from NA was 6:1 and the ratio of in vitro CB in these two tissues was 1.3:1. This, together with the fact that CBM from 1-NOL were formed at almost identical rates by lung and liver microsomes, suggested that 1-NOL may not be required for the in vitro CB of 1-Naphthalene. Inclusion of GSH into incubations with 1-NOL decreased CB but did not result in the formation of the GSH adducts that were isolated during the microsomal metabolism of NA. Contrary to NA, i.p. dosing of 1-NOL did not result in bronchiolar necrosis or depletion of GSH. In vivo CB levels were also lower than that noted after NA administration. These results indicated that 1-NOL (a) need not be an intermediate in the formation of CBM from NA in vivo or in vitro and (b) played an inconsequential role in the NA-induced lung lesion.

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535 Comparison of Several Indicators of Cytotoxicity in Rat Hepatocytes in Primary Culture. C.A. McQueen, B.N. Merrill, and G.M. Williams. American Health Foundation, Valhalla, NY.

Hepatocytes in primary culture are used as an in vitro system for assessing chemical cytotoxicity. A comparison was made of four indicators of cytotoxicity: leakage of intracellular enzymes, cell survival, dye exclusion and inhibition of protein synthesis. Monolayer cultures were prepared from hepatocytes isolated from F344 rats. The cultures were exposed to 2-aminofluorene, acetaminophen, coumarin, hydralazine, propylene glycol or tartrazine for up to 18 hours. All the chemicals tested except propylene glycol were toxic at least at the highest concentrations tested. Leakage of lactate dehydrogenase (LDH), cell survival, and trypan blue exclusion were all reliable indicators of cytotoxicity. After 18 hours exposure to 10^-2M coumarin, there was a 50% increase in the amount of LDH in the medium. These cultures contained only 2% of the cells in the unexposed flasks and no dye excluding cells. However, the sensitivity and usefulness of each endpoint was dependent on the chemical tested. For example, 10^-2M acetaminophen interfered with the determination of LDH. Preliminary studies have also indicated that the cytotoxicity of 2-aminofluorene could be monitored by inhibition of protein synthesis. These results demonstrate the utility of several indicators of cytotoxicity and illustrate the value of using multiple endpoints.


Exposure of suspensions of isolated hepatocytes to tert-butyl hydroperoxide (TBHP) resulted in decreased glutathione (GSH) concentration, increased malondialdehyde production and cell death. Hepatocytes isolated from mice were more susceptible to TBHP-induced injury than rat hepatocytes as a 50% decrease in cell viability occurred at 0.2mM and 1.0mM TBHP in mouse and rat hepatocytes respectively. Time course experiments revealed an abrupt decrease in viability (95 to 10%) following 15 to 30 minutes exposure to TBHP (2mM) in cells from both species. GSH content in rat hepatocytes decreased to 50% of control 5 minutes after the TBHP addition (2mM) and subsequently recovered by 15 minutes. In contrast, 2mM TBHP decreased GSH concentration in mouse hepatocytes by 95% within 5 minutes and showed no tendency toward recovery. GSH concentration in mouse hepatocytes was significantly depressed after 30 minutes exposure to 0.35mM TBHP whereas rat hepatocyte GSH was not significantly decreased until exposed to 0.75 mM TBHP. These data demonstrate a marked species difference in the susceptibility of mouse and rat hepatocytes to peroxidative injury induced by TBHP.

Acetylcholine, choline acetyltransferase, and acetylcholinesterase are found in gametes of various animals. Recent studies have shown that acetylcholine may play a role in fertilization. Prazoxin, the active metabolite of parathion, is a potent inhibitor of acetylcholinesterase. The effect of prazoxin on in vitro fertilization was studied using 8622 mouse eggs and cauda epididymal spermatozoa. Various concentrations of prazoxin were incorporated in the capacitation and/or insemination medium. The presence of prazoxin during capacitation did not reduce sperm motility. To score fertilization, eggs were harvested at 6 hr or 24 hr after insemination and immediately washed and fixed. The presence of second polar body, pronuclei, sperm tails, or first cell cleavage were recorded to determine the percentage of fertilization. Prazoxin inhibited fertilization by at least 50% at concentrations of 5 μM and 50 μM. Prazoxin did not appear to affect the first cell cleavage once the two pronuclei were formed. The rate of polyspermy of treated groups was not different from the control.

539 INHIBITION OF STEROIDOGENESIS IN THE TESTES OF RATS TREATED WITH HEXAFLUOROCETONIC (HFA). P. J. Gillies and K. P. Lee, E. J. du Pont de Nemours & Co., Inc., Haskell Laboratory for Toxicology and Industrial Medicine, P. O. Box 50, Elkton Road, Newark, DE

Rats exposed to HFA develop testicular atrophy (TA). Previous studies suggested that the TA may be associated with an inhibition of steroidogenesis. To investigate this hypothesis, steroidogenesis was examined in Leydig cell enriched fractions (LC) isolated from the testes of rats dosed dermally with 130 mg/kg/day HFA • sesquihydrate for 14 days and from pair-fed control rats. Relative to controls, LC from HFA treated rats exhibited decreased incorporation of [14C] acetate (78%) and [3H] mevalonate (44%) into steroids. Testosterone was decreased 50% in the testes of HFA-treated rats. Incubation of LC from control rats with 1.0 μM HFA did not result in a similar inhibition of steroidogenesis. Analyses of LC by electron microscopy revealed morphological changes consistent with an inhibition of steroidogenesis e.g. marked reduction and segregation of the smooth endoplasmic reticulum from mitochondria, large lipid droplets, and fewer mitochondria. To determine if the inhibition of steroidogenesis was due to decreased levels of circulating gonadotrophic hormones, the effects of HFA on LH and FSH were investigated. HFA did not significantly affect these hormones.

538 A QUANTITATIVE MORPHOLOGICAL STUDY OF THE STAGE SPECIFICITY OF SPERMATOCYTE DAMAGE IN ETHYLENE GLYCOL MONOMETHYL ETHER (EGM) INDUCED TESTICULAR TOXICITY. E.M. Casey and W.H. Butler. British Industrial Biological Research Association, Woodmansterne Road, Carshalton, Surrey, SMS 4DS, U.K.

EGM is a water soluble derivative of ethylene glycol used extensively in the chemical industry, particularly in the manufacture of paints and varnishes. Administration by various routes including inhalation has been shown to produce testicular atrophy in many species. A detailed morphological study with quantitation of spermatocyte and spermatid populations was carried out in the rat to identify the specific site of cellular damage. Groups of rats given a single oral dose of EGM at 250 or 500 mg/kg body weight were killed 1, 2, 4, 8, 16 & 32 days after dosing. The spermatocyte was the only cell type affected and degeneration was restricted to those cells in post zygotene stages of meiotic development. Whereas 500 mg EGM/kg affected all spermatocytes in post zygotene development, 250 mg EGM/kg exhibited further specificity directed at cells in early pachytenes (Stages I & II using the classification of Leibond & Clermont) and in late pachytenes through to division (Stages XIII & XIV). Spermatocytes in the intermediate phase of pachytene (Stages IIII- XI) and in pre-pachytene development were unaffected as were spermatids and spermatogonia. (Supported by Ministry of Agriculture, Fisheries & Food, U.K.)

540 DEPRESSION OF RAT SERUM FOLLICULAR STIMULATING HORMONE (FSH) AND LUTEINIZING HORMONE (LH) AFTER EXPOSURE TO 1,2-DIBROMO-3-CHLOROPROPANE (DBCP). E.M. Smaller and W.N. Pipper, Dept. of Pharmacology, Univ. of Neb. Med. Ctr., Omaha, NE 68105. Sponsor: W.O. Berndt.

DBCP is an effective nematicide. Its use was banned in 1977 following reports of infertility in male workers involved in its production. Plasma testosterone levels have been reported to remain within the normal range despite elevated plasma FSH levels and normal or elevated plasma LH levels in humans exposed to DBCP. Because the testicular lesion produced in rats by single exposure to DBCP is believed to resemble that observed in humans with repeated exposure, the rat has been proposed to be an appropriate model for studying the mechanism of DBCP-mediated testicular toxicity. The present study was designed to determine the effects of DBCP on serum FSH and LH levels in rats undergoing sexual maturation. Serum gonadotropin levels were measured by radioimmunoassay at 12, 24, 48, 72, and 144 hours following administration of a single dose of DBCP (200 mg/kg orally) to 34 day old Sprague-Dawley rats. Within 12 hours of DBCP administration, serum LH and FSH levels decreased to 15% and 75% of control values, respectively. Levels of serum FSH declined further to 60% of control values at 48 hours, whereas serum levels of LH remained at 15% of controls at this time period. Levels serum levels of FSH and LH returned to control values at 144 hrs. (Supported by March of Dimes Grant #15-44).
541 ADVERSE EFFECTS OF BUTYL BENZYL PHthalate ON Bone Marrow AND THE MALE REPRODUCTIVE SYSTEM.

A 14 day dietary study was conducted in adult, male, Fischer 344 rats at levels of 0.0, 0.625, 1.25, 2.5 and 5.0% butyl benzyl phthalate (BBP) to evaluate potential effects of this plasticizer on the hematopoietic and male reproductive systems. Total body, thymus, testis, epididymis, prostate and seminal vesicle weights were reduced at 2.5 and 5%, while pituitary weight was unaffected. Histological evaluations revealed dose-dependent atrophy of the testis, prostate and seminal vesicles at 2.5 and 5%, atrophy of the thymus and epididymis at 5%, and the presence of immature sperm cells in the lumen and tubular necrosis of the epididymis at 2.5 and 5%. Plasma testosterone was decreased at 2.5 and 5%, while FSH and LH were increased. Abnormalities were not observed in the circulating components of blood, and clotting tests (prothrombin time, activated partial thromboplastin time) were near normal. However, bone marrow counts (cellularity) were reduced at 2.5 and 5%. These data document a direct toxic effect of BBP on testis with secondary effects on other reproductive organs; pituitary and hypothalamic responses do not appear to be affected. The reduced bone marrow cellularity suggests that longer exposures to BBP may affect circulating blood components or compromise clotting abilities.

542 VALIDATION OF A NEW REPRODUCTIVE TOXICOLOGY PROTOCOL USING DIETHYLSTILBESTROL (DES) AS A MODEL COMPOUND.

A new protocol designated “Fertility Assessment by Continuous Breeding” provides a mechanism for evaluating chemical effects on male and female fertility and reproduction. The present study evaluated this test system using DES as a model reproductive toxicant. The protocol employs young adult CD® mice and is comprised of 5 possible tasks: task 1, 2-week dose finding; task 2, continuous breeding; task 3, determination of affected sex; task 4, offspring assessment; and task 5, hormone analyses. Only task 2 and 3 were conducted in this study. Task 2 employed dietary levels of 0, 1, 10 and 50 ppm DES during the 7-day pre-mating, 90-day cohabitation and 21-day segregation periods. Task 3 utilized the control and high-dose mice from task 2 in a crossover mating trial (control x treated) to determine whether one or both sexes were affected by treatment. DES (50 ppm) altered reproduction in female CD® mice as evidenced by significant decreases in the fertility index, number of litters per pair, number of live pups per litter and a lower proportion of pups born alive per litter. This study clearly demonstrates the potential of this new protocol in the rapid, sensitive, and accurate detection of reproductive toxicants. (Supported by Contract No. N01-ES-2-5014).


The NTP is currently evaluating a new testing system entitled “Fertility Assessment by Continuous Breeding” which studies effects of chemical exposure on fertility and reproduction in male and female CD-1 mice. The test involves 4 inter-related tasks: task 1, dose finding; task 2, continuous breeding; task 3, determination of the affected sex; and task 4, offspring assessment. Tasks 1, 2 and 4 were performed in this study. In task 2, male and female mice were given free access to drinking water containing 0, 0.25, 0.5 or 1.0% EG and mice were housed as breeding pairs for 14 weeks; there were slight, but significant, decreases in the litters per pair, live pups per litter and mean pup weight in the 1.0% group compared to the controls (no effect at 0.25 or 0.5%). For task 4, offspring (70 days of age) from 0 and 1% groups were cohabited for 7 days; there were significant decreases in fertile matings and litter size. Incidental observations in the F1 offspring (1% group) included skeletal anomalies of the skull, the thoracic vertebrae, sternebrae and ribs. (Supported by Contract No. N01-ES-2-5013 and N01-CP-45615).

544 THE TESTICULAR TOXICITY OF ETHYLENE GLYCOL MONO-METHYL ETHER (EGME) AS CHARACTERIZED BY MATING SUCCESS, SPERM PARAMETERS, AND HISTOPATHOLOGY, R.E. Chapin, S.L. Dotson, N.B. Ross, and J.C. Lamb, IV, National Toxicology Program, NIHES, Post Office Box 12233, Res. Tr. Park, NC 27709.

EGME has been examined as part of a program to identify predictive indicators of male reproductive toxicity. ECME was dissolved in distilled water and administered p.o. to adult male F344 proven-fertile for 5 d at 0, 50, 100 or 200 mg/kg/d; controls received distilled water. Males were each cohabited with 2 female F344 rats each wk for 8 wk after dosing. Females were sacrificed by CO2 asphyxiation 10-14 d after removal from the male's cage. Pregnancy rates (SP) in the control groups were 75-93%. SP in the low dose EGME animals did not differ from controls. Treatment with 100 mg/kg EGME decreased SP to 53% at wk 5; otherwise, this group was not different from controls. The first effect of 200 mg/kg EGME was seen at wk 4; the pregnancy rate at wk 5 was 5% and rose slowly thereafter, but was not back to control levels later. EGME decreased fertility, but did not appear to induce dominant lethal mutations in offspring. In other males treated similarly, but not mated, there were doses- and time-dependent increases in headless sperm and decreases in sperm number. These data support previous findings that EGME, at 100 mg/kg, is selectively toxic to spermatozoa, while at higher doses, there is an apparent effect on spermatogonia or Sertoli cells.
Last year we reported that we were unable to detect reproduction problems in rats in a 2-litter reproduction study in a dog kennel in Allegan, MI, where reproduction problems have occurred for 10 yr (THE TOXOCOLOGIST 3:15, 1983). There was a history of fluorosis in that kennel associated with intake of dog food containing 460 ppm P<sup>2</sup>. Although high-fluoride chow has not been fed since 8/81, and the local water has not been used since 6/81, the reproduction problems continue. In a 2-way factorial study, initiated 3/82, 20 "proven" Sheלתies were brought into the kennel from outside the county and assigned to 4 groups (4 females, 1 male). Ten dogs (Gps. A&B) are given dog chow supplemented with rock phosphate to produce a P<sup>-</sup> concentration of ca. 460 ppm, while 10 (Gps. C&D) receive regular chow. Ten dogs (Gps. A&C) are given well water while 10 (Gps. B&D) receive distilled water. Reproduction problems (low fertility, birth defects, perinatal deaths) have been seen in all 4 treatment groups although the adults appear generally healthy and results of hematology, urinalysis, blood chemistry, and hormonal analyses have shown no obvious abnormalities. Thus the reproduction problems have been duplicated in the study dogs. The causative factor(s) remains unknown but does not appear to be either F<sup>-</sup> or local well water. Since study dogs are confined to the kennel and runs, direct contact with soil and/or foliage also appears to be eliminated as a possible cause.

2,2,2-Trifluoroethanol (TFE) is used as volatile working fluid in waste heat recovery devices and is a known testicular toxin. Male F-344 rats were given intraperitoneal injections of TFE at 0, 2, 10 and 50 mg/kg for 4 days and five rats per dose level were sacrificed at 1, 7 and 21 days posttreatment. Testicular weight as a proportion of body weight was significantly lower (p < 0.05) than controls in the rats receiving 50 mg/kg at 7 and 21 days. Dose-related mild to severe degeneration of testicular epithelium was observed with reduction in spermocyte numbers as early as 1 day after treatment. The degeneration was more widespread at 7 and 21 days after treatment, and the presence of spermatid giant cells was noted. Electron microscopy of testicular tissue from rats 21 days after 50 mg/kg TFE revealed only minor changes in organelles of the Leydig and Sertoli cells. Serum and testicular testosterone concentrations were significantly lower than controls 1 day after the 50 mg/kg treatment and the testicular concentration was significantly higher at 7 and 21 days. These studies have shown that the initial effect on testicular epithelium is apparently a direct one and not mediated through early damage to Sertoli or Leydig cells. (Research was supported by U.S. Department of Energy Contract DE-AC04-76EV010103.)


Sucrose polyester (SPE) is a nonabsorbable mixture of hexa-, hepta-, and octaesters of fatty acids with sucrose, which has physical properties similar to conventional dietary fats. Since SPE replaces a portion of edible fats the caloric intake of the consumer is reduced, which is seen as a tool in combating the problem of overweight in Western populations. Because SPE is neither absorbed nor metabolized it forms a bulk lipid phase in the small intestine resulting in effects on the enterohepatic circulation of lipid-soluble materials, such as cholesterol. We determined the effects of SPE on reproduction and development, by continuously feeding 1, 5, or 10% of the diet to groups of 30 female and 15 male, CD rats for two generations. The growth of the rats during each 91 day period after weaning was not different, but the rats fed either 5 or 10% SPE increased their feed intake significantly to compensate for the reduced energy density of the diet, and consequently had lower feed efficiencies. SPE had no significant effect on reproduction in either generation, including live births, birth weights, number weaned, and weaning weights. There was no increase in resorptions or abnormal fetuses in either generation as a result of the SPE treatments. Thus, it is apparent that SPE, even at an exaggerated level of 10% of the diet, poses no reproductive or teratogenic hazard for the consumer.

TESTICULAR LESIONS AND TESTOSTERONE FLUCTUATIONS IN RATS AFTER INTRAPERITONEAL INJECTION OF 2,2,2-TRIFLUOROETHANOL. T.C. Marshall, F.F. Hahn and B. Parker. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM

High doses of theebromine (TBR) have been previously reported to induce testicular atrophy and aspermatozoa in rats. This study evaluated the effects of a regressive period following TBR-induced testicular atrophy on reproductive parameters. TBR was fed for 7 weeks at 0.2, 0.6 and 0.8% of the diet to Sprague-Dawley rats. At 7 weeks, histopathology was evaluated in a subgroup from each treatment group via unilateral orchectomy. Hematology parameters and TBR in plasma and testes were determined in subgroups. Remaining rats were mated with proven breeder female rats and peri/postnatal parameters were determined. For the next 12 weeks all rats were fed the control diet. Orchiectomized rats were sacrificed for histopathology; hematology and chemistry parameters were again determined in subgroups. The remaining rats were mated and peri/postnatal parameters evaluated. Results indicated that after 7 weeks' atrophy was induced only at the 0.6 and 0.8% TBR level; only 2 litters were produced at 0.6% TBR and none at 0.8%. After the 12 wk. regression period, fertility and reproductive function were similar between control, 0.2 and 0.6% TBR groups. Histopathology demonstrated a significant improvement in testicular morphology in the 0.6% group. These results indicate that after a sufficient recovery period from TBR-induced testicular atrophy, normal offspring can be produced from previously infertile rats.

INHIBITION OF CHLOROFORM FORMATION BY CHLORINE DIoxide IN WATER.  D.H. Suh and M.S. Abdel-Rahman. Toxicol. Lab., Pharmacol. Dept., UMDNJ-NJ Medical School, Newark, NJ

Chlorination of drinking waters leads to the generation of trihalomethanes. Therefore, chlorine dioxide (ClO₂) which does not form trihalomethanes is being considered as an alternative disinfectant. In a previous study, it has been demonstrated that blood chloroform (CHCl₃) levels were significantly decreased with the treatment of ClO₂ in vivo. Studies were conducted to investigate mechanism of inhibition of CHCl₃ formation by ClO₂ (5 mg/l) in the presence of hypochlorous acid (HOCI) (5, 10, 20 mg/ml), using sodium citrate (1 mM) as an organic substance. When citrate was reacted with HOCI, 8-keto-glutaric acid (8-KG), mono-, di- and tri-chloroacetone were produced as reaction intermediates and CHCl₃ as a final product. There was a linear relationship between the concentrations of HOCI and the formation of CHCl₃. When ClO₂ was substituted for HOCI, neither CHCl₃ was formed nor citrate concentration was changed. Also, CHCl₃ formation was inhibited by ClO₂ in the presence of HOCI and citrate. GC/MS analysis indicates that this inhibition is related to the reaction of ClO₂ with 8-KG to form malonic acid which is not a substance for CHCl₃ formation. Chlorine dioxide also oxidizes other intermediates such as mono- and di-chloroacetone to form acetic acid.


Polycythemia is a hematologic disease characterized by erythrocytosis and occasionally leukocytosis. Various types of polycythemia occur in animals as a result of either hyperconcentration, elevated erythropoietin, or more rarely, autonomous myeloproliferation. An inbred strain of Wistar rats develops about a 40% incidence of polycythemia following transplacental exposure to methylmercury (MeHg), ethyleneurea (EU), and sodium nitrite (NO₂⁻). Dams are fed 10 ppm MeHg in the diet for 13 weeks prior to parturition, and gavaged with 50 mg/kg EU and 25 mg/kg NO₂⁻ on days 17-19 of gestation. Offspring typically develop clinical signs of polycythemia at 2-6 months of age with hematocrits ranging from 60-80%. Death results in 1-4 months after onset depending on the severity of the condition and secondary complications. Original investigators noted several features of this disease in rats that were similar to human polycythemia vera, a fatal myeloproliferative disorder involving neoplasia of bone marrow stem cells. Red blood cell mass and marrow cell culture results indicate this disease is an absolute polycythemia that is erythropoietin sensitive. Further information will be presented which will support either secondary polycythemia due to elevated serum erythropoietin levels or primary polycythemia due to myeloproliferation. Accurate characterization and classification of this polycythemic disease in rats is essential to determine its appropriateness as a much needed animal disease model for human polycythemia.


Treatment of rats with PFDA results in altered serum thyroid hormone levels, changes in resting heart rate (HR), and changes in body temperature. A Gould 2400 recorder with an ECG-Biotach amplifier was used to measure in vivo HR in rats under light ether anesthesia 7 days following a single i.p. dose of PFDA (75mg/kg). The HRs were lower in PFDA-treated rats than in pair-fed controls. In addition, body temperatures were measured rectally 8 days following PFDA treatment (75mg/kg) and were found to be significantly lower than body temperatures in pair-fed controls. In order to measure serum thyroid hormone levels, rats were sacrificed and blood collected at 0.5, 1, 2, 4, 6, 8, and 10 days following a single i.p. injection of PFDA (75mg/kg). The serum levels of thyroxine (T₄) and triiodothyronine (T₃) were measured by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). As early as 12 hours following PFDA treatment, serum T₃ and T₄ levels were reduced by 35% and 70%, respectively, and remained depressed throughout the 10 day study. The data suggest that early PFDA-induced changes in serum thyroid hormone levels may be partly responsible for the bradycardia and hypothermia observed 7-8 days following PFDA treatment.

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PHOTOTOXICITY OF PSORALENS PLUS UVA LIGHT (PUVA) IN MAMMALIAN CELLS IN CULTURE. E.G. Lee, E.J. Yurkow, M.A. Gall and J.B. Laskin. UMDNJ-Rutgers Med. Sch./Rutgers U. Joint Prog. in Toxicology, Piscataway, NJ 08854

Psoralens (Ps) are naturally occurring plant toxins that inhibit growth of mammalian cells when activated by 365 nm light (UVA). In this study we compared the ability of 8-methoxypsoralen (8-MOP) and 4,8,15-trimethylpsoralen (TMP) to inhibit DNA, RNA and protein synthesis in mouse sarcoma (S-180) and human epithelial (Hela) cells. Macromolecular synthesis was monitored by 3H-thymidine, -uridine and -leucine uptake. In both cell lines, PUVA was found to inhibit nucleic acid synthesis in a concentration dependent manner and was maximal 5 min after exposure to the Ps. The concentration of Ps required to inhibit DNA and RNA synthesis by 50% (IC₅₀) in the cells was similar. The IC₅₀'s for DNA synthesis inhibition by TMP and 8-MOP were 0.03 µM and 0.82 µM for S-180 cells and 0.17 µM and 7.6 µM for Hela cells, respectively. No inhibition of protein synthesis was found for either toxin. It is concluded that DNA was the target for Ps action. Increased sensitivity of the cells to TMP was correlated with the greater lipidsolubility of TMP when compared to 8-MOP. Although differences in sensitivity between S-180 and Hela cells were apparent, TMP and 8-MOP have a similar mechanism of action in both cell types. Partially supported by a Grant from Mary Kay Cosmetics.

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The effects of tri-n-butyl phosphate (TBP) were investigated in the Sprague-Dawley (SE) rat over a period of 18 weeks.

Groups of randomized female (ave. wt. 206±10g) and male SD rats (294±13g) were divided into low dose, high dose and control groups (12 rats/sex/group). TBP was administered by gavage once a day for 5 days a week over an 18-week period. Low dose animals received 0.70 mL/kg throughout the experiment and high dose animals received 0.30 mL/kg for the first 6 weeks. For the remaining 12 weeks, the high dose level was increased to 0.35 mL/kg/day.

Histopathological examination of tissues revealed that all female and male rats orally administered TBP developed diffuse urothelial hyperplasia of the urinary bladder. Some low and high dose males showed also focal nodular hyperplasia with mitotic figures. Similar changes were not found in the control animals.

CONTAMINATED GROUNDWATER IN CALIFORNIA: AN EMERGING HEALTH CONCERN. P.E. Bertaux and D.P. Spath. California Department of Health Services, Berkeley, CA.

The contamination of underground water supplies by pesticides and industrial chemicals is a problem whose wide extent is just becoming clearly evident in California. Pesticides in excess of recommended action levels found in well water used for drinking include: 1,2-dichloro-3-chloropropane (DBCP), ethylene dibromide (EDB) and 1,2-dichloropropane (1,2-D). These compounds have been demonstrated to be carcinogenic to animals. Industrial chemicals, such as trichloroethylene (TCE), 1,1,1-trichloroethane (TCA), and tetrachloroethylene, which have resulted from improper disposal of wastes, have also caused groundwater contamination. Epidemiological studies are being conducted to investigate the possible association of high DBCP levels in drinking water with the elevated rates of stomach cancer and childhood leukemia in one location and an apparent cluster of birth defects in another location where water has been contaminated with TCA. Groundwater provides about 40% of California's drinking water needs. Some measures being taken are mandated monitoring programs and provision of grants or loans to water companies to enable them to drill deeper wells in order to tap deeper untainted aquifers, or to install activated charcoal filters or air stripping equipment. Preventive measures have been taken in banning or restricting the use of certain pesticides.

CHARACTERIZATION OF CARBON DISULFIDE BINDING TO BLOOD AND OTHER BIOLOGICAL SUBSTANCES. C.W. Lam and V. DiStefano. Div. of Toxicology, Univ. of Rochester, Rochester, NY. Sponsor: T.W. Clarkson.

Free and bound CS₂ are present in subjects exposed to CS₂. In rats exposed to 2 mg/liter (~60 ppm) for 4 hr, concentrations of acid-labile CS₂ (AL CS₂, a form of bound CS₂ which can be recovered from biological samples by acid treatment at elevated temperature) in plasma and RBCs increased linearly with time of exposure. About 90% of the blood AL CS₂ in plasma of exposed rats was found in the precipitate. A similar result was obtained with RBC lysates precipitated with ammonium sulfate. CS₂ was found to bind to human hemoglobin, albumin, γ-globulin and horse heart cytochrome C. Studies with radio-labeled CS₂ indicated that CS₂ binds to glycine, but not to N-acetyl cysteine or glucose. These studies suggest that CS₂ binds to amino groups, but not to sulfhydryl or hydroxyl groups in vitro. Radioisotope studies also showed that CS₂ binds significantly to human plasma and serum but to a much smaller extent to a 5% bovine serum albumin solution. Substantial portion of the bound CS₂ could not be released from the samples upon acid treatment at elevated temperature. These are the first studies showing the existence of non-acid-labile bound CS₂ in plasma.

These studies were supported by NIHES Grants ES-02039 and ES-07026.
557 ROLE OF RED BLOOD CELLS IN THE TRANSPORT OF CARBON DISULFIDE IN BLOOD. C.W. Lam and V. DiStefano, Div. of Toxicology, Univ. of Rochester, Rochester, NY. Sponsor: T.W. Clarkson.

CS₂ is present in exposed subjects in free and bound forms. In rats exposed to 2 mg/liter of CS₂ for 4 hr, concentrations of free CS₂ in the RBCs approached a plateau within 2 hr of exposure. Free CS₂ concentrations in the plasma reached a steady state level within 15 min of exposure. More than 90% of the free CS₂ in blood was present in the RBCs regardless of the length of exposure. When an aqueous solution of CS₂ was added to plasma and then mixed with an equal volume of RBCs, about 90% of the CS₂ diffused into the RBCs. When CS₂-treated RBCs were mixed with an equal volume of plasma, about 10% of the CS₂ diffused into the plasma. It is apparent from these results that RBCs are the major carriers of CS₂ in blood. We found that 98% of the free CS₂ in RBC lysate was associated with the hemoglobin. Free CS₂ was readily partitioned into olive oil (RBCs/Oil = 1/6) and to a much small extent into phosphate buffer (RBCs/Buffer = 39/1). Extraction of CS₂ from CS₂-toaded RBCs into albumin solution increased linearly with the increase of protein concentrations. These results indicated that RBCs may play an important role in the transport of CS₂ from lung to other tissues and vice versa.

These studies were supported by the NIEMH Grants ES-02039 and ES 07026.

559 EFFECTS OF REDUCED AND OXIDIZED GLUTATHIONE ON THIOUREA BINDING. H.W. Leung, Division of Occupational & Environmental Health, Graduate School of Public Health, San Diego State University, San Diego, CA. Sponsor: J.E. Holson.

Certain sulphur compounds have been reported to offer protection against the toxicity of thiourea. The present study was initiated to delineate the role of glutathione in modifying thiourea-induced toxicity. Results indicated that 35S-thiourea did not bind directly with either reduced glutathione (GSH) or oxidized glutathione (GSOS) in a cell-free system. 35S-thiourea distributed rapidly in rat blood, and the pattern or rate of distribution was unaffected by the addition of GSH. Chromatographic separation of the plasma revealed a single radioactivity peak, which coincided with the elution volume (Ve) of free 35S-thiourea. However, when the plasma fraction incubated with GSOS was chromatographed, three radioactive peaks were observed: one associated with the void volume (Vv), the second at Vv/Ve= 2.8 and the third coincident with free 35S-thiourea. The data suggest that GSOS may be involved in modifying thiourea binding to tissue protein. Its role in relationship to the protective effect of GSH is discussed. (Supported by San Diego State University Foundation Faculty Grants-in-aid 21134)

558 THREE-MONTH SUBCHRONIC TOXICITY STUDY WITH MV-678 IN RATS. M.W. Sauerhoff, R.E. Ouellette, G.M. Zwicker, and R. I. Freudenthal. Stauffer Chemical Company, Environmental Health Center, Farmington, CT.

A three-month study was conducted to determine the subchronic toxicity of MV-678 when administered to rats by gavage. Twenty-five Charles River Sprague-Dawley derived rats of each sex were assigned to each of five dose groups (4 treated groups and 1 control group). MV-678 was administered by gavage once each day, 5 days a week at dose levels of 2, 10, 30, or 200 mg/kg/day. Controls received corn oil (5 mg/kg) only. Data collected for the study included body weights, food consumption, clinical observations, hematol and blood chemistry values, urinalysis, and gross necropsy and histopathology observations. Oral exposure to MV-678 Technical for up to three months produced clinical signs, blood chemistry changes, and increased liver weights in male and female rats given 200 mg/kg/day of MV-678. The 30 mg/kg/day females also had blood chemistry changes and slightly increased liver weights. The hematologic changes observed in the treated females were not considered toxicologically significant at 30 mg/kg/day and less. Microscopic examination of tissues revealed mild liver cell hypertrophy in the 30 and 200 mg/kg/day females and the 200 mg/kg/day males, but not in the 10 mg/kg/day males. Thus, no toxicologically significant effects were apparent in male or female rats given 2 or 10 mg/kg/day of MV-678 for three months.

560 SEQUENCE OF ACUTE TOXIC INJURY INDUCED IN RAT KIDNEY BY DIMETHYLNITROSAMINE (DMN). G.C. Hard and R. Mackay. Fels Research Institute, Temple University School of Medicine, Philadelphia, PA.

A single dose of the renal carcinogen DMN produces acute toxic injury in the rat kidney which is intermediate in time-course between rapidly acting nephrotoxins exemplified by mercuric chloride, and the sustained chronic injury produced by such compounds as cisplatinum or uranyl nitrate. To provide an explanatory basis for this distinctive pattern of renal injury, the sequence of ultrastructural changes over a 3 week period was determined following one intraperitoneal injection of 60 mg/kg DMN in protein-deprived Wistar rats. The sequential involvement of different target cell types during the acute response suggested that DMN was metabolized in the P2 segment of the proximal tubule and that a reactive intermediate might be released to produce subsequent cytotoxic injury, firstly in the resident cortical fibrocytes, and then in adjacent capillary endothelium. Development of overt cytotoxicity in the P2 segment of proximal tubules coincided with endothelial necrosis and red cell extravasation, indicating ischemia as a likely basis for the delayed epithelial injury. Infiltration of the cortex by mononuclear phagocytes which followed the focal vascular damage was related to erythropagocytosis. Resolution of the renal lesion by 3 weeks was associated with endothelial and tubule regeneration and clearance of extravasated erythrocytes and phagocytes from the interstitium.
561 TOXICITY OF MONOCHLORACETIC ACID. M. Berardi and R. Snyder, Joint Graduate Program in Toxicology, Rutgers University and UDMDNJ/Rutgers Medical School, Piscataway, N.J. 08854.

Monochloracetic acid (MCA) caused the death of three workers exposed dermally to the molten (60°C) form. A marked fall in blood pressure, rapid respiration and unconsciousness preceded death. Following a single oral or sc dose, mice exhibit tremors, respiratory depression, unconsciousness, and occasionally tonic, clonic convulsions prior to death, and the mean time to death is 5 hours. Some survivors show a front paw paralysis with crossing of the front limbs, sometimes accompanied by tremors and Straub tail. These symptoms occur as early as 16 hours and the damage appears to be permanent. In rats given 1.0 μCi 1-14C-MCA orally, radioactivity in plasma, liver, lung, kidney, heart, testis, and spleen peaked at 1-2 hours, and declined rapidly (t 1/2 = 2-7 hours). Radioactivity in brain, although lower than in other organs, continued to rise up to 8 hours and plateaued from 16-24 hours. Radioactivity in cerebral cortex, striatum, thalamus, hippocampus, cerebellum, pons, medulla, and spinal cord is evenly distributed, but is about 2.5 times higher in the hypothalamus. MCA may exert action on the hypothalamus by interfering with central control of blood pressure or other vital functions, and act at another site in the CNS to cause paralysis. (Supported by a grant from Hercules, Inc.)

563 SPECIES DIFFERENCES IN THE METABOLISM OF AMPHETAMINE BY ISOLATED HEPATOCYTES. C.B. Green, R.S. LeValley and C.A. Tyson, SRI International, Menlo Park, CA 94025. (Supported by NIH GM 28158)

Amphetamine (AMP) is metabolized in the liver by either aromatic hydroxylation to p-hydroxyamphetamine (pHA) or by oxidative deamination to phenylacetone (PA) and benzoic acid (BA), the major pathway being species dependent. Hepatocytes were isolated from rat, rabbit, dog and squirrel monkey livers, incubated with 10-6M (7-3H)AMP, and metabolites analyzed by reverse phase HPLC. As found in vivo, the major metabolite produced by rat hepatocytes was pHA, increasing with time to about 85% of the administered dose. As much as 15% of the dose was found in BA fraction and less than 10% was PA. The predominant pathway of AMP metabolism in rabbit hepatocytes was oxidative deamination, also mimicking in vivo results, with about 70% of the radioactivity converted to products of this pathway by 60 min. Dog hepatocytes formed pHA in a time-dependent manner. At 240 min, pHA and PA each represented 4-5% of the dose. The metabolic profile from squirrel monkey liver cells was similar to the dog, although less of each metabolite was formed. The major metabolites for all species except rat were BA and its conjugates, with the quantity produced correlating to the relative rates of AMP metabolism. In 240 min, rabbit, rat, dog and squirrel monkey hepatocytes metabolized 72, 50, 20 and 15% of AMP, respectively. In general, species differences in AMP metabolism (relative rates and profiles) were reproduced by hepatocytes isolated from these 4 species.

562 SUBCHRONIC TOXICITY STUDIES WITH ALEYLARYL PHOSPHATES. B.G. Hammond, M.W. Stevens, F.R. Johnsen, G.J. Levinekas, Monanto Co., St. Louis, MO; D.E. Johnson, TRDC, Mattawan, MI.

Alkyaryl phosphates are used as flame retardants in industrial hydraulic fluids. The subchronic toxicology of dibutylphenyl phosphate (DBFP) and a phosphate blend: 36% triphenyl phosphate, 41% nonylphenylidiphenyl phosphate, and 23% cumylphenylidiphenyl phosphate were evaluated in CD rats. Phosphate esters were mixed into the diet for 13 weeks to provide dosages of 50, 150 & 500 mg/kg (DBFP) or 100, 500 & 2000 mg/kg (blend). Body weights were recorded weekly; hematology and clinical parameters were examined at 6 and 13 weeks. All animals were necropsied; organ weights were recorded and histopathology was performed on high-dose and control groups as well as target organs for all groups. A second 13-week study was undertaken at 5 mg/kg DBFP or phosphate blend because of effects observed in initial studies.

In the first study, dose-related mortality occurred in high-dosage animals (blend). Slight to moderate changes in clinical chemistry and hematology parameters were observed in animals from all dosage groups (blend, DBFP). Histopathologic changes were observed in the kidney, bladder, ovary (DBFP), or kidney, liver, ovary (blend), of animals in all dosage groups. In the second study, 5 mg/kg was determined to be a "no-effect" level for clinical and histopathologic changes.

564 IN VITRO METABOLISM OF 2,4-DICHLOROPHENOL (2,4-DCP) IN A RAT LIVER MICROSOMAL SYSTEM. G.R. Siegel and D.R. Buhler, Dept. of Agric. Chem. and Environ. Hlth. Sci. Ctr., Oregon State Univ., Corvallis OR.

Metabolism of 14C-labeled 2,4-DCP was investigated with microsomes from untreated male Sprague-Dawley rats and from rats which had been pretreated with either phenobarbital (PB) or α-naphthoflavone (BNF). HPLC of ether extracts of control incubation mixtures indicated the presence of a single metabolite corresponding to a few percent of the parent compound which was identified as 3,5-dichlorocatechol, as determined by comparison with standard by HPLC and mass spectral analysis. Production of the metabolite was found to be NADPH dependent, decreased by PB pretreatment and increased by BNF pretreatment. A second, minor metabolite was observed under conditions of BNF induction. The effects of two in vitro inhibitors, SKF-525A and α-naphthoflavone (ANF), were also examined; both (0.1 mM) decreased catechol formation. Enzymatically mediated, NADPH dependent covalent binding to microsomal protein of radioactivity from 14C-2,4-DCP was also observed. Pretreatment by both PB and BNF increased covalent binding, with PB having a greater effect. Neither SKF-525A nor ANF had any effect on covalent binding. These results suggest that formation of the catechol from 2,4-DCP is mediated by a cytochrome P-450 system; induction data indicate P-448 involvement. Results also suggest that covalent binding may not be due to an epoxide intermediate. (Supported by U.S. EPA Cooperative Agreement CR-809385-02).

To clarify the role of GST in the metabolism of DNFB, FAM and Fluorocate (FAC), we investigated the substrate specificity of GST and FSD in the anionic protein fraction of DBAE sephadex purified mouse liver cytosol. FAM was defluorinated in the FSD assay at a rate of only 1% that observed for FAC. Both alkyl fluorinated substrates underwent no measurable non-enzymatic dehalogenation. In contrast, DNFB, was spontaneously defluorinated in a non-enzymatic reaction with glutathione, a cosubstrate for both FSD and GST, forming the thioether dehalogenated conjugate. This spontaneous defluorination occurred at a rate approximately 40 times the spontaneous dehalogenation rate observed with the chlorinated congener, 1-chloro-2,4-dinitrobenzene (CDNB). DNFB was also enzymatically dehalogenated in the GST assay forming the thioether product at approximately twice the rate observed for CDNB. Although FAM and FAC both inhibit GST activity, the inhibition occurs only at high concentrations suggesting a non-specific effect. Antibody raised in the rabbit to FSD precipitated 100% of the FAM and FAC defluorinating activity while leaving GST activity toward CDNB and DNFB unchanged. The results indicate that FSD is responsible for FAM and FAC defluorination activity while DNFB is defluorinated by GST. This work was supported by grants ES0200103 and GM07146.

566 STEREOSELECTIVITY IN THE MICROSONAL CYTOCHROME P-450-DEPENDENT OXIDATION OF STYRENE TO STYRENE 7,8-OXIDE (SO) IN RATS AND RABBITS. G.L. Foureman, C. Harris, O. Hernandez, A. Bhatia and J.R. Benda. NIEMS/NH, Research Triangle Park, North Carolina.

The stereoselective oxidation of [14C]-styrene to R- or S-SO was studied in hepatic microsomes from untreated, phenobarbital (PB)- or 3-naphthoflavone (3NF)-induced rats, and in pulmonary and hepatic microsomes from rabbits. Metabolically generated SO was almost quantitatively (>95%) converted to its four diastereomeric glutathione (GSH) adducts. The SO-GSH conjugates were separated by HPLC, allowing us to determine the relative amounts of R-SO and S-SO formed. The stereoselectivity of the reaction depended on the source of the microsomes. Hepatic microsomes from control rats produced a 1.5-fold excess of R-SO (1.5±0.10, 21; S-SO/R-SO, mean ± SD, n), those from 3NF-treated rats a 1.5-fold excess of R-SO (0.86±0.06, 17) and those from PB-treated rats a slight excess of S-SO (1.09±0.09, 16). PB but not 3NF treatment significantly increased the rate of styrene metabolism by rat hepatic microsomes. Both rabbit pulmonary and hepatic microsomes produced excess R-SO (1.5-fold and 1.2-fold, respectively) during the oxidative metabolism of styrene. Thus, liver microsomes from untreated rabbits and untreated rats differed in the predominant SO enantiomer formed. These data indicate that cytochrome P-450 isozymes vary in the stereoselective oxidation of SO, a relatively small hydrocarbon substrate of the monooxygenase system.

567 ABSORPTION, DISTRIBUTION AND EXCRETION OF SPAN 20 IN RATS. S. Giovanetto, L. Martis, E. Lengel and E. Woods. Travenol Laboratories, Morton Grove, IL.

Span 20 is a nonionic surface active agent used primarily as a water-in-oil emulsifier in a number of cosmetic and pharmaceutical preparations. In the present study, disposition kinetics of Span 20 containing [14C]-sorbitan monolaureate was studied in rats following oral administration of 2.1 g/kg of the surfactant. Peak radioactivity of 4.01 ± 0.71 (n=4) percent of dose per 100 ml occurred within 30 min of treatment and declined to background level within 24 hours. Significant initial accumulation of radioactivity was found in the liver, kidneys, lungs, heart and spleen. The maximum 14C concentrations as a percentage of dose in the liver and kidneys occurred within 4 hours and were 1.7 and 0.81, respectively. Urinary excretion was the major route of elimination of [14C]-sorbitan monolaureate. Forty-eight hours after the treatment, 87.2 ± 0.8 and 5.6 ± 1.0 (n=4) percent of the dose were excreted in urine and feces, respectively. These data indicate rapid absorption and elimination of the surfactant following oral administration in rats.

568 2-NITROPROPANE (2NP) METABOLISM IN UNINDUCED MOUSE LIVER MICROSONES. A.R. Markou, A.P. Eelsen, Toxicology Program, Dept. of Environ. and Indus. Health, The University of Michigan, Ann Arbor, MI.

2NP is an important chemical in industry where there is ample opportunity for human exposure. Animal studies showing nitrite in major organs, methemoglobinemia and tumors after 2NP exposure suggested that biotransformation may be necessary for toxicity. Using liver microsomes from phenobarbital and 3MC pretreated rats, Ulrich, et al. (Biochem. Pharm. 27:2301, 1978) demonstrated cytochrome P-450 (P-450) mediated denitrosation of 2NP to nitrite and acetone. Activity in uninduced rat, however, was reported to be very low making this novel reaction of purely academic interest with little application as a potential mechanism of 2NP toxicity in control animals.

Our work with uninduced mouse liver microsomess has shown, however, a significant amount of P-450 mediated denitrosation. Activity (n moles nitrite released/min/mg protein) measured in five mouse strains ranged from 6 in PL/J mice to 10 in BALB mice. A significant decrease in activity with 3MC 525A, alpha-naphthoflavone and metyrapone was observed. Nitrite release was also seen in a cumene hydroperoxide supported system indicating involvement of an oxidative mechanism. The NADPH dependent reaction indicated two pH optima (7.6 and 8.8). Kinetics of this reaction at these pH's indicated different Ks values but similar Vmax's. These results suggest two isozymes of P-450 may be involved in denitrosation of 2NP in control mice.
METABOLISM, DNA BINDING AND CYTOTOXICITY OF AFLATOXIN B₁ IN TRACHEAL EXPLANTS FROM SYRIAN HAMSTERS. R.A. Coulombe, B.W. Wilson and D.P.H. Haich, California Primate Research Center, University of California, Davis, CA 95616.

Metabolism, DNA binding and cytopathological effects of aflatoxin B₁ (AFB₁) were studied in isolated tracheal explants from Syrian golden hamsters. Explants were exposed to 0.1, 0.5 and 1.0 μM [14C]AFB₁ in Dulbecco’s modified Eagle medium for 24 h, then analyzed for DNA binding and metabolism. Binding (pmol AFB₁/mg DNA) was dose-related (16.3±1.9 to 180.8±16.7), and analysis of the culture medium revealed the metabolic contributions aflatoxical (APL) and aflatoxin Q₁ (AFQ₁). Ultrastructural analysis of sections of tracheal epithelium revealed dose-related morphologic changes primarily in the nonciliated cells, but no discernible distribution of label with respect to cell type was detected by autoradiography. In addition, a dose-dependent supernatant (SN) prepared from hamster trachea was shown to activate AFB₁ to metabolites mutagenic to S. typhimurium TA 98, but was ca. 70 times less active on a per mg protein basis than was SN from hamster liver. These results demonstrate the metabolic capabilities of tracheal epithelium in the activation of AFB₁, with results contributing to the formation of respirable particles, implicating the body in the activation of AFB₁. The study was supported by the NIH (Grant HL07013).

IDENTIFICATION OF THE PROXIMATE PEROXISOME PROLIFERATOR(S) DERIVED FROM DE-2-(2-ETHYL-HEXYL)PHTHALATE (DEHP). A.M. Mitchell, J.C. Lhuenut, J.W. Bridges and C.R. Elcombe, Imperial Chemical Industries PLC, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK E.N.S.B.A.N.A., Campus Universitaire, 2100 Diegem, France, and Roberts Institute, University of Surrey, Guildford, Surrey, UK.

The proximate peroxisome proliferator(s) derived from DEHP have been identified using rat hepatocyte cultures. DEHP metabolites were added to cultures at 24 hr intervals. After 3 days of exposure to the hepatocytes were harvested and homogenized. Peroxisome proliferation was determined as CN⁻ insensitive palmitoyl CoA oxidation (PCO) activity. 2-Ethylhexanol and mono-(3-carboxyl-4-ethylphthalate (I) and mono-(3-carboxyl-5-ethylphthalate (V) had little effect upon peroxisomal PCO. However, MEHP and mono-(2-ethyl-5-hydroxxyethyl)phthalate (IX) and mono-(2-ethyl-5-oxoethylphthalate (VI) increased PCO by 7-11 fold. Electron microscopy on whole cells confirmed the biochemical data. Thus it appears that the ω-1 oxidation products are of prime importance in the proliferation of hepatic peroxisomes. The identification of the proximate proliferator has allowed studies on the mechanisms of, and species differences in, peroxisome proliferation without the confounding influence of biotransformation.

PHARMACOKINETICS OF ETHANOL IN THE FERRET. D.E. McLain, D. A. Roe and J. G. Babish, Division of Nutritional Sciences, Cornell University and Department of Preventive Medicine, NYS College of Veterinary Medicine, Cornell University, Ithaca, NY.

Select doses of ethanol (25% w/v) were administered to female ferrets (Mustela putorius) by gastric intubation to determine dosage corresponding to peak blood alcohol levels (BAL) greater than 100 mg/100 ml blood (mg%) for an extended duration. Low doses (6 ml/kg) resulted in peak BAL of 202 mg% in 1.5 hours and 50 mg% after 12 hours. Intermediate doses (8 ml/kg) resulted in peak BAL of 253 mg% in 1.4 hours and 89 mg% after 12 hours. High doses (12 mg/kg) did not increase peak BAL proportionally, resulting in BAL of 128 mg% after 12 hours, and increased the volume of distribution significantly. Computer estimate of kinetic parameters concluded that metabolism in this range of BAL was not a first-order process. Moreover, while decreasing enzyme velocity with increasing substrate concentration precluded calculation of Michaelis-Menten parameters, the data suggested possible metabolic alterations at dosage levels employed. (Supported in part by a grant from the Distilled Spirits Council to the United States.)
573 EFFECTS OF CENTRILOBULAR AND PERIPORTAL HEPATOXINS ON CYTOSOLIC AND MITOCHONDRIAL ALCOHOL AND ALDEHYDE METABOLIZING ENZYMES. R.G. Little, J.J. Hjelle, C.D. Klaassen and D.R. Petersen. Dept. of Pharmacol., Univ. of Colorado, Boulder, CO and Dept. of Pharmacol., Toxicol. and Therap., Univ. of Kansas Med. Ctr., Kansas City, KS.

Male Sprague-Dawley rats were treated with either the periportal hepatotoxicant allyl alcohol (AA) or aflatoxin B1 (ATX) or the centrilobular hepatotoxins 1,1-dichloroethylene (DCE) or bromobenzene (BB) in order to determine if select cytosolic and mitochondrial alcohol and aldehyde metabolizing enzymes exhibit heterogeneous intralobular distribution. Histopathological examinations substantiated significant hepatotoxic-induced liver damage. Alcohol dehydrogenase (ADH) specific activity was significantly reduced by AA, DCE and BB (42, 46 and 46% of control, respectively). Aldehyde reductase specific activity was decreased to 24% of control by DCE but was relatively unaffected by the other hepatotoxins. The high and low affinity (for acetaldheyde) forms of cytosolic and mitochondrial aldehyde dehydrogenase (ALDH) were not consistently lowered by the various hepatotoxins. From these data we conclude that (1) ADH is not heterogeneous distributed in the lobule, (2) aldehyde reductase inhibition is hepatotoxin specific (i.e. DCE) and (3) mitochondrial and cytosolic ALDHs are probably not located predominately in one area of the lobule. (Supported by USPHS Grants AA 03527, ES 03192 and ES 07079).


Heterogeneous intralobular distribution of xenobiotic biotransformation pathways is known to exist. This study was undertaken to determine if microsomal aldehyde dehydrogenase (ALDH) also exhibits this heterogeneity. Rats received either the periporal hepatotoxins allyl alcohol (AA, 0.05 ml/kg), or aflatoxin B1 (ATX, 5 mg/kg) or the centrilobular hepatotoxins 1,1-dichloroethylene (DCE, 500 mg/kg) or bromobenzene (BB, 0.8 ml/kg). Serum sorbitol dehydrogenase and alanine aminotransferase activities were greatly elevated by each treatment substantiating significant hepatotoxicity. NADPH cytochrome c reductase specific activity and recovery of homogentate reductase activity in the microsomal fraction were not affected by the hepatotoxins. As expected, cytochrome P-450 concentration was lowered by the centrilobular hepatotoxins only (decrease of 32 and 61% for DCE and BB, respectively). Microsomal ALDH specific activity using propionaldehyde as substrate (5 mM) was decreased 48 and 57% by the centrilobular hepatotoxins DCE and BB and not by AA or ATX. The findings suggest that microsomal ALDH predominates in the centrilobular portion of the liver lobule. (Supported by USPHS grants AA 03527, ES 03192 and ES 07079).


Ethylene glycol (EG) is metabolized to various toxic intermediates, the initial steps involving oxidation to glyceraldehyde (GA) via alcohol (AD) and aldehyde dehydrogenases. 1,3 Butanediol (BD), a potent AD inhibitor, impedes the synthesis of GA and subsequently oxalic acid, which cause metabolic acidosis and renal oxalosis, without the serious side effects of ethanol, the current treatment of choice. In order to assess the therapeutic value of BD in EG toxicosis, dogs were orally dosed with commercial antifreeze at 6 ml/kg at 0 hr and treated IV 7 times at 6 hr intervals with 5.5 ml/kg BD (20% in PSS) beginning at 8, 12, 18, and 21 hr. Blood samples were periodically assayed for GA concentration by HPLC (Hewlett et al, JAOAC 66:276, 1983). Three dogs dosed only with EG died at 4, 18, and 37 hr. One dog receiving only the BD treatment regimen showed no signs of toxicity. Five dogs received both EG and BD treatment: of 2 treated at 8 hr (C,E), 1 survived and 1 died at 27 hr. 2 treated at 12 and 21 hr (C,E) survived; 1 dog (D) died soon (27 hr) after treatment was initiated at 18 hr. Four of the 5 dogs showed dramatically decreased serum GA concentrations after BD treatment (A,B,C,E). GA concentration plateaued in dog D after 2 treatments. These data suggest that BD is effective in inhibiting AD dependent GA formation in vivo and that this methodology would be useful in objectively evaluating the efficacy of other AD inhibitors in EG toxicity.


Methanol and ethanol are significantly metabolized to formaldehyde and acetaldehyde respectively in the presence of hepatic 100,000g supernatant or mitochondrial fractions, ascorbic acid (4 mM) and 1,10-phenanthroline (10 mM). The specific activity of methanol oxidation is 1720 nmol/min/mg protein in the 100,000g fraction and 790 in the mitochondrial fraction. The specific activity of ethanol oxidation is 1590 nmol/min/mg protein in the 100,000g fraction and 820 in the mitochondrial fraction. The activity is enzymatic in that it is linear with time, proportional to protein concentration, and sensitive to temperature. In order to determine if ascorbic acid was operating through one of the known alcohol oxidizing enzymes, inhibitors of alcohol dehydrogenase and catalase were employed. The ascorbic acid system is sensitive to catalase inhibitor; sodium azide and 3-amino-1,2,4-triazole 4-Methylpyrazole, an inhibitor of alcohol dehydrogenase, had little effect. When ascorbic acid was incubated with purified catalase, methanol was oxidized to formaldehyde indicating that catalase may be an enzymatic component in the ascorbic acid system. The mechanism by which ascorbic acid is involved in the oxidation of these alcohols and the nature of the "oxidizing species" will be discussed. (Supported by Hoffmann-La Roche Grant 23007.)
577 ASCORBIC ACID, THIAMINE, OR CYSTEINE EFFECT ON ETHANOL OR ACETALDEHYDE INDUCED INCREASES IN ANTIPYRYNE HALF-LIFE.
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Sponsor: R.B. Larson.

The inhibition by ethanol (20% or 40% and acetaldehyde (ACDH) of mixed-function oxygenase activity has been demonstrated in vivo by monitoring changes in salivary half-life (t1/2) of antipyrine (AP). The ability of L-ascorbic acid (C), thiamine-HCl (B1) or L-cysteine (Cys) to prevent ETORH- and ACDH- increased t1/2 of AP was studied in adult (200-300g) male rats. ETORH (43 mmole/kg po) or ACDH (6 mmole/kg ip) was administered 20 min prior to AP (100 mg/kg ip). At dosages of 2 mmole/kg C and Cys or 0.24 mmole/kg B1 given 90 min prior to ETORH the t1/2 of AP remained unchanged from control (166 ± 5 min) compared with 243 ± 12 min in the presence of ETORH alone. When C or B1 was given with respect to ACDH the t1/2 of AP remained unchanged from control compared with 213 ± 11 min in the presence of ACDH alone. Cys pretreatment had no effect on ACDH-increased AP t1/2. Thus C or B1 pretreatment prevented both ETORH- and ACDH-increased AP t1/2. Cys pretreatment only prevented ETORH-increased AP t1/2.

578 SKIN ABSORPTION OF PESTICIDES BY DECONVOLUTION
H.L. Fisher,1 B. Most,2 L.L. Hall,1 Environmental Protection Agency, 1 Northrop Services, Inc.,2 Research Triangle Park, NC

A method for the calculation of dermal absorption and metabolism by deconvolution modeling using urinary excretion data from i.v. and dermal dosing is described. Using published average data (1) from 6 human subjects on skin- to- urine and blood-to-urine transfer of 12 pesticides, the skin-to-blood transfer rates for each compound are estimated. From a temporal profile of urinary excretion, a time profile of the dermal absorption is produced. Dermal absorption rate was largest within 8 hours of dosing for all pesticides examined. Only carbaryl showed a lag (3.5 hr) in penetration. This may indicate a prolonged transit time through skin or a chemical transformation in skin has occurred. After onset, absorption occurred rapidly with 45% of the 120 hour cumulative absorption occurring in 8 hours. For parathion and diazinon, over 50% of the 120 hour total absorption occurred in the first 4 hours. The deconvolution technique described here permits the calculation of the temporal aspect of dermal absorption for linear systems. Estimates of chemical transformation occurring dermally may be obtained.


579 DERMAL ABSORPTION AND DISPOSITION OF 1, 3-DIPHENYLGLUANIDINE IN RATS. P.V. Shakhov,1 Y.M. Ioannou,2 H.L. Fisher,3 and L.L. Hall.3 NIEHS,2 USEPA,1 Research Triangle Park, NC

Dermal absorption, distribution, and metabolism of 1, 3-diphenylgluanidine (DPG), widely used in food packaging and as an accelerator in processing rubbers, was studied in adult male Sprague-Dawley rats. DPG shows 10% penetration through shaved back skin of the rats in 5 days. The first-order dermal absorption rate constant as determined by least square method was 0.021 ± 0.002 days⁻¹ (T 1/2 = 33.6 days). Approximately 13% of the absorbed dose remained in the body in 5 days. Retention in skin, muscle, liver, intestine and fat contributed most to the body burden of DPG derived radioactivity in 5 days. All tissues showed tissue to plasma ratios greater than 1, with liver and intestine ratios of 21 at 5 days. Approximately 61% of the absorbed dose was eliminated into urine and 27% into feces in 5 days, showing rapid clearance of absorbed DPG from the body. HPLC analysis of urine revealed two major peaks (parent compound and metabolite(s)). For up to 72 hr 95% was excreted as parent while after this time no parent was seen. The extraction of radioactivity from the treated skin patch shows only parent compound on HPLC. Although DPG shows very slow dermal penetration and rapid clearance from the blood, this route of exposure needs to be considered in chronic occupational exposure risk assessments.


Studies by us and others have shown that morpholine derivatives, widely used in rubber manufacturing, are sensitizing agents in guinea pigs and humans. Our purpose was to investigate the biochemical basis of this sensitization reaction. We examined the reaction of representative amino acids, Cys, Lys, and Gly, with: morpholine (M), 4,4'-dithiodimorpholine (DTS), morphylinylmercaptobenzothiazole (MMT) and 2-mercaptobenzothiazole (MBT) under physiological conditions. Following incubations for 24,48,72 and 168 hrs, products were separated via silical gel thin layer chromatography (TLC) on F-254 plates. New compounds were observed for MMT with all three amino acids after 24 hr. Additional new compounds were found for: Cys/MMT and Cys/DTS (24 hr); Lys/MMT (72 hr); Lys/MMT and Cys/MMT (168 hr). No reaction was observed with H. Following extraction, the new compounds were examined spectrosopically. Results suggest sensitizing nature of DMS is probably due to reaction of its disulfide with -SH and -NH2 amino acid moieties. The -SH group of the MMT may react either via oxidation and/or thioester formation with the carboxylate group of the amino acids. These latter adducts may be the allergens responsible for the MMT sensitization. Supported by USPHS 0159.
SKIN PENETRATION OF CHEMICALS IN VITRO: METABOLIC VIABILITY AND SPECIES DIFFERENCES. J. Kao and J. Whitaker, Biology Div., ORNL, Oak Ridge, TN

The rate limiting barrier in skin penetration is generally considered to be the nonviable stratum corneum; but metabolic viability was shown to be the major determinant in the in vitro penetration of topical benzo(a)pyrene [B(a)P] in mouse skin. Using a skin permeability chamber culture system, species differences in the penetration of chemicals was examined as a function of metabolic viability. Metabolic viability was assessed by 13C-glucose metabolism, and in studies with B(a)P (5 μg/200 mm²), the percent penetration in 15 hrs was 18.9, 1.5 and 0.6 in mouse (M), rabbit (R) and guinea pig (G) skin, respectively. No differences between viable and nonviable tissue were observed with R and G skin, but penetration in nonviable M skin (1.7%) was drastically reduced. In contrast, testosterone penetration in viable skin from all 3 species was significantly (p<0.01) lower than the corresponding nonviable skin. The percent penetration in viable tissue was 49.6, 10.9 and 4.5 with the corresponding values for nonviable tissue being 66.6, 26.4 and 6.9 for M, R and G skin, respectively. These results showed that skin penetration involves both diffusional and metabolic processes. Their relative importance will depend on the physicochemical properties of the chemical and the metabolic capability of the skin towards the compound in question. (Supported by OMER, U.S. DOE under contract W-7405-eng-26 with UCC.)

SUBCHRONIC DERMAL TOXICITY OF PARA-TERTIARY BUTYL BENZOIC ACID IN RATS. S. Z. Cagen, T. H. Gardiner, D. R. Patterson, and C. C. Lu, Toxicology Research, Shell Development Company, P.O. Box 822, Houston, TX.

Para-tertiary butyl benzoic acid (ptBB) has had applications in the manufacture of resins, polymers, and corrosion inhibitors and this study was designed to evaluate the subchronic dermal toxicity of aqueous solutions containing the diethanolamine salt of ptBB. Male and female Fischer 344 rats were treated topically 5 days/week for up to 13 weeks with dosing solutions resulting in daily exposures of 0.0, 17.5, 35.0, 70.0 or 140.0 mg/kg ptBB. These doses did not produce overt clinical signs of toxicity, however, treatment with the two highest doses resulted in decreases in body weight. Treatment with all ptBB containing solutions resulted in dose-related increases in liver wt/body wt and kidney wt/body wt ratios, and treatment of male rats with the two highest doses resulted in reduced testes weight. Treatment-related pathologic changes were confined to three organ systems: liver (cytoplasmic vacuolation), kidney (papillary necrosis, tubular dilation), and testes (tubular degeneration, interstitial edema, giant cell formation). The testicular effects were observed in rats exposed to only the two highest doses of ptBB but were associated with marked changes in testicular function; sperm counts per testis decreased by 92 to 99% in these groups.

We have previously reported (R. Chen, M. Brabee, and R. Conolly, The Toxicologist 3 (1), 1982) the headspace disappearance of VDC in rat hepatic mitochondria and microsomes. We now report the identification by GC-mass spectrometry of monochloroacetate in rat hepatic mitochondria and microsomes exposed in vitro to VDC. Metabolites were isolated from incubates by solvent extraction and then methylated. A Hewlett-Packard 5993 GC-mass spectrometer with SE 30 capillary column was used to generate mass spectra. The identification of monochloroacetate as a mitochondrial and microsomal metabolite of VDC was confirmed by comparison of the mass spectra of its methylated derivative with that of authentic methylchloroacetate. The production of monochloroacetate from rat hepatic mitochondria and microsomes was NADPH dependent. Phenobarbital pretreatment of the rats from which mitochondria and microsomes were prepared increased monochloroacetate production in microsomes but not in mitochondria. (Supported by NIHNS Training Grant 5 T32 ES07062.)
METABOLISM OF INHALED DIAZONE METHANE IN VIVO IN MALE RATS. M. L. Gargas and M. E. Anderson, Wright-Patterson AFB, OH

Diazomethane is metabolized by two pathways: one that yields CO and one that requires glutathione (GSH) and produces CO₂. Both yield 2 moles of halide ion. We studied the kinetic properties of the two pathways in vivo by exposing male rats to various inhaled concentrations of CH₂Cl₂, CH₂BrCl, and CH₂Br₂ and determining end-exposure carboxyhemoglobin (HBCO) and plasma bromide. The CO pathway was high affinity, low capacity, and the GSH pathway was much lower affinity but high capacity. The rate of halide release from the GSH pathway was related as CH₂BrCl > CH₂Br₂ > CH₂Cl₂. Presumably bromide is a preferred leaving group but steric hindrance in the initial reaction with GSH is important with CH₂Br₂. We also studied the effects of pyrazole (which inhibits microsomal oxidation) and 2,3-epoxypropanol (which depletes GSH) on diazone metabolism. Pyrazole abolished CO production from CH₂Br₂. GSH depletion did not change the yield of halide ion from the high affinity CO-pathway. It did increase the steady state HBCO levels with CH₂Cl₂ and CH₂BrCl, but not with CH₂Br₂. It appears that the putative forml chloride (FC) intermediate from CH₂Cl₂ or CH₂BrCl has a longer life than the forml bromide from CH₂Br₂ and a significant portion of the FC (~20-30%) may react with other cellular nucleophiles instead of spontaneously decomposing to CO.

1,4-BIS [(3,5-DICHLOROPYRIDYL)OXY]BENZENE: EFFECTS OF STRUCTURAL ANALOGS AS INDUCERS OF HEPATIC DRUG-METABOLIZING ENZYMES: I. Lambert, M. Kelly, J. Merrill and S. Safe, Dept. of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX, 77843

1,4-BIS [(3,5-dichloropyridyl)oxy]benzene (TCPBOP) resembles phenobarbital (PB) in its mode of induction of the hepatic drug-metabolizing enzymes in mice. The structural features of this molecule include: a linear tricyclic aromatic ether ring system, an internal 1,4-disubstituted benzene ring and two 3,5-dichloropyridyl substituents. Several analogs of TCPBOP have been synthesized and these include, 1,4-bis [(3-chloropyridyl)oxy]benzene, 1,4-bis [(5-chloropyridyl)oxy]benzene, 1,4-bis [(pyridyl)oxy]benzene and 1,4-bis [(5-bromopyridyl)oxy] benzene. Dose-response induction of mice hepatic microsomal cytochrome P-450, aldrin epoxidase and aminopyrine N-demethylase gave EC₅₀ values for TCPBOP and related homologs. The results illustrate that changes in the structure of pyridyloxy ring markedly affect enzyme induction activity. The order of activity for the substituents was 3,5-dichloropyridyl > 5-chloropyridyl > 5-bromopyridyl > 3-chloropyridyl > pyridyloxy. The results confirm the induction potency of these compounds and support a receptor-mediated mechanism of action. (Supported by the Texas Agricultural Experiment Station.)

585 THE PHARMACOKINETICS OF 14C-1,3-DICHLOROPROPENE IN RATS AND MICE FOLLOWING ORAL ADMINISTRATION, P.K. Dietz, E.A. Hermann and J.C. Ramsey, Toxilogical Research Laboratory, Dow Chemical Co., Midland, MI 48640.

The fate of 14C-cis,trans-1,3-dichloropropene (1,3-D) was determined after oral dosing of 1 or 50 mg/kg to male Fischer 344 rats and 1 or 100 mg/kg to male B6C3F1 mice. Urine, feces and expired air were collected till 48 hrs post-dosing when all animals were sacrificed. Tissues and remaining carcasses were then analyzed for residual radioactivity. Urine was the major route of excretion, with 51-61% (rats) and 63-75% (mice) of the administered dose excreted over 48 hrs. Feces and expired CO₂ accounted for roughly 18% and 6% of the administered dose in rats and 15% and 14% of the administered dose in mice, respectively. At the end of 48 hrs, 2-6% of the original dose remained in the carcasses of both species. LC separation of urine excreted by rats and mice indicated two minor and two major metabolites. The predominant metabolite was identified as N-acetyl-S-(3-chloroprop-2-enyl) cysteine with its sulfone or sulfoxide tentatively identified as the remaining major metabolite. No evidence of major dose-dependencies were noted in either species over the range of administered dose levels. These data indicate that conjugation with glutathione is a major route of 1,3-D metabolism by rodents.

586 NON-PROTEIN SULFHYDRL CONTENT AND MACROMOLECULAR BINDING IN RATS AND MICE FOLLOWING ORAL ADMINISTRATION OF 1,3-DICHLOROPROPENE, F.K. Dietz, D.A. Dittenber, H.D. Kirk and J.C. Ramsey, Toxilogical Research Laboratory, Dow Chemical Co., Midland, MI 48640.

The non-protein sulfhydryl (NPS) content and covalent binding to macromolecules was determined in tissues of rodents following oral administration of 1,3-dichloropropene (1,3-D). Male Fischer 344 rats and B6C3F1 mice were given single oral doses of 0, 0.5, 2.5, 5, or 100 mg 1,3-D/kg for NPS studies and 0, 0.5, 1, 5, 10, 50 or 100 mg 14C-1,3-D/kg for binding studies. Forestomachs (FS), glandular stomachs (GS), livers, kidneys and bladders were removed at 2 hrs post-dosing for analysis. NPS levels in the FS of rats and mice were depleted to 17-27% of control values at 100 mg/kg and 19-37% of controls at 50 mg/kg. Significant depletion in the FS was also noted at 25 mg/kg (27% and 51% of control values in rats and mice, respectively). Effects on NPS in the GS and liver were also dose-dependent but less severe. Binding in the FS and GS was greatest at dose levels which caused the most depletion of tissue NPS. Limited binding was noted in the livers, kidneys or bladders. Since conjugation of 1,3-D with glutathione (GSH) appears to be a detoxification mechanism, the results of this study suggest that the sensitivity of the FS in rats and mice to the toxic effects of orally administered 1,3-D would be increased at dose levels of 25-100 mg/kg, where GSH levels are severely depleted.
589 EFFECT OF DISULFIRAM AND DIETHYLDITHIOCARBAMATE ON ATPASE AND NUCLEOTIDASE ACTIVITIES OF RAT LIVER CELL MEMBRANES. D.J. Brown and A. Hunter. East Carolina University School of Medicine, Greenville, NC. Sponsor: John P. DaVanzo

Disulfiram (DS), a drug used in alcoholic therapy, modifies the transport of ouabain and bromosulfophthalein in isolated hepatocytes. In the following study the effect of DS and its bio-transformation product, diethylidithiocarbamate (DDC), on Na+,K+-ATPase, Mg++-ATPase, and 5'-nucleotidase activities in rat liver cell membranes was examined. DS (0.25 or 0.50 mmol/kg) in corn oil (5 ml/kg) or DDC (0.50 mmol/kg) in distilled water (5 ml/kg) were given ip to adult male Sprague-Dawley rats. At various times (3, 6, 12, 24, and 48 hrs) after administration the animals were sacrificed and plasma and bile canicular membranes were isolated. Enzyme activities were determined by measuring inorganic phosphate. DS significantly elevated bile canicular membrane ATPase and nucleotidase activities from control levels 6 hrs after treatment. The increased enzyme activities were corroborated by increased release in hepatocytes 6 hrs after DS administration. Plasma membrane Mg++-ATPase was decreased in comparison to control values 12 hrs after DS administration. DDC reduced bile canicular membrane Mg++-ATPase activity, 6,12 and 24 hrs after administration and plasma membrane Mg++-ATPase activity 6 hrs after treatment. In summary, DS and DDC alter ATPase and nucleotidase activities in rat liver cell membranes.

590 CORTICOSTERONE INHIBITION OF BENZO(a)PYRENE METABOLISM IN COMPARISON WITH METYRAPONE AND 4-p-NAPHTHOFLAVONE. M. Bogdandzy, A.E. Roberts, B.R. Brown, and R.A. Schatz. Tox. Prm., Northeastern U. Boston

Studies in our lab demonstrate that acute alcohol inhibits benzo(a)pyrene (BaP) metabolism in rats and that the effect requires an intact adrenal. A physiological study of alcohol stress is increased plasma levels of corticosterone (CS). We compared the ability of CS and a specific inhibitor of P450 (metyrapone, MT) and P448 (4-α-naphthoflavone, αNF), to inhibit BaP metabolism in vitro. Arylhydrocarbon hydroxylase (AHH) was measured in control, P450 (phenobarbital, PB) and P448 (3-methylcholanthrene, 3MC) induced microsomes. AHH activity in control microsomes in the presence of CS and MTY were 70 and 66% of control, respectively, at inhibitor/substrate ratio (ISR) = 5. In PB (P450 enhanced) microsomes, these values were 54 and 29% control. αNF either stimulated or had no effect on AHH in control or PB induced microsomes. In 3MC (P448 enhanced) microsomes, AHH activity for CS, MTY, and αNF were 69, 123, and 265% control. In contrast, analysis of BaP metabolite patterns shows that CS does not alter overall BaP diol formation in control, Pb, or 3MC microsomes. MTY, and αNF inhibited diol formation in Pb and 3MC induced microsomes, respectively. Thus, CS most closely resembles MTY in the inhibition of BaP metabolism. The results suggest that CS will preferentially inhibit pathways leading to detoxification of BaP without affecting activation. Supported by BBRC grant 507 RR 05830-03.

591 POLYCHLORINATED TERPHENYLs AS INDUCERS OF HEPATIC DRUG-METABOLIZING ENZYMES: B. Leese, M.A. Denomme, J. Gyrkors, K. Homonoko, B. Chittim and S. Safe. Dept. of Chemistry, University of Guelph, Guelph Ontario, Canada, N1G2L1. Dept. of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843

Polychlorinated terphenyls (PCT) are industrial formulations which have been widely identified as contaminants in fish, wildlife and human adipose tissue. Laboratory studies indicate that the commercial PCT mixtures induce several hepatic drug-metabolizing enzymes in rodents, however, some of this activity may be due to PB impurities. This study reports the effects of o-, m- and p-terphenyl and several PCT congeners as inducers in the immature male Wistar rat. o-terphenyl induce microsomal aminopyrine N-demethylation whereas the p- and m-terphenyl isomers were inactive. The enzyme induction activities of several PCT congeners did not exhibit any remarkable structure- activity dependence; 2,4-dichloro-o-terphenyl and 2,2,4,4-tetrachloro-o-terphenyl induced aminopyrine N-demethylation and 2,3,4,5-tetrachloro-o-terphenyl induced ethoxyresorufin O-deethylation. All of the remaining congeners (7) were inactive at a dose level of 300 mol/kg. The results for the PCT congeners and mixtures confirm their relatively weak effects as inducers of the drug-metabolizing enzymes. (Supported by the Natural Sciences and Engineering Research Council of Canada.)

592 2,2-DIMETHYL-5-t-BUTYL-1,3-BENZOIXDIOXOLE: AN UNUSUAL INDUCER OF MICROSMAL ENZYMES. J.C. Cook and E. Hodgson. Interdepartmental Toxicology Program, N. C. State University, Raleigh, NC 27695.

Cytochrome P-450 induction, in the case of the methylenedioxyphenyl (MDP) compound, isoasafrole, appears to be regulated by the Ah locus. 2,2-Dimethyl-5-t-butyl-1,3-benzodioxole (DBBD), a MDP analog whose methylene hydrogens have been replaced by methyl groups, does not form an inhibitory complex with cytochrome P-450 nor induce this cytochrome. However, DBBD treated male Dub: ICR mice show an increase in NADPH-dependent cytochrome c reductase (1.6x) and epoxide hydrolase (1.9x) activity. This separation of cytochrome P-450 induction from epoxide hydrolase and NADPH-dependent cytochrome c reductase appears to be unique among inducers of xenobiotic metabolizing enzymes. In similar experiments, mice were treated with "phenobarbital (PB) + DBBD" and "3-methylcholanthrene (3-MC) + DBBD" and the following parameters were measured: cytochrome P-450 content, NADPH cytochrome c reductase, ethylmorphine and benzphetamine N-demethylase, 7-ethoxycoumarin O-deethylase, benzo(a)pyrene hydroxylase, ethoxyresorufin O-deethylase, and SDS-PAGE. PB + DBBD treatment gave results identical to those with PB alone. In contrast, cytochrome P-450 content, benzo(a)pyrene hydroxylase, and ethoxyresorufin O-deethylase activities in 3-MC + DBBD treated mice were lower than in 3-MC treated animals. SDS-PAGE confirmed that 3-MC induction of cytochrome P-450 was reduced by DBBD, suggesting that it may be an antagonist to the Ah cytosolic receptor.

The binding of certain compounds, most notably isocyanides and the metabolites of methylene dioxymphenyl compounds, to intact microsomes gives rise to the Type III optical difference spectra which are characterized by two pH dependent peaks in the Soret region. Previous studies with piperonyl butoxide, using reconstituted systems with isolated cytochrome P-450 isozymes, showed that only one fraction, A1, produced the typical Type III spectrum. The other fractions bound a piperonyl butoxide metabolite after the addition of NADPH without producing characteristic Type III spectra. In the current studies, ethyl isocyanide was used as the ligand for the dithionite reduced isozymes. Although each of the isozymes interacted with ethyl isocyanide to produce two pH dependent peaks, the pH equilibrium point for the isozymes differed from each other. As in microsomes, the spectral binding constant, Kd, for the two Soret peaks differed from each other for each of the fractions. The isozymes interacted with other isocyanides to produce similar Type III spectra. Comparative studies of Type III spectrum formation and methylenedioxymphenyl compounds using these isozyme fractions are in progress.


MV-678 [1-[6-Methoxy-4,8-dimethylhydrazino]-4-[1-methylthyl]benzene], a recently developed insect growth regulator, increased the hepatic mixed function oxidase enzymes which metabolize endogenous and exogenous chemicals. In an initial set of experiments, male and female rats received 0, 50, or 800 mg/kg/day of MV-678 by gavage for three days, and in a second set of experiments, male rats received 0, 50, or 800 mg/kg/day of MV-678 by gavage for 30 days. A significant increase in both absolute and relative liver weight, microsomal protein content, cytochrome P-450 content, NADPH-cytochrome P-450 reductase activity, and ethylmorphine N-demethylase activity was observed in male and female rats at the high dose level at three days. Similar increases were observed in the 800 mg/kg/day males at 30 days. Reversibility of induction was determined by measuring the same parameters in animals treated for 30 days after a 15 or 30 day recovery period. At 15 days post-dosing, all biochemical parameters at the high dose level, except relative liver weight and microsomal ethylmorphine N-demethylase activity, had returned to control levels. No significant differences between the control and high dose group animals were noted at 30 days recovery.


The cytochrome P-450 system is comprised of a family of isozymes that are localized within specific cell-types in the lung. The relative distribution of the different isozymes in these cell-types is presently unknown. Using 2-acetylaminoflourene (AAF; 0.94 μM), which is known to be metabolized by a number of different cytochrome P-450 isozymes in liver, the distribution of 7-, 9-, 5-, 5a, 1- and N-hydroxylation activities were examined in isolated rabbit lung cells fractionated by centrifugal elutriation into 7 populations. Macrophages obtained by lavage were also examined. High correlations (r>0.9) were seen between the rates of formation of 9-, 5, 5a, 1- and N-hydroxy AAF indicating that these metabolites were formed within the same population of cells although not necessarily the same cell-type. In contrast, 7-hydroxylation did not correlate well with any of the other pathways (<0.5). Pretreatment with TMBD selectively induced 7-hydroxylation in all cell populations possessing activity. Macrophages exhibited cytochrome P-450 activity, 9-hydroxylation being the most active pathway. In contrast, the endothelial cell fraction showed little or no ability to metabolize AAF.


2,6 dichloro-4-nitroaniline was given by gavage daily to female Sprague Dawley rats (135-150 g) at 1000 mg/kg for 4 days in an effort to correlate an induction of cytochrome P-450 with an increase in the 2 and 4-hydroxy products of biphenyl hydroxylase as previously reported by Gallo et al. In the present study treated animals had an increased relative liver weight of 184% of control. The 4-hydroxy product of biphenyl hydroxylase increased 4 times control whereas the 2-hydroxy product increased 10 times control. However, no increase in cytochrome P-450, measured as a function of carbon monoxide binding, could be detected (0.68 and 0.81 nmoles P-450/mg protein in control and treated animals respectively).

The results of preliminary studies designed to elucidate the mechanism of this apparent paradox suggest the possibility of an induction of a subspecies of cytochrome P-450 or the direct hydroxylation of the biphenyl by a hydroxy radical in situ.

(This has been supported by a Fellowship from the Procter and Gamble Co. awarded through the Society of Toxicology).
597 DIETHYL ETHER ANESTHESIA DECREASES THE EXCRETION OF BILIRUBIN. R.L. Dills and C.D. Klaseen, Dept. of Pharmacol., Toxicol. & Therap., Univ. of Kansas Medical Center, Kansas City, KS.

Bilirubin excretion is reduced during diethyl ether anesthesia by 32% in rats. In an attempt to determine the mechanism of this phenomenon, we compared the effect of diethyl ether on hepatic uridine-5'-diphosphoglucuronic acid (UDPGA) concentration and bilirubin excretion. Rats were anesthetized with urethane or diethyl ether. Bile was collected through a biliary cannula and was analyzed by high-pressure liquid chromatography for bilirubin and its two conjugates, the monoglucuronide (BMC) and diglucuronide (BDG). The biliary concentration of BDG in the diethyl ether group was one-third the concentration of those given urethane whereas BMC levels were similar. Hepatic UDPGA was found to be only slightly depleted by urethane but greatly depleted by diethyl ether throughout the exposure period. A second group of rats was administered diethyl ether for surgery and allowed to recover from the anesthesia for two hours. During recovery, bile samples were analyzed for bilirubin and for liver UDPGA. BMC concentrations were found to change only slightly over the two hour recovery period. BDG levels rose significantly during this period and paralleled the increase of hepatic UDPGA. Thus, the decrease in bilirubin excretion observed during diethyl ether anesthesia appears to be due to a decrease in hepatic UDPGA concentration. (Supported by USPHS Grants ES 07079 and ES 03192).


The effects of oral and dermal administration of single doses of 100-1,000 mg/kg DEF on esterases and liver metabolism enzymes were studied one day following dosing. 0.0-Diethyl O-4-nitrophenyl phosphorothioate (parathion) and trp-cresyl phosphate (TCP) were also used. Brain acetylcholinesterase was inhibited with topical doses of 500 and 1,000 mg/kg DEF and with parathion. Plasma cholinesterase and liver carboxylesterase activities were decreased in all groups. Neurotoxic esterase (NTE) was decreased with topical doses of 200-1,000 mg/kg DEF and with TCP. Oral doses of DEF increased cyt. P-450 content, 70 to 200% while dermal application caused a 200 to 325% increase. Aniline hydroxylase and p-chloro- n-methylaniline demethylase were increased by DEF treatments. Liver microsomal proteins from hens treated with phenobarbital (PB), 3-methyl cholanthrene (3MC), or DEF were analyzed using SDS-polyacrylamide gel electrophoresis. A striking increase in a 49K protein band in microsomes from PB and DEF hens was seen, while a 55K band was increased from 3MC-treated hens. In conclusion dermally applied DEF was more effective in inhibiting esterases and inducing cyt. P-450 than oral DEF; toxicity was directly related to the dose and route of administration. (Supported in part by NIEHS ES02717 & NIHGM 080062).

598 BIOTRANSFORMATION BY LIVER, KIDNEY AND INTESTINE OF CATTLE. John B. Watkins, Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN, Sponsor T.R. Bosin

Wide differences in the ability of various species to biotransform xenobiotics exist, and definitive data regarding this capacity in livestock are needed. The present study has determined the ability of liver, kidney and intestine from cattle to metabolize xenobiotics. Fresh tissue samples were frozen at -70°C until assayed. Microsomes and cytosols were prepared, and assays were performed under standard conditions. Data from rat liver were similar to established values. Cattle liver had 0.69 nmol P450/mg protein. N-Demethylation of benzphetamine and ethylmorphine by liver was 5.2 and 11 nmol/min/mg, respectively, while styrane oxide hydration was 9 nmol/min/mg. Glucuronidation of diethyldiethylstilbestrol, estron, N-phenethyl and testosterone by cattle liver was 3.1, 0.16, 7.1 and 0.015 nmol/min/mg, respectively. Enzymatic activities in kidney and intestine were 2-10% of hepatic values. In contrast, conjugation of chlorodinitrobenzene, ethacrynic acid and sulfobromophthalain with glutathione were similar in all three tissues (850, 18,0, and 0.50 nmol/min/mg, respectively, in liver). Thus, biotransformation in cattle occurs predominantly in liver; however, notable activities were detected in kidney and ileum. (Supported in part by BRSG 5 S07 RR5371 to Indiana Univ and Contract DE AG04-76ET3826 to New Mexico State Univ).

Studies were done to evaluate the direct effects of calcium (Ca) and zinc (Zn) on microsomal steroid and xenobiotic metabolism in the inner (zona reticularis) and outer (zona glomerulosa + zona fasciculata) zones of the guinea pig adrenal cortex. Benz(a)pyrene (BP) hydroxylase, benzphetamine (BZ) demethylase, and steroid 21-hydroxylase activities were greater in inner than outer zone microsomes but 17a-hydroxylase activity was greater in the outer zone. Neither Ca nor Zn, at concentrations of 1-50 uM, affected BP or BZ metabolism in either zone. However, both metals decreased 21-hydroxylase activity in both zones. In the absence of metals, inner zone microsomes converted progesterone and 11β-hydroxyprogesterone primarily to 21-hydroxylated metabolites. The presence of Ca or Zn produced concentration-dependent decreases in the production of 21-hydroxylated products and increases in the formation of 17β-hydroxylated metabolites. Neither metal affected the pattern of steroid metabolism by outer zone microsomes because of the predominance of 17β-hydroxylation even in the absence of metals. The results indicate that Ca and Zn exert relatively selective inhibitory effects on microsomal 21-hydroxylation and, as a result, produce greater changes in steroid metabolism in the inner than outer adrenal zone. (Supported by USPHS CA-22152)

602 INTERACTION OF ALCOHOLS WITH CARBON DISULFIDE METABOLISM. R.J. Rubin, B. Taffe, and P. Igner. Johns Hopkins Univ., Baltimore, MD 21205

Carbon disulfide (CS2) has been shown to be metabolized by microsomes to carboxyl sulfide (COS) which in turn is metabolized to CO2 by a soluble enzyme, carbonic anhydrase (CA). We have investigated: (1) the kinetics of the in vitro metabolism of CS2 by microsomes isolated from control rats fasted overnight, (2) the effect of the in vitro addition of Methanol (M), Ethanol (E) or Tsopropanol (I) to the microsomal incubation, and (3) the influence on the in vitro kinetics of pretreatment of rats with E or I. The reactions contained washed microsomes, a NADPH generating system, and 50 mM acetazolamide (to inhibit CA). The generated COS was determined by head space analysis using thermal conductivity gas chromatography. Lineweaver-Burk plots of the control data revealed a biphasic reaction with a high affinity/low velocity (HA/LV) component and a low affinity/high velocity (LA/HV) component. M at concentrations up to 100 mM had no effect on either component; both E and I markedly inhibited the HA/LV site while not affecting the LA/HV site. I was a more potent inhibitor than E. At 18 hrs. after oral administration of E (6ml/kg) or I (5mg/kg), the HA/LV component was markedly induced with little effect on the LA/HV component. These results are interpreted to indicate that there are at least 2 different P-450's for the metabolism of CS2, each having different affinities and maximum velocities and that certain alcohols can either directly inhibit or induce one of them. (Supported by ES02927).
The effect of butylated hydroxytoluene (BHT) on the disposition of aflatoxin B₁ (AFB₁) was investigated. Male Fischer F344 rats, fed a control chow or chow supplemented with 0.5% BHT for 10 days, were dosed by oral intubation with [¹⁴C] AFB₁. Animals were sacrificed at various time points and their tissues and excreta analyzed for radioactivity. Treatment with BHT enhanced the elimination of radioactive products in the urine and decreased binding to liver nuclear DNA. Lactating rats, treated in the same manner and sacrificed 6 hours after an oral dose of [¹⁴C] AFB₁, exhibited similar tissue distribution and decreased binding to liver nuclear DNA. The mammary glands of BHT-treated dams contained higher levels of APM₁, suggesting that their pups are exposed to higher levels of APM₁ in the milk. The alteration in the disposition and DNA binding of AFB₁ is related to the induction by BHT of hepatic enzymes involved in the detoxification of AFB₁. The results of these studies suggest that BHT pretreatment will protect an individual from the carcinogenic effects of AFB₁. (Supported by Stauffer Chemical Company Fellowship and NIEHS Training Grant PHS ES07039).

LONG-TERM DEPLETION OF REDUCED GLUTATHIONE IN MICE USING L-BUTHIONINE-S,R-SULFOXIMINE. J.D. Sun, S.S. Ragsdale, J.M. Benson and R.F. Henderson. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM

Previous methods to decrease in vivo levels of reduced glutathione (GSH) have been unable to do so for extended periods of time. The purpose of this study was to characterize the long-term in vivo depletion of GSH using L-buthionine-S,R-sulfoximine (BSO). Male CD-1 mice were given 0, 10, 20 or 30 mM BSO in their drinking water. At various times for up to 28 days, animals were sacrificed and GSH was measured in lungs (LG), lung lavage fluid (LF), liver (LV), kidneys (KD), and blood (BD). In addition, LG and LF alkaline phosphatase, lactate dehydrogenase, glucose-6-phosphate dehydrogenase, glutathione reductase and glutathione peroxidase, and LG and LV cytochrome P₄₅₀ levels were measured. Results showed BSO lowered GSH levels in a time and dose-dependent manner. As a percentage of controls, GSH levels in LG, LF, LV, KD, and BD were lowered to 72 ± 3%, 30 ± 4%, 43 ± 2%, 73 ± 1%, and 66 ± 4%, respectively. No effect was seen on the various indicators of lung damage, and levels of LG and LV P₄₅₀ remained unchanged as compared to controls. This indicates that BSO is effective in lowering in vivo levels of GSH without affecting other important toxicological parameters. Thus, BSO may be useful for long-term studies involving GSH and chemical toxicities. (Research was supported by U.S. DOE Contract DE-AC04-76EV01013.)

INTERACTION OF FORMALDEHYDE WITH GLUTATHIONE. P.H. Ayres, T.C. Marshall, J.A. Bond, J.D. Sun and C.H. Hobbs. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM

A recently proposed mechanism for formaldehyde (CH₂O) induced toxicity is the depletion of glutathione (GSH). Our objective was to study the interaction of CH₂O with GSH in aqueous solutions, cell-free tissue homogenates, and in intact isolated perfused lung and liver. CH₂O was prepared by heating paraformaldehyde solution at 110°C for 5 days. CH₂O was added (0-500 μg/ml) to a buffered solution (pH 7.4) containing 1 μM GSH or homogenates of lung and liver from Fischer-344 rats and incubated at 37°C for 4 hrs. GSH was analyzed by fluorescence spectroscopy at 0.5-hr intervals. Isolated lungs and livers were perfused with Kreb's-Henseleit buffer (pH 7.4) containing CH₂O (150 μg/ml) for 45 min. GSH in tissues was determined at the end of each perfusion. Addition of CH₂O to an aqueous solution of GSH, tissue homogenates, or perfused lung failed to decrease levels of GSH. Perfusion of isolated livers with CH₂O caused a depletion of GSH to 45 ± 10% of controls. Lack of an interaction of CH₂O with GSH in a buffered solution indicates that CH₂O does not cause a depletion of GSH through direct binding to GSH. Depletion of GSH by CH₂O in the isolated perfused liver, but not in tissue homogenates, implies that metabolic processes in intact cells are responsible for the interaction of CH₂O with some component of the GSH system. (Research was supported by U.S. DOE Contract No. DE-AC04-76EV01013.)

EFFECT OF COBALT ON BILIARY EXCRETION OF BILIRUBIN AND GLUTATHIONE. K.J. Stelzer and C.D. Klaassen, Dept. of Pharmacol., Toxicol. and Therap., Univ. of Kansas Medical Center, Kansas City, KS.

Adult male rats received cobaltous chloride (250 μmol/kg, sc) at various times (1-72 hrs) prior to assessment of hepatic heme oxygenase activity, bile flow, bilirubin concentration, bilirubin glucuronides, and hepatic and biliary glutathione levels. Hepatic heme oxygenase activity reached a peak 24 hrs after treatment but returned to control level by 72 hrs. Biliary concentrations of the mono- and di-glucuronides of bilirubin (BMG and BDG) were substantially increased at 24 hrs and returned to control levels more slowly than heme oxygenase. Bile flow was not significantly changed at any time. Levels of hepatic reduced and oxidized glutathione (GSH and GSSG) tended to increase after cobalt, but were not statistically significant. Biliary GSH and GSSG were increased within 1 hr after Co treatment and were twice control values at 3 hrs after treatment. These levels declined to control by 6 hrs. These results demonstrate that increased biliary excretion of bilirubin glucuronides following cobalt treatment may be associated with elevated liver heme oxygenase activity. However, changes which occurred in biliary excretion of glutathione in response to cobalt treatment did not correlate with hepatic glutathione levels. (Supported by USPHS ES 07079, ES 01142 and ES 03192).
Rats were fed for three weeks diets that contained either 20% coconut oil, 20% corn oil, or a mixture (18% coconut oil plus 2% corn oil) as the sole source of dietary fat. Hepatic glutathione (GSH) S-transferase activity was then determined with 1-chloro-2,4-dinitrobenzene (CDNB) or 1,2-dichloro-4-nitrobenzene as substrates. Feeding dietary coconut oil produced a 21±3% decrease in soluble transferase activity compared to corn oil feeding. GSH-transferase activity in the microsomal fraction was not affected. Km for soluble GSH-transferase was higher if 20% coconut oil was fed, indicating that optimum affinity of the enzyme for substrate is dependent on a source of dietary polysaturated fatty acids. Ultrafiltration to remove compounds with molecular weights <50,000 did not eliminate the differences in soluble transferase activity due to dietary fat. Separation of the transferases by fast protein liquid chromatography indicated that relative quantities of the various transferases were affected equally by the dietary treatments. The results suggest that type of dietary fat is important in the detoxification of carcinogens or other toxins that are conjugated with glutathione.

Studies were undertaken to determine if static levels of liver and kidney GSH and related enzyme activities, in rats, were altered by feeding a semi-purified diet as compared to commercial rations. Male, Sprague-Dawley rats were randomly assigned to groups fed either commercial rations (Purina* #5012) or a semi-purified diet containing 30% corn yeast admixture for up to six weeks. Hepatic GSH content was decreased in animals fed semi-purified diets for six weeks compared to animals maintained on commercial rations for three weeks. Rats fed GSH levels were not influenced by dietary regimens. γ-Glutamylcysteine synthetase activity was decreased with time but not with dietary treatment. The ability to maintain GSH in the reduced state, mediated by glutathione reductase activity, was inconsistently influenced by dietary composition. Hepatic, but not renal, capacity to degrade GSH by γ-glutamyl transferase activity increased with time. Glutathione peroxidase and glutathione-S-transferase activities were not influenced by dietary composition. These results provide evidence that dietary composition may influence the status of biochemical systems. Furthermore, these changes may alter the disposition of foreign chemicals. (Supported by NIH/NES-02425).


Depletion of non-protein sulfhydryls (NPS) by 1,2-dibromoethane (DBE) may result from direct conjugation by glutathione S-transferases (GST) or the conjugation of mixed function oxidase (MFO) products. This study determined the role of these pathways in NPS depletion in rat liver, forestomach and glandular stomach. Comparison of NPS depletion following administration (30 mg/kg, p.o.) of DBE and d4DBE revealed that depletion in liver by d4DBE was less (85±7% of control) than that due to DBE (68±5%). No such difference was observed in forestomach or glandular stomach. This isotope effect suggests that NPS depletion in liver might be due to both MFO and GST while only direct GST acts in the stomach. Using microsomal and cytosolic preparations from these tissues the metabolism of 14C-DBE was examined. Liver had the greatest GST activity (2990 pmol/min/mg protein in cytosol and 120 pmol/min/mg protein in microsomes). Cytosolic GST activities in forestomach and glandular stomach were only 155±17 and 104±8 pmol/min/mg protein, respectively. Forestomach and glandular stomach microsomes had no GST or MFO activity. However, liver microsomes did metabolize DBE (1334±4 pmol/min/mg protein). These results suggest that liver MFO activity contributes to DBE-induced NPS depletion, while in stomach this depletion is due to GST activity alone. (Supported by ES07091.)

Studies were undertaken to determine if static levels of liver and kidney GSH and related enzyme activities, in rats, were altered by feeding a semi-purified diet as compared to commercial rations. Male, Sprague-Dawley rats were randomly assigned to groups fed either commercial rations (Purina* #5012) or a semi-purified diet containing 30% corn yeast admixture for up to six weeks. Hepatic GSH content was decreased in animals fed semi-purified diets for six weeks compared to animals maintained on commercial rations for three weeks. Rats fed GSH levels were not influenced by dietary regimens. γ-Glutamylcysteine synthetase activity was decreased with time but not with dietary treatment. The ability to maintain GSH in the reduced state, mediated by glutathione reductase activity, was inconsistently influenced by dietary composition. Hepatic, but not renal, capacity to degrade GSH by γ-glutamyl transferase activity increased with time. Glutathione peroxidase and glutathione-S-transferase activities were not influenced by dietary composition. These results provide evidence that dietary composition may influence the status of biochemical systems. Furthermore, these changes may alter the disposition of foreign chemicals. (Supported by NIH/NES-02425).

Sulfobromophthalein inhibition of glutathione and methylnitroence secretion into bile. N. Ballatori and T.W. Clarkson, Div. of Toxicology, Univ. of Rochester School of Medicine, Rochester, NY.

A recent study has shown that sulfobromophthalein (BSP) can inhibit glutathione (GSH) and methylnitroence (M) secretion into bile at a dose that has no effect on liver levels of GSH or M (A.J.P. 244:G435, 1983). In order to investigate the mechanism through which BSP is exerting its effects we have examined the biliary secretion of GSH and M after the bolus iv administration of various doses of BSP, of BSP-5G (the GSH conjugate of BSP), and of DBSP (a non-metaabolizable analogue). The effects of BSP on GSH secretion were dose dependent; at a dose of 120 μmol/kg the rate of GSH secretion fell to zero. DBSP also inhibited GSH secretion, although the inhibition was not as complete as that observed after BSP administration; at a dose of 180 μmol/kg, GSH secretion fell to 18% of control. BSP-5G, on the other hand, had no effect on GSH secretion into bile when given at a dose of 120 μmol/kg. At doses of 240 and 360 μmol/kg, the rates of GSH secretion fell by approximately 20% and 50%, respectively. In all experiments the changes in M secretion were parallel to the changes in GSH secretion. The results suggest that the BSP-induced inhibition of GSH secretion into bile is due to a combination of two factors: 1) the depletion of a small and specific pool of hepatic GSH that was destined for biliary secretion, and 2) the direct inhibition of GSH transport into bile. (Supported by: GM25329, ES07026, ES01247, ES01248)
DISPOSITION OF [14C]-KATHON BLOCCIE IN MALE SPRAGUE-DAWLEY RATS. J.D. DeBecty, F.H. Deckert, R.B. Steiner, M.E. Scribner, J.N. Moss, A.H. Hayes and J.M. Smith, Toxicology Department, Rohm and Haas Company, Spring House, PA 19477.

The disposition of Kathon® bloccie, an aqueous solution (1%) of a 3:1 mixture of [3-14C]-5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one, was examined in rats following a single iv dose of 0.8 mg/kg. Urine, feces, expired air, blood and plasma, liver, kidneys, and testes were sampled at selected times up to 96 hr. All samples were analyzed for total 14C: total recovery of 14C was 94 to 111% of the administered dose. Radiolabeled was distributed rapidly to all tissues. Elimination of 14C from most tissues was biphasic with a terminal half-life of 4 days. Over 50% of the 14C was excreted in less than 24 hr and, by 96 hr, feces, urine, and CO2 accounted for 35, 31, and 4% of the dose, respectively. Total 14C was rapidly eliminated from plasma and was described by the following equation: C = 1.7e-1.8t + 0.6e-0.3t + 0.1e-0.01t. However, total 14C in blood remained constant at 30% of the dose from 6 to 96 hr indicating sequestration of Kathon and/or metabolites by red blood cells. 14C levels in testes were 100-fold less than blood at 96 hr. These results indicated that 14C-Kathon was rapidly eliminated from male rats via the feces and urine following an iv dose, suggesting that Kathon or its metabolites undergo biliary excretion. The data also indicated that under these conditions, the testes did not accumulate significant levels of Kathon equivalents.

DOWICIL 200 PRESERVATIVE: PHARMACOKINETICS AND METABOLISM IN FISCHER 344 RATS. J. M. Wechsler, Jr., P. E. Kastl, F. A. Smith and M. D. Drygza, Toxicology Research Laboratory, Health & Environmental Sciences, USA, The Dow Chemical Company, Midland MI.

DOWICIL 200 Preservative (cis-1-{3-chloroallyl}-3,5,7-tri-aza-1-azoniadamantane chloride) (D200) is a broad-spectrum antimicrobial used for the preservation of cosmetics. Studies to investigate the absorption, tissue distribution and metabolism of 14C-D200 following either dermal, oral or iv administration revealed that only about 1 to 2% of the dermally applied 14C was absorbed, whereas absorption following oral administration was nearly complete. The percentage of the dermally applied 14C absorbed did not appear to be dose or concentration-dependent. Orally or intravenously administered 14C-D200 was extensively metabolized and the metabolites rapidly excreted in the urine or as CO2 in the expired air. Results suggested a moderate dose-dependency in the disposition of orally administered D200 when radiolabeled in the chloropropene side-chain. The disposition of "absorbed" 14C derived from D200 radiolabeled in the chloropropene side chain was not markedly different between all three routes of administration. However, the fate of absorbed 14C from D200 radiolabeled in the hexamethylenetetramine ring appeared to be different for oral vs. dermal administration.


The disposition and metabolism of 2-[14C]-tris(2-chloro-2-propyl)phosphate, the major component of FYROL PFC, was determined in male and female CD® rats after a single oral (20 or 200 mg/kg) or intravenous (20 mg/kg) dose. Urine, feces, and expired air were collected for 192 hrs. Excretion of 14C was rapid, with an average of 92% of an oral dose (200 mg/kg) being excreted within 48 hours of dosing. The major routes of excretion were urine and feces. Terminal half-lives of plasma radioactivity (t 1/2) were approximately 46 hours and 52 hours in male and female rats, respectively. Residual tissue 14C at 192 hours was less than 1% of that administered. Urinary metabolites were separated by TLC or GLC, and identified by GC/MS. Approximately 71.5% of rat urinary 14C was identified as 0,0-bis(2-chloro-2-propyl)-0-(2-propionic acid) phosphate. Other metabolites included bis[1-chloro-2-propyl]-0-hydrogen phosphate (18.1%) and 1-chloro-2-propanol. A possible metabolic pathway for the biotransformation of tris(1-chloro-2-propyl)phosphate in rats is presented.


Tri(2-ethylhexyl)trimellitate (TEHT) is used as a plasticizer in polyvinylchloride resins. Little is known about its metabolism. The objective of this work was to study the metabolism and disposition of radiolabeled TEHT in male Sprague-Dawley rats. Rats received a single dose by gavage of 100 mg/kg [14C]TEHT. About 75% of the dose was excreted in the feces. The remainder was excreted in the urine as metabolites (15%) or was expired as 14CO2 (1.9%). Radioactivity was excreted in the urine largely in the form of 2-ethylhexanol (2-EH) metabolites and mono(2-ethylhexyl)trimellitate. Radioactivity was excreted in the feces as unchanged TEHT (85% of the fecal radioactivity), small amounts as mono- and di(2-ethylhexyl)trimellitate and as unidentified polar metabolites. Less than 0.2% of the dose remained in the tissues at the time of sacrifice (144 hrs). Kinetics of elimination were estimated from breath and urinary excretion data. Elimination of 14CO2 was biphasic with halflives of 4.3 and 31 hrs. Excretion of radioactivity in urine was biphasic with halflives of 3.4 and 42 hrs. These studies show that TEHT was hydrolyzed to a limited extent in the gastro-intestinal tract to 2-EH and mono- and di-esters of trimellitic acid, and was largely excreted unchanged in the feces.

The disposition of benzylbutyl phthalate (BBP), a widely used plasticizer, was examined after oral and i.v. administration to rats. Male Fischer-344 rats (n=3) were dosed with 14C BBP, 2, 20, 200 or 2000 mg/kg PO (30 μCi/kg) and urinary and fecal excretion was determined. In 24 hrs, 61-72% of the dose was excreted in the urine and 13-15% in the feces at 2-200 mg/kg. At the 2000 mg/kg dose 16% of the 14C was excreted in the urine and 57% in the feces. The major urinary metabolite (36-58% of urinary 14C) coeluted with monobutyl-phthalate (MBP) on TLC and HPLC. Three hours after i.v. administration of BBP (20 mg/kg), 53-61% of the dose was excreted in the bile of anesthetized rats. Bilberry metabolites were MBP, 19% of bilary 14C, MBP-glucuronide and unidentified metabolite(s). The half-lives of BBP, MBP and total 14C in blood (20 mg/kg, i.v.) were 23 min, 13.5 and 14.6 hr, respectively. This study indicates that BBP is rapidly metabolized and the major route of excretion of metabolites is biliary. These metabolites are reabsorbed and ultimately eliminated in the urine.

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TISSUE DISTRIBUTION AND EXCRETION OF TRI-(2-ETHYLHEXYL)TRIMELLITATE IN RATS. L. Martin, E. Fried, and E. Woods. Travenol Laboratories, Morton Grove, IL

The disposition kinetics of tri-(2-ethylhexyl) trimellitate (TEHTM), a new plasticizer for polyvinyl chloride plastic, was studied in rats following intravenous administration of carbonyl-14C-tri-(2-ethylhexyl)trimellitate using an oil-in-water emulsion as the vehicle. The distribution half-life, elimination half-life, and clearance values, estimated from the plasma concentration of radioactivity data obtained following i.v. administration of 10.5 mg/kg of TEHTM (59.9 μCi/kg), were 46.2 min, 5.34 days, and 40.5 ml/kg/hr, respectively. Urinary excretion of 14C-TEHTM was slow with a renal clearance of 13 ml/kg/hr. Significant accumulation of radioactivity was found in the liver, lung, and spleen at all times, accounting for 72% of the administered dose at 24 hr. Fourteen days following treatment, 3.8% of the radioactivity was excreted in the urine and 16.0% was found in the feces. Bilary excretion was the major route of elimination of TEHTM.

619 IN VITRO IMMUNOTOXIC EFFECTS OF MULTIPLE CONCENTRATIONS OF FORMALDEHYDE ON LYMPHOCYTE CULTURES. C.L. Leach, R.P. Sharma and S.G. Oberg, Toxicology Program, Utah State University, Logan, UT.

Formaldehyde (HCHO) has been shown to have immunotoxic effects via inhalation in rats. Cultured lymphocytes from rats exposed in vivo showed a general trend of stimulation followed by depression or return to normal in lymphocyte blastogenesis assays, using phytohemagglutinin M (PHA), lipopolysaccharide (LPS) and the mixed lymphocyte culture (MLC). In vitro exposures of lymphocytes were conducted to ascertain whether in vivo effects were due to primary effects of HCHO. Spleen cells from F-344 rats were cultured with PHA, LPS and alloimmune lymphocytes for the MLC. Cytotoxic function of lymphocytes was evaluated using the 51Cr-release assay. Concentrations of 0.001, 0.01, 0.1, 1, 10 and 100 μg/ml of HCHO were utilized. Results of the PHA and LPS indicated a general depression, but at intermediate dose, stimulation was observed. The MLC and the 51Cr-release assays were relatively unchanged. 3H-thymidine and 3H-leucine incorporation of spleen cells in the presence of the same HCHO concentrations were performed on daily basis for 6 days to monitor the effects of HCHO on DNA and protein synthesis. Results of the leucine incorporation indicated a dose-dependent decrease in protein synthesis except at day 2 of the 0.01 μg/ml concentration where stimulation was observed. Thymidine incorporation was relatively unchanged.

620 EVALUATION OF INHALATION HAZARD OF ORGANIC CHEMICAL AIR CONTAMINANTS. Catherine Aranyi and William J. O'Shea. IIT Research Institute, Chicago, IL. Sponsor: James D. Pentera

The potential health hazards of exposure to TLV concentrations of acetaldehyde, acrolein, propylene oxide, benzene, toluene, phenol, chloroform, 1-methyl chloroform, carbon tetrachloride, methyl chloride, ethylene dichloride, ethylene trichloride, perchloroethylene, allyl chloride, benzyl chloride, and chlorobenzene, compounds which may be present in the ambient or work room atmosphere, were investigated. The effects of single and multiple 3-hr inhalation exposures were evaluated in mice by monitoring changes in their susceptibility to experimentally induced streptococcal aerosol infection and pulmonary bacterial activity to inhaled K-pneumoniae. When significant changes in these parameters were found further exposures were performed at reduced vapor concentrations until the no-effect concentration was reached. Multiple (5 consecutive) daily exposures were then performed at this concentration. Significant changes in both experimental parameters were observed after single 3-hr exposure to TLV concentrations of methylene chloride, ethylene dichloride, ethylene trichloride, perchloroethylene, and toluene. (Supported by EPA Grant No. CR 80743.)
621 EFFECT OF CYCLOPHOSPHAMIDE (Cy) ON HOST RESISTANCE AFTER PRIMARY AND SECONDARY INFECTIOUS CHALLENGE. J. Fenters, P. Barbera and K. Ketels. IIT Research Institute, Chicago, IL 60616

Cy, a potent immunosuppressive agent, depletes B-cells and is cytotoxic for T-cells. Cy was selected to validate and extend the data base for our host resistance panel which consists of bacteria, viruses, and a parasite. This panel is used to examine potential immunomodulatory effects of test agents. A complex interaction of T- and B-cells, macrophages, and interferon protect the host against these infections. Alteration in one or more of these entities could result in increased host susceptibility to infection. Both primary and secondary infectious challenge were examined to determine the extent of possible immunomodulation. Female B6C3F1 mice were injected with 30 mg Cy/kg daily for 14 days. After primary infectious challenge, Cy had no effect on mortality due to Streptococcus, Listeria, and herpes simplex type 2 virus (HSV-2), whereas a significantly increased host susceptibility was found to influenza virus, Trichinella, and herpes type 1 virus. After secondary infectious challenge with Streptococcus a significant increase in susceptibility was found in Cy-treated mice. There was no effect on host resistance to influenza, Listeria, and HSV-2. These studies show the importance of using more than one assay and that these assays should be used to assess the overall effect of complex immune interactions in an intact animal. (These studies were supported by NIEHS Contract N01-ES-50000).

623 THE EFFECT OF ORGANOPHOSPHORUS AND CARBAMATE CHOLINESTERASE INHIBITORS ON IN VITRO IMMUNE RESPONSES. G. A. W. Waithnow and P. B. Ferney, Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN.

The organophosphorus compounds, paraoxon (PXN) and tetraethyl pyrophosphate (TEPP), and the carbamates, physostigmine and neostigmine, were incubated in vitro with human peripheral blood lymphocytes either alone or in the presence of the lectin, phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM). The cholinesterase inhibitors did not alter the lectin-induced responses of the lymphocytes as measured by the incorporation of 3H-thymidine (3H-TdR) except in a few cases of PXN and physostigmine exposure where decreases in 3H-TdR incorporation could be observed. These changes could be attributed to the cytotoxic nature of the two compounds. The use of cholinesterase inhibitors in alloantigen-stimulated mixed lymphocyte reactions (MLR) yielded similar results. These findings provide further evidence that serine esterases may not be essential for lymphocyte stimulation in vitro.


The fetal period is, in general, the life stage most sensitive to harmful effects of xenobiotic compounds. This is readily apparent in the case of cytotoxic agents which form adducts with the DNA of replicating cells. The purpose of the present studies was to determine whether exposure of the fetus to nonteratogenic levels of the cytotoxic drug cyclophosphamide (Cy) would result in lesions of the immune system of adult animals. Pregnant C57BL/6J mice were given up to 5 μg/g of Cy during late fetal organogenesis or early histogenesis. There were no obvious morphological differences in treated or control offspring nor were body or organ weight to body weight ratios consistently affected. In one experimental series female offspring exposed to a total of 4 μg/g Cy from day 9-12 demonstrated reduced NK cell activity whereas males similarly exposed were not affected. Divergent results in the response to nitrogen stimulated lymphoblastogenesis were also observed between male and female offspring in this and other experiments. No other effects were noted. Studies to identify whether early or late developmental Cy exposure alters immune function will be discussed.

624 NATURAL KILLER CELL FUNCTION IN CHEMICALLY-TREATED RATS. P. A. Talcott, G. A. Whitbeck, and L. D. Koller. Veterinary Medicine, University of Idaho, Moscow, ID.

Splenic natural killer (NK) cell-mediated cytotoxicity was assessed in rats exposed to selenium (Se) and to the N-nitroso compounds, ethylnitrosourea (ENU) and diethyl nitrosamine (DEN) in vivo. NK cells are large granular lymphocytes that are thought to have a major role in immune surveillance of the neoplastic process. It, therefore, would be beneficial to monitor the NK system in a host that was exposed to known carcinogens, such as DEN and ENU, as well as to a compound such as Se which is suspected of being a tumor preventive agent. Weanling, female Sprague-Dawley rats were administered 0.5, 2.0 or 5.0 ppm Se in the drinking water for 10 weeks. Additional groups of rats were injected ip with 1.5, or 10 mg/kg DEN twice weekly for 10 weeks, while others received 0.15% or 0.316% ethylurea (EU) in the feed alone or in combination with 1 or 10 ppm sodium nitrite (NO2) in the drinking water.
625 IMMUNOTOXICITY OF TWO N-NITROSO COMPOUNDS VIA MULTIPLE IMMUNE ASSAYS IN THE RAT. G.M. Henningson, J.H. Exxon, L.D. Koller, C.A. Osborne, P.A. Talcott, and D. Schultz. Veterinary Medicine, University of Idaho, Moscow, ID

The "one rat" model system was used to measure a wide range of immune indices in rats exposed to the N-nitroso compounds, ethylnitrosourea (ENU) and diethylnitrosamine (DEN). Many N-nitroso compounds are potent carcinogens that may either require metabolic activation, such as DEN, or act directly as does ENU. Ethylnitrosourea (EU) and sodium nitrite (NO2) can combine at pH 7 in the mammalian stomach to form ENU. Four week old female Sprague-Dawley rats were injected IP with 1, 5, or 10 mg/kg DEN twice weekly for 10 weeks. Additional groups of rats were administered either 1 or 10 ppm NO2 in the drinking water alone or in combination with feed containing 0.15 or 0.316% EU for 10 weeks. Each rat was subsequently tested for immune responsiveness through comparisons with unexposed controls as well as positive controls which were immunosuppressed with either dexamethasone or cyclophosphamide. Humoral immunity was measured by a CLHA method to detect IgG to keyhole limpet hemocyanin. Cell-mediated immunity was measured by delayed-type hypersensitivity to bovine serum albumin. Prostaglandin and interleukin 1 levels were measured to ascertain macrophage function. Natural killer (NK) cell cytosis and interleukin 2 production were also assessed. Reticuloendothelial organs were weighed and collected for histopathological examination. This information will be presented to describe the immunopathological effects of an indirect and direct acting N-nitroso carcinogen in the rat.

627 CHEMICALLY-INDUCED CHANGES IN ANTIGEN DISTRIBUTION CAN AFFECT ANTIBODY (AB) PRODUCTION. P.J. Bick, P.J. McNeary, and M.P. Holsapple. Dept. of Microbiology and Immunology, Medical College of VA/VCU, Richmond, VA. Sponsor: A.E. Munson

We have demonstrated that subchronic exposure (S.C.) to diethylstilbestrol (DES) produced only a marginal suppression (20%) of the Ab response to sheep erythrocytes (sRBC), a T-dependent antigen administered i.p. (Bick et al., Immunopharmac., In Press). Spleen cells from DES treated animals responded normally to both sRBC and DPNFCC, a thymus-independent antigen, in vitro. These results indicated a minimal effect on humoral immunity and are in marked contrast to earlier studies demonstrating 80% suppression when the sRBC was administered i.v. (Luster et al., J. Reticulo. Soc. 28:561). The objective of this investigation was to use DES as a probe to correlate its effects on RES function, traditionally a marked stimulation, with antigen distribution and subsequent antibody production. Mice were injected, either i.v. or i.p. with 3HCr-labeled sheep erythrocytes on day 11 of a 14-day exposure to DES. RES activity and antibody production were measured 24 h after the final DES treatment (day 15). Results indicate a much greater DES-induced change in antigen distribution and antibody formation when the antigen was injected i.v. An accurate interpretation of a chemical's effects on antibody production cannot be made without an evaluation of that chemical's effects on RES activity and antigen distribution. (Supported by NIH contract ES-15001, Training Grant IIT2ES50708)


The immune system of the rat has recently been characterized. Data indicate that immune responses in the rat are comparable, and in some cases, more responsive than in the mouse. For instance, natural killer cells in most species of mice are relatively inactive and insensitive to endogenous and exogenous stimuli but also afford a good model for human NK cell activity, since the rat NK cell closely parallels that of man in regard to morphology, tissue distribution and function with age. Immune cells from rats have also been investigated extensively for interleukin 1 (monokine) and 2 (lymphokine) activities. The rat manifests good cell mediated and macrophage responses as well as adequate humoral, interferon and prostaglandin activities. Comparable pharmacologic and toxicologic data are amassed for the rat, this species is used for both carcinogen studies, and the rat is specified by the FDA as a research animal in which clearance of drugs and other chemicals are tested for approval in man. The information presented will define normal values for immune responses in the rat, for antibody, delayed-type hypersensitivity, phagocytosis, interleukin 1 and 2, natural killer cells, and prostaglandin activity. These parameters are validated by known immunomodulators. Assessment of both primary and immune indices in a "one rat" model system are advantageous for immunopharmacologic/toxicologic investigations.

628 CARCINOGENICITY AND IMMUNOTOXICITY OF CHLORINATED PHENOLIC DRINKING WATER CONTAMINANTS. J.H. Exxon and L.D. Koller. Veterinary Medicine, University of Idaho, Moscow, ID

The use of chlorine for disinfection of drinking and waste waters produces a number of chlorinated organic compounds in the finished product. One group of chemicals which form spontaneously are chlorinated phenols ranging from the monochlorophenol to pentachlorophenol. One study has shown that 2-chlorophenol (2CP)-exposed mice had enhanced skin tumor incidence following treatment with 3-methylcholanthrene. Phenol and phenols in general have been reported to be immunosuppressive. In the present study, tumor incidence and latency to the transplacental carcinogen, ethylnitrosourea (ENU), were studied in rats exposed prenatally to 2CP or pentachlorophenol (PCP). The immune status was also assessed in 2CP and PCP exposed rats by measuring antibody synthesis, delayed-type hypersensitivity (DTH) and macrophage function. Tumor incidence was increased and tumor latency was decreased in 2CP and PCP treated rats also given ENU compared to those which received only ENU. PCP-treated animals had depressed antibody synthesis and DTH response, but enhanced macrophage function. No effects of 2CP on immune parameters were observed. These results suggest that 2CP and PCP are not carcinogetic per se but may act as cocarcinogens in the presence of other tumorogenic compounds. Suppression of host immunity by certain chlorinated phenols may be a contributing factor to the cocarcinogenic effect.
629 EFFECTS OF DIMETHYL NITROGSAMINE (DMN) ON CELL-MEDIATED IMMUNITY AND BONE MARROW OF B6C3F1 MICE. L. B. Schook, J. K. Pullen, S. S. Duke, and M. P. Holsapple, Dept. of Microbiology and Immunology, Medical College of Virginia/VCU, Richmond, VA 23298. (Sponsored by A. E. Munson).

Female B6C3F1 mice were exposed (i.p.) to 1.0, 3.0, or 5.0 mg/kg DMN daily for 14 days. The mixed lymphocyte response (MLR) and the lymphoproliferative responses to T-cell mitogens (ConA and PHA) were significantly suppressed. In contrast, the delayed hypersensitivity response (DHR) to KLH as measured by the influx of endogenously labelled ([3H]-I-ludr) monocytes was increased (300% in the 5 mg/kg treated mice). However, the DHR to KLH was decreased by 60% (5 mg/kg) when measured by the influx of exogenously labelled ([3H]-Cr) monocytes. The tested DHR in the DHR was interpreted to reflect an effect on bone marrow. DMN produced little change in the total number of recovered marrow cells, while exposure to cyclophosphamide caused a 43% reduction. A dose-related increase in [3H]-thymidine uptake and the number of CFU-GM was demonstrated with Hs cells from DMN-treated mice after 7 days in culture. Contradictorily, DMN treatment decreased the number of cells in S-phase with a concomitant decrease in the number of cells expressing Ia antigens. Thus, exposure to DMN increases the progenitor populations (CFU) while decreasing the number of cells expressing Ig antigen which may contribute to a decline in the accessory function by macrophages reflected by the DMN-induced suppression of the DHR and MLR. (Supported by NIEHS ES-1-5001.)

630 SEPARATION OF CARCINOGENIC INITIATION AND IMMUNOSUPPRESSION BY POLYCYCLIC AROMATIC HYDROCARBONS. K. L. White, Jr., H. H. Lysy, P. J. McNerney, and M. P. Holsapple, Department of Microbiology and Immunology, Medical College of Virginia/VCU, Richmond, VA 23298. (Spons. by A.E. Munson).

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous in the environment. It has been established that metabolism of PAHs, such as Benzo(a)pyrene (BAP), by the mixed function oxidase (MFO) system generates the ultimate carcinogen. We have observed that subchronic exposure to 10 mg/m2 Dimethyl-dibenzanthracene (DMBA) and Methylcholanthracene (MCA) suppressed the in vivo humoral immune response (IgM antibody Torming cell, AFC) to SRBC in B6C3F1 mice, a mouse strain whose MFO is highly inducible by PAHs. Surprisingly, immunosuppression was greater in DBA/2 mice, an inbred strain whose MFO is not inducible by the PAHs. In vitro studies, using the Mitchell-Dutton assay, demonstrated that BAP was immunosuppressive in culture and that a MFO activation system was neither required nor enhanced this activity. Subchronic exposure to Benzo(a)anthracene and Dibenzo(a,h)anthracene suppressed the in vivo humoral response in B6C3F1 mice. Neither compound suppressed the in vitro AFC response even in the presence of an MFO system which activated cyclophosphamide. Studies using radiolabelled PAHs are in progress to quantitate the formation of hydroxylated metabolites in vitro. Our data suggest that the immunosuppression by the PAHs is not mediated by the metabolites which initiate carcinogenicity. (Supported by NIEHS ES-1-5001 and ES02520).

631 IMMUNOSUPPRESSION BY SELECTED HEXACHLORODIBENZO-P-DIOXINS (HDDOs) IN ADULT FEMALE B6C3F1 MICE. M.P. Holsapple, J.A. Munson, P.J. McNerney, and A.E. Munson, Deps. of Pharmacol. and Toxicol. and Microbiol. and Immunol., Medical College of VA, Richmond, VA

Pentachlorophenol (PCP) is a widely used pesticide which is known to be contaminated by polyhalogenated dibenzo-p-dioxins other than 2,3,7,8-TCDD, the prototype of the class. Subchronic (14 day) exposure to technical grade PCP at 10, 30, and 100 mg/kg (p.o.) daily suppressed the peak (day 4) IgM Ab response to SRBC by 44%, 53%, and 72%, respectively. In contrast, subchronic exposure to pure (dibenzo-dioxin-free) PCP had no effect, suggesting that the suppression by technical PCP was due to the dibenzo-p-dioxins. Subchronic exposure to 1,2,3,6,7,8-HCCD at 0.2, 1.0, and 4.0 µg/kg concentrations, reflecting those found in technical grade PCP, did suppress the peak IgM Ab response to SRBC by 30%, 47%, and 52%, respectively. Direct addition of 0.1 µg of either 1,2,3,6,7,8-HCCD or 1,2,3,7,8,9-HCDC to spleen cell suspensions of untreated mice suppressed (>80%) the Ab response to SRBC with no effect on viability. Preincubation with a crude liver homogenate preparation which readily activated cyclophosphamide, abolished the activity of the HCCDs. These results indicate that the immunosuppression by the HCCDs is due to the parent compound and that metabolism is a detoxifying process. (Supported by NIEHS ES-1-5001 and NIH ES02520).


Benzopyrenes are produced during the pyrolytic combustion of petroleum fuels. These studies compared the immunotoxicological effects of BAP with Benzol(e)pyrene (BE) a non-carcinogenic isomer of BAP. Animals received BAP or BE for 14 days in corn oil s.c., at equal doses up to 40 mg/kg. Humoral immunity was evaluated by measuring IgM antibody response to SRBC, basal serum immunoglobulins, mitogenic response to LPS, and serum complement. Cell-mediated immunity (CMI) was evaluated using the delayed hypersensitivity response to KLH and SRBC, popliteal lymph node response to SRBC, lymphocyte blastogenic response to Con A, and the acute inflammatory response to carrageenin. RES clearance of 51Cr labeled SRBC was used to measure macrophage function. Neither BAP nor BE produced biologically relevant changes in CMI. The AFC response to BAP was suppressed in a dose-dependent manner; BE produced no effect. Basal Ig levels and serum complement were unaffected. The B- and T-cell mitogen response was depressed by both treatments. A dose-dependent reduction in spleen macrophage phagocytosis was observed only with BAP. These studies confirmed that BAP, but not BE, has the major immunosuppressive activity. (Supported by the API).

Pentachlorophenol is an antimicrobial agent used chiefly for the preservation of wood. Subchronic exposure to Technical Grade Pentachlorophenol (PCP) significantly inhibited mouse complement mediated lysis of sensitized rabbit erythrocytes when measured in a microtiter hemolytic assay. Serum complement levels of B6C3F1 female mice treated daily for 14 d by gavage with 100 mg/kg PCP were reduced 70% of control animals when evaluated on the day following the last PCP exposure. In animals treated for 14 d and allowed a 15 d recovery period a 64% suppression of functional complement levels was observed. Mice which were killed to recover for 30 d had only 52% of the hemolytic complement activity as compared to controls. Animals treated with 10 or 30 mg/kg did not show this marked effect; neither did animals treated with a comparable dose of EC-7, a PCP preparation purified to reduce dioxin contamination. Animals treated with either 1,2;3;5;6,7;8 or 1,2;3;7,8,9 hexachlorodibenzo-p-dioxin (HDDO), two contaminants of PCP, had serum complement levels which were 50-60% of control animals when evaluated after 14 d of exposure to 0.1 and 0.5 μg/kg of HDDO. The data suggest that dioxins affect the complement system of mice and may contribute to the suppression observed with technical grade PCP. (Supported by NIHES ES-1-S001).


The aim of this study was to determine the sensitizing potency of morpholine (M), 4,4'-Dithiodimorpholine (DTDM), Morpholylmercaptobenzothiazole (MMBT) and 2-Mercaptobenzothiazole (MBT) in guinea pigs. A modification of the method employed was used. In order to increase the sensitivity of the assay, the concentration of morpholine used was increased to 10% and a higher concentration of MBT was used. All of the compounds studied in this experiment were sensitized either in petrolatum or in petrolatum alone. All of the guinea pigs treated with DTDM and MMBT were sensitized; 70% of the animals were sensitized to MBT and none of the animals treated with morpholine were sensitized. When the animals were subsequently tested for cross-sensitization, the following results were observed: 66% of the DTDM-sensitized animals reacted with MBT; 30% of the animals sensitized to DTDM reacted with MBT; 80% of the MMBT-sensitized animals reacted to MBT and 10% to MBT and to morpholine; 30% of the MBT-sensitized animals reacted to MBT. The rank order sensitization potential in guinea pigs as observed from this study is: 1) DTDM, 2) MMBT, 3) MBT.

635 EFFECT OF REPEATED PARATHION EXPOSURES ON THE PRIMARY IGM RESPONSE AND BONE MARROW STEM CELLS IN C57BL/6 MICE. P.M. Bertholomew, G.P. Casale, W.J. Duggan, J. Bella Paza, V.S. Gallochio, H.A. D'Capua and S.D. Cohen, Dept. of Thera. Radiology, Yale Univ. Sch. of Med., New Haven, CT 06510 and Toxicology Program, Sch. of Pharm., Univ. of Conn., Storrs, CT 06268.

Parathion (PT)-induced immunosuppression has been demonstrated only after single doses that caused severe cholinergic stress. Studies with adrenalectomized mice and cell cultures support the hypothesis that PT, and/or a metabolite, may have a stress-independent, direct action on the immune system. We have demonstrated that mice made tolerant to PT were immunosuppressed 24 hours after 18 daily doses of PT, cholinesterase activities were inhibited 82, 72, 61 and 38% in brain, lung, diaphragm and liver, respectively, yet no signs of cholinergic toxicity were observed. Numbers of specific IgM plaque forming cells and viable, nucleated spleen cells were decreased 63 and 39%, respectively. These decreases were accompanied by a 33% increase in bone marrow granulocytic macrophage colony forming units (CFU (pooled data)). The data suggest that PT and/or a metabolite, may act directly on the peripheral splenic lymphocyte population. Increased CFU may be a compensatory mechanism to correct splenic cellular deficiencies. (Supported in part by NIHES grant ES02524 and by fellowships from Richardson-Vicks, Inc. and Sandz, Inc.)

636 PARAOXON (PX)-INDUCED SUPPRESSION OF THE IN VITRO RESPONSE OF MURINE SPLEEN CELLS TO SHEEP RED CELLS (SRBC). W.J. Duggan, G.P. Casale, S.D. Cohen, R.A. DiCapua, Toxicology Program, Sch. of Pharm., Univ. of Connecticut, Storrs, CT 06268.

Recent work in our laboratory demonstrated marked parathion (PT)-induced suppression of humoral immunity, in mice. Our findings suggest that stress may have been a component of the observed immunosuppression, but did not preclude the possibility that PT and/or a metabolite acted directly on the immune system. We tested the latter possibility by assessing the effects of PX on the in vitro response of mouse spleen cells to SRBC. PX (5X10^-7 M to 1X10^-3 M) was added to C57BL/6 spleen cell cultures immunized with SRBC. After 5 days incubation, cultures were harvested and antibody-forming cells (AFC) were enumerated. The number of AFC was reduced 30% to 49% by 1X10^-7 M to 1X10^-3 M PX. 5X10^-7 M PX had no significant effect. Cell viabilities were not altered by PX treatments. PX inhibited (50%) spleen leucocyte alpha-naphthyl acetate (ANA) and acetylihydroxyamine (AICH) hydrolysis at 7.2X10^-7 M and 2.1X10^-4 M, respectively.

The data indicate that PX has a direct, immunosuppressive effect which may be mediated by inhibition of a cell-associated esterase(s).

Supported by NIHES grant ES02524.

One possible epigenetic mechanism by which carcinogens may promote the outgrowth of transformed cells is by immunosuppression. We have demonstrated that the carcinogenic polycyclic aromatic hydrocarbon DMBA has an acute effect on both cellular (CM1) and humoral (HMI) mediated immunity. As chronic immunosuppression may be more important in carcinogenesis, immune function assays quantitating HMI and CM1 were performed 4 and 8 weeks following DMBA exposure. Adult B6C3Fl female mice were administered DMBA subcutaneously at 5, 50, and 100 mg/kg in 10 equal doses over 2 weeks (corn oil vehicle control). Both the IgM and IgG splenic antibody producing cell response (i.e., HMI) to sheep erythrocytes were depressed up to 95 and 75% respectively at 4 weeks. Splenoocyte lymphoproliferative responses to B-cell (LPS) and T-cell (PHA and ConA) mitogens were depressed up to 88 and 93% respectively at 4 weeks. Alloantigen-induced proliferation of splenocytes in a mixed lymphocyte culture was depressed up to 90%. The generation of cytotoxic T-lymphocytes against P815 tumor cells was depressed up to 88%, while natural killer cell cytolytic killing was up to 84% at 4 weeks. Similar suppressions in HMI and CM1 were noted at 8 weeks. Thus, DMBA exposure results in chronic immunosuppression which could potentially contribute to tumor outgrowth.


Suppression of CMI has been suggested as an epigenetic factor in carcinogenesis. Several PAHs, among them potent carcinogens, were examined to test this hypothesis in assays quantitating CMI. Adult female B6C3Fl mice were subcutaneously administered benzo[a]pyrene or benzo[e]pyrene (BaP or BeP; 50, 200 or 400 mg/kg), 3-methylcholanthrene (MCA; 100, 200 or 400 mg/kg) or 7,12-dimethylbenz[a]anthracene (DMBA; 5, 50, or 100 mg/kg) divided into 10 equal doses over two weeks. Spleen cells were used in T-cell mitogen (PHA)-induced lymphoproliferation assays and in 4 hour 51Cr-release assays quantitating natural killer (NK) cell or cytotoxic T-lymphocyte (CTL)-mediated lysis of YAC-1 and P815 tumor cells, respectively. MCA and DMBA suppressed all aspects of CMI tested with up to a 50 and 81% inhibition of CTL and up to a 42 and 55% inhibition of NK activity, respectively. BeP affected only PHA-induced lymphoproliferation (60% decrease). Preliminary studies indicated significant suppression of CTL activity in mice exposed to dibenz[a,h]anthracene (75%), while noncarcinogenic PAHs (dibenzo[a,h]anthracene, BeP and perylene) had no effect. Thus, CMI parameters implicated in tumor immuno-surveillance can be suppressed following exposure to carcinogenic PAHs. These data are consistent with an immune-related component in PAH-induced carcinogenesis.

SENSITIZATION POTENTIAL OF INHALED TDI IN GUINEA PIGS. C. Collins, C. Bier, W. Friedman, C. Breckenridge, J. Wallace, S. Cornhill, BioResearch Laboratories, Montreal, Quebec. American Cyanamid Company, Wayne, New Jersey. University of Louisville, Kentucky

Toluene diisocyanate (TDI) induces dermal and pulmonary sensitization in man and guinea pigs. Groups of 8 guinea pigs were exposed to TDI vapor for 3 h/day at target concentrations of 0 and 4.23 mg/l (0.6 ppm) on study days 1-5. All animals were subjected to pulmonary challenge by inhalation of TDI (5.06 ppm) on study days 18 and 19 and of an aerosol of TDI-guinea pig serum albumin complex (TDI-GPAS) on study days 25, 26, and 27. Pulmonary response was assessed by respiratory rate measurements. The animals were challenged topically and intradermally with TDI and TDI-GPAS, respectively, on day 28, and the sites were assessed on days 29 and 30. Antibody titers (IgE and IgG2) were measured in serum samples collected pre-exposure and on days 15, 22, and 30.

There was no increase in respiratory rate (as indicator of sensitization) in either group when challenged with TDI alone. However, respiratory rate increased in 7/8 test animals during exposure to TDI-GPAS. These animals did not show a similar response to GPAS alone.

An ophthalmic response to challenge with intradermal injection of TDI-GPAS occurred in 7/8 test animals but was not evident with topical TDI. Positive antibody titers following exposure were observed in 6/8 test animals following exposure. This experimental model may be suitable for screening potential sensitizers, provided sensitizing doses are selected carefully and respiratory monitoring is performed under well-controlled conditions.

CELL CYCLE PERTURBATION OF DNA AND RNA CONTENT IN THE BONE MARROW AND ALVEOLAR MACROPHAGES OF RODENTS AFTER SUBCHRONIC INHALATION EXPOSURE TO FORMALDEHYDE. C.E. Dallas, D.H. Nellard, J.C. Theiss, and E.J. Farbush. Environmental Sciences, University of Texas School of Public Health, Houston, TX.

Fluctuations in the cell cycle of selected rodent cells after subchronic inhalation of formaldehyde were studied to help examine potential preneoplastic events due to exposure to this known nasal carcinogen. DNA and RNA content were measured from bone marrow cells of B6C3Fl mice, A-strain mice, and Sprague-Dawley rats, thus providing an inter-species examination of potential systemic effects of formaldehyde exposure. Additionally, measurements were conducted on alveolar macrophages from Sprague-Dawley rats to determine effects at or near the site of exposure. All animals were exposed to either 0.5, 3, or 15 ppm formaldehyde for 4 hours/day, 5 days/week, for up to 24 weeks. Biometric measurements of acridine orange-stained cells for DNA and RNA content analysis were performed with a flow-cytometer. Significant increases in the RNA content of G1 phase cells, the most sensitive of the indicators, started occurring after 4 weeks of exposure in the bone marrow cells of A-strain mice inhaling 15 ppm. Increases in B6C3Fl bone marrow G1 cells were less conclusive, while Sprague-Dawley rats exhibited no changes after 16 weeks. After just 1 week of exposure at all dose levels, alveolar macrophages from the rats demonstrated significant increases in RNA content.
USE OF FLOW CYTOMETRY (FCM) TO ANALYZE BONE MARROW PERTURBATIONS INDUCED BY ETHYLENE OXIDE (EO). R. Popp, S. Lock, D. Popp, R. Mann and R. Hend, Jr., Oak Ridge National Laboratory, Oak Ridge, TN 37831.

Bone marrow responds quickly and often specifically to stress, but many cell populations make up the marrow and it has been difficult to characterize the response of specific cells. With FCM it is possible to perform single cell analysis and identify subpopulations. Mice exposed to 255 ppm EO in inhalation chambers for 6 hr/day were removed after 1,2,4,8 and 14 days and 4,6,8 and 10 weeks (5 days/week) exposure. Bone marrow was flushed from the tibia and femur. One aliquot was stained with propidium iodide for cell cycle analysis and another was reacted with fluorescein conjugated monoclonal antibody for B cell analysis. The preparations were analyzed for forward and 90° scatter and fluorescence on an Ortho 50H. Computer analyses of scattergrams and histograms of these parameters permit identification of granulocytes and lymphocytes in the bone marrow, quantitation of B cells, and analysis of cell proliferation. EO exposures cause a loss of granulocytic elements from the bone marrow, most severe by day 2, followed by replacement and hyperproliferation, signaled by a relative deficit of lymphocytes most pronounced by 8-10 weeks. The results show that FCM analysis of mixed cell populations is useful in identifying the effect of EO on specific cells.

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EFFECTS OF DIMETHYLNITROSAMINE (DMN) ON HOST RESISTANCE AND IMMUNITY. P. Thomas, R. Fugmann, C. Aranyi and J. Pentero. ITT Research Institute, Chicago, IL 60616.

DMN, a known carcinogen and alkylating agent was evaluated for effects on host resistance and immunity in the B6C3F1 mouse. Endpoints measured were mortality following challenge with Listeria monocytogenes, Streptococcus zooepidemicus, Influenza and Herpes simplex type 1 virus (HSV-1) and susceptibility to B16-F10 tumor and Trichinella spiralis. Effects of DMN exposure on lymphocyte mitogenesis, natural killer cell function, antibody response, delayed hypersensitivity, and macrophage cytosis also were evaluated. DMN exposure for 14 days increased host susceptibility to infectious agents where immune protection is mainly antibody-mediated or nonspecific (Influenza, Streptococcus). Susceptibility to infection by agents thought to invoke cellular immunity was unaffected (Listeria, HSV-1, Trichinella) or decreased (B16-F10 tumor). In general, the results of the in vitro immune function tests correlated with those of the in vivo host resistance assays. The results of these studies illustrate the necessity for employing a panel of host resistance and immune function assays when screening compounds for immunotoxicity.

(Supported by NIERS Contract No. M01-ES-1-5000).


Adverse effects of EO exposure involve the hematopoietic system. This study was designed to identify perturbations in bone marrow and peripheral blood elements of mice during exposure to EO. Mice exposed in 1.5m³ inhalation chambers to 255 ppm EO for 6 hr/day were removed for analysis after 1,2,4,8 and 14 days and 4,6,8 and 10 weeks (5 days/week). Prior to sacrifice, blood was removed from the orbital sinus for white and red blood cell counts, blood and plasma hemoglobin determination and hematocrit. A blood film was made for differential count. Bone marrow was flushed from femurs and tibias and counted. Aliquots were used for CFU-S (stem cell) and mutation assays and an aliquot was used for flow cytometry (FCM) analysis. Perturbations of peripheral leukocytes occur after only 6 hr of exposure. Hematocrit, red cell number and hemoglobin are generally depressed with transient compensatory bursts and bone marrow cellularity and C FU-S are below normal. However, white cell numbers fluctuated dramatically during the exposure period. There is a shift in differential toward granulocytes, at times resulting in severely depressed numbers of lymphocytes in the peripheral blood. The FCM analysis shows an early depletion of granulocytes in the bone marrow followed by replacement and a relative lymphocyte deficit, especially pronounced at 10 weeks.

Research sponsored by DOE under contract with UCC and EPA Contract No. DW930018-01-0.

METHYLmercury EFFECTS ON CELL CYCLE KINETICS. D.G. Vogel, P.S. Rabinovitch, and N.K. Motter, Dept. of Pathology, Univ. of Washington, Seattle, WA. Sponsor: M.R. Juchau

Methylmercury (MeHg) affects many cellular processes. MeHg effects on cell cycle kinetics are being investigated to help identify its mechanisms of action. Normal human fibroblasts cultured in Eagle's minimal essential medium and 10% calf serum were synchronized in G1 by serum deprivation and then released from G1 by the addition of media containing serum, 3 μM BrUdR, and various concentrations of MeHg. Subsequent flow cytometric analysis of ethidium bromide/mithramycin or Hoechst 33258 stained cells allowed quantitation of the proportion of cells in G1, S, G2, and the next G phase. After first exposure to MeHg the cell cycle time was lengthened due to a prolonged G1. At 3 μM MeHg a G1 25% longer than the control was produced. The G1/S transition rate was also decreased in a dose-related manner. At longer time periods (days rather than hours) the G1 effect was not detectable, but there was an increase in the G2 percentage which was directly related to MeHg concentration and length of exposure. After 8 days at 5 μM MeHg 45% of the population was in G2. Thus a short term (hours) response lengthened the G1 phase. A later (days) response accumulated cells in the G2 phase. The G1 phase response may relate to inhibition of protein synthesis. The G2 response suggests that continued MeHg exposure affects mitosis and is consistent with previous hypotheses regarding mechanisms of MeHg action.

(NIERS ES 00677, ES 07032, and NIA AG 01751.)
We have previously reported that heavy metals such as cadmium chloride (CdCl$_2$) inhibit membrane bound enzymes and the uptake of dopamine (DA) and norepinephrine by rat brain synaptosomes (RBS) in vitro. The present studies were initiated to study the in vivo effects of CdCl$_2$, mercuric chloride (HgCl$_2$) and methyl mercuric chloride (CH$_3$HgCl$_2$). Male Sprague-Dawley rats were treated with different doses of the heavy metals by i.P. or P.O. for 14 days. RBS were prepared from control and treated animals and 3H-DA uptake was determined. Rats receiving CdCl$_2$, HgCl$_2$ and CH$_3$HgCl$_2$ showed a general pattern of decreased uptake of 3H-DA by RBS. The decrease in all cases was dose-dependent. I.P. administration was more effective than P.O. treatment. CH$_3$HgCl$_2$ was more effective in decreasing the uptake of 3H-DA as compared to HgCl$_2$ and CdCl$_2$. A 50% decrease of 3H-DA uptake was observed with 0.1, 0.5 and 0.75 mg/kg for CH$_3$HgCl$_2$, HgCl$_2$ and CdCl$_2$ respectively when the rats were treated I.P. whereas ID$_{50}$ values for P.O. treated rats were 0.75, 0.5 and 1.0 mg/kg for CH$_3$HgCl$_2$, HgCl$_2$ and CdCl$_2$ respectively. These data suggest that the heavy metals may induce neurotoxicity by interfering with the uptake of neurotransmitters such as dopamine. (Supported by NIH NBS grant RR 008169).

**ACUTE TOXICITY OF TRIMETHYLITIN IN THE MONKEY.**


Trimethyltin (TMT), a neurotoxic organometal, affects areas of the limbic system, especially the amygdala, entorhinal cortex and hippocampus. Few data exist regarding the effects of TMT in primates. In this study, adult cynomolgus monkeys (Macaca fascicularis) were injected i.v. with 1.10 mg TMT/kg and observed for clinical signs. Hyperactivity and hyperreactivity to acoustic stimuli appeared within 48-72 hrs. Tremor appeared by 96 hrs, first as a movement tremor but rapidly progressing to a continuous tremor of the extremities. Hyperactivity was replaced by severe ataxia, lethargy and coma. Animals were sacrificed by perfusion with saline and buffered glutaraldehyde. Tissues were taken for LM and EM. Aside from mild centriobular degeneration of the liver significant damage was confined to the brain. Degenerating pyramidal cells were seen in the proximal CA-3 and CA-4 subfields of Ammon's horn, while the fascia dentata was spared. Mild neuronal damage was observed in the neocortex, brainstem and dorsal column of the spinal cord. Ultrastructurally, degenerating neurons contained numerous lysosomes, autophagic vacuoles, and small membrane-packed organelles. Histologically, injury was less than predicted from clinical observations or rodent studies, emphasizing the need for neurochemical studies of TMT poisoning.

**THE INFLUENCE OF CARBON MONOXIDE EXPOSURE ON BRAIN LEAD KINETICS IN DEVELOPING RATS.**

J.M. Frazier. The Johns Hopkins University, Baltimore, MD.

In order to investigate the interactions between carbon monoxide (CO) exposure and brain lead (Pb) kinetics in developing rats, the following study was designed. Adult female hooded Long-Evans rats were exposed to 1000 ppm Pb, as lead acetate in drinking water, for 70 d prior to mating and Pb exposure was continued through gestation and lactation. During gestation and lactation dams and their offspring were continuously exposed to 150 ppm CO. At weaning, dams were sacrificed and offspring were split into two groups with Pb exposure continuing at 1000 ppm in drinking water in one group and Pb exposure discontinued in the other group, giving a total of 8 experimental groups (Pb prior to weaning, CO prior to weaning, Pb post weaning). Offspring were sacrificed at 21, 40, 60 and 120 days of age, brains removed and Pb, Zn and Cu concentrations determined by atomic absorption spectrophotometry. Preweaning exposure to Pb reduced brain Zn concentrations at 21 d but not at 40, 60 or 120 d. Brain Pb concentrations in offspring removed from Pb exposure at weaning decreased to control levels by 120 d. CO exposure increased brain Zn concentration at 21 d and depressed brain Pb concentrations at all time points. None of the treatments had any effect on brain Cu concentrations. These data indicate a significant interaction between CO exposure and brain Pb and Zn concentrations.
RESISTANCE TO CADMIUM-INDUCED HEPATOTOXICITY IN IMMATURE RATS. P.I. Goering and C.D. Klaassen, Dept. of Pharmacol., Toxicol. and Therap., Univ. of Kansas Medical Center, Kansas City, KS.

Because the concentration of metallothionein in perinatal rat liver is 10-20 times higher than levels present in liver of untreated adult rats, it was of interest to determine if immature rats are less susceptible to the hepatotoxic effects of cadmium (Cd) seen in adults. Male Sprague-Dawley adult rats received a hepatotoxic dose of 4.0 mg Cd/kg iv, and 10-day-old rats received 4.0, 5.0 or 6.0 mg Cd/kg, iv. Ten hr following Cd injection, plasma enzyme activities in adults were elevated (aspartate aminotransferase, 50-fold; sorbitol dehydrogenase, 87-fold) and histologic examination showed extensive hepatic injury; however, no damage was evident in 10-day-old rats, even at the 6 mg Cd/kg dose. 10 days after challenge (3.5 mg Cd/kg, iv, 7 uCi Cd/mg Cd) the concentration of Cd was higher in liver, heart and brain and lower in kidney of 10-day-olds compared to adults. A marked difference in the hepatic subcellular distribution of Cd was observed in 10-day-olds with a higher amount in cytosol and less in the particulate fraction. Gel filtration chromatography indicated most cytosolic Cd was bound to metallothionein. These data support the hypothesis that prolonged levels of metallothionein are important in tolerance to Cd toxicity and that tolerance results from an altered hepatic subcellular distribution of Cd. (Supported by USPHS Grants ES 01142 and ES 07079).

MORPHOMETRIC ANALYSIS OF CADMIUM-INDUCED CYTOTOXICITY IN CULTURED HEPATOCYTES. E.M.B. Sorensen and B. Acosta. Department of Pharmacology, University of Texas, Austin, TX.

Primary liver cell cultures from neonatal rats were subjected to morphometric analysis at the optical level to determine whether quantitative information could be obtained to monitor morphologic damage to hepatocytes exposed to cadmium in the presence or absence of calcium. Both time and dose effects were studied. Cells were arbitrarily categorized into four groups by morphologic changes which resulted from cadmium exposure. Type I cells were normal morphologically, Type II cells were slightly damaged, Type III cells were markedly damaged, and Type IV cells were disintegrated. The following trends were observed to be statistically significant for cells exposed to 200 uM cadmium: the percentage of Type I cells decreased (and Type II cells increased) as time increased from 5 to 60 minutes, Type III cells increased from 5 to 30 minutes but decreased from 30 to 60 minutes, and Type IV cells remained unchanged at 5 and 30 minutes but increased from 30 to 60 minutes. After 5 minutes exposure, significantly more Type III and significantly fewer Type I cells were present when hepatocytes were exposed in the absence of calcium. This later result was not observed at 30 to 60 minutes at 200 uM. With only minor exceptions, cadmium concentration was increased at both 30 and 60 minutes, the percentage of Type I cells decreased, Types II and III cells increased, and Type IV cells remained unchanged. These results indicated that morphometric analysis at the optical level can be used to quantify cytotoxic damage in cultured hepatocytes.

ALTERATIONS OF SERUM FATTY ACID COMPOSITION OF CHICKS DURING LEAD TOXICITY. W.E. Donaldson and D.M. Latta. Toxicology Program, NCIC, Raleigh, NC.

A series of 3-week experiments was conducted to study the effects of dietary and injected lead (as Pb acetate-3 HgD) on fatty acid composition of total serum lipids of chicks as measured by gas-liquid chromatography. In Exp 1, diets deficient or adequate in methionine (MET) were fed with 0 or 1000 ppm Pb added to the diet. Pb decreased the concentrations of 16:1 and 18:2 fatty acids (no. carbon atoms:no. double bonds) and increased the concentration of 20:4. MET decreased 16:1 and the Pb x MET interaction was significant. The ratio 18:2/20:4 was decreased by Pb. In Exp 2, chicks fed 0 or 2000 ppm Pb were injected i.p. with 0 or 52 mg Pb/100 g body wt. Dietary Pb significantly increased 20:4 and decreased 18:1 and the ratio 18:2/20:4. Injected Pb was without effect at 4 hr post-injection. Neither dietary Pb (2000 ppm) nor Pb injection affected the 18:2/20:4 ratio of red blood cell ghosts although dietary Pb approached significance (p=0.067). In Exp 3, diets containing 2000 ppm Pb, 60 ppm Cd, 500 ppm Hg or 10 ppm Se were compared to a diet with 0 addition. Pb reduced the ratio 18:2/20:4 while Cd and Hg increased it and Se was without effect. The data indicate that chronic Pb exposure stimulates metabolic conversion of 18:2 to 20:4 while acute exposure does not. The effect of Pb on 18:2/20:4 is not observed with Cd, Hg or Se which suggests that the serum 18:2/20:4 ratio may serve as an aid in diagnosis of Pb toxicity.
653 EFFECTS OF DIETARY METHIONINE AND CHOLINE ON LEAD TOXICITY IN CHICKS. D. M. Latta and W. E. Donaldson. Toxicology Program, NCSU, Raleigh, NC.

Three factorial (2x2x2) experiments were conducted to study the effects of dietary methionine (MET) and choline (CHO) on lead (Pb) toxicity in growing chicks. The basal diets were based on glucose-soybean meal (Exp 1) or glucose-soybean meal-isolated soy protein (Exp 2 & 3). Pb was fed at 0 or 1000 ppm (as Pb acetate-3 H2O) in all experiments. In Exp 1 MET was 65% vs. 100% while Pb was 43% vs. 100% in nutritional requirements. In Exp 2 & 3, MET was 44% vs. 100% while Pb was 33% vs. 100% of nutritional requirements. Pb significantly reduced body weight while added MET significantly increased body weight in all experiments. Added CHO increased body weight only in Exp 2 & 3. The Pb x MET interaction was significant in all experiments in that growth depression from Pb was less severe in MET-deficient diets. Pb depressed hemoglobin levels in MET-deficient chicks but the depression was less in MET-deficient chicks. CHO did not affect hemoglobin. Non-protein sulfhydryl groups were measured as an estimation of liver glutathione (GSH). Pb increased GSH in livers and the increase was greater in MET-deficient vs. MET-deficient chicks. CHO did not affect liver GSH. The data suggest that the amelioration of Pb toxicity by MET results from its use for protein or peptide synthesis rather than its use as a source of methyl groups.

655 ROLE OF METALLOTHIONEOIN IN RESISTANCE TO CADMIUM-INDUCED TESTICULAR NECROSIS IN INBRED MICE. C. J. Chellman, G. L. Diamond and Z. A. Sahik. Division of Toxicology, University of Rochester, Rochester, NY.

It has been suggested that decreased testicular Cd accumulation is the critical factor which determines resistance to Cd-induced testicular damage (A/J) and the other susceptible (129/J). Mice of both strains were injected i.v. with either 10 (129/J) or 45 (A/J) moles CdCl2/kg to achieve equal testicular Cd concentrations (4 moles Cd/g). 24 hr after injection there was no microscopic evidence of damage in A/J testes, while 129/J testes showed marked interstitial hemorrhage and seminiferous tubule necrosis. 2 hr after injection 105,000 x g testicular cytosol was prepared and fractionated on Sephadex G-75 Superfine. Resistant A/J testes had 2.7 times more of the total tissue Cd bound to metallothionein (MT) than susceptible 129/J testes (1156 ± 129 vs. 451 ± 43 moles Cd/g). Therefore, resistance of A/J testes to Cd appears to be determined not by decreased testicular Cd accumulation, but by increased sequestration of Cd by MT. However, no significant strain difference was found when basal concentrations of MT were determined in whole testes (43 μg MT/g). This suggests that resistant testes may possess an MT with enhanced Cd affinity, or have elevated MT concentrations in a subpopulation of cells which accumulate Cd in vivo. (Supported by NIH Grants ES 01247 (Pilot Project) and ES 01445, and by NIH Training Grant ES 07026).


Triethyllead (TEL) and trimethyllead (TML) compounds have been shown to be neurotoxic inducing various neurobehavioral alterations in rats. The present report is to present neuropathological changes induced by these compounds. Adult Fischer-344 rats were injected (s.c.) with TEL chloride (7.8 mg/kg) or TML chloride (22.0 mg/kg) and were sacrificed 7 or 28 days after injection. Although the histopathology was only minimal in TEL-treated animals, electron microscopy revealed, however, significant neuronal swelling in the hippocampal granule neurons as well as hypertrophy and accumulation of mitochondria in many dorsal root ganglion neurons as early as 7 days after TEL exposure. 28 days after intoxication, an increased number of small mitochondria were observed in these neurons. In contrast, TML-treated animals showed very prominent histopathological changes in the brain stem and spinal cord neurons after 7 days of intoxication. Many of these large neurons appeared to be chromatolytic. Electron microscopy also revealed and confirmed lytic changes of these neurons. Unlike TEL, TML induced only minimal pathological changes in the dorsal root ganglia at this time. Our present findings provide definitive demonstration of pathological lesions induced by TEL and TML. This information is useful for the understanding of toxic impacts of these compounds.

656 EFFECTS OF LEAD ON NONPROTEIN SULFHYDRYL CONTENT OF VARIOUS TISSUES OF CHICKS. C. McGowan and W. E. Donaldson. Toxicology Program, NCSU, Raleigh, NC.

The effects of lead (Pb) on concentrations of nonprotein sulfhydryl groups, presumably reduced glutathione (GSH), in various tissues and organs of chicks were measured. Chicks were fed diets with either 0 or 2000 ppm Pb for 3 weeks. Selected 3-week-old chicks were injected (i.p. with either 0 or 52 mg Pb/100 g body weight. All Pb was administered as Pb acetate-3H2O. Pb for injection was dissolved in 5% acetic acid. Tissues were obtained 4 hours post-injection of Pb except as noted. In Exp. 1, chicks fed 0 Pb were injected i.p. with saline or methionine sulfoximine (MS) an inhibitor of GSH synthesis. MS significantly reduced GSH in liver as compared with saline controls. However, if saline or MS treated chicks were given Pb injections, liver GSH was markedly higher in both groups than in saline controls. In Exp. 2, liver, kidney, whole bone, bone marrow and muscle GSH was measured in chicks fed 0 and 2000 ppm Pb. One group of 0 Pb chicks was injected with Pb. Pb injection increased liver and kidney GSH, lowered muscle GSH and did not affect blood or marrow. Dietary Pb increased GSH in all tissues except marrow. Exp. 3 was a time-course of Pb effects (0, 0.5, 1, 2 and 4 hours post-Pb injection) on tissue GSH. Pb elevated GSH in all tissues by 0.5 hours, but the levels declined toward control values by 4 hours only in muscle and blood. The data suggest translocation of GSH among tissues as one response to acute Pb insult.
657 THE EFFECT OF MERCURY ON THE DOMESTIC CHICKEN AS INFLUENCED BY DEHYDRATION AND DURATION OF TREATMENT. R.E. Griscom, J.P. Thaxton, Interdepartmental Toxicology Program, NC State University, Raleigh, NC 27650. Sponsor: F.E. Guthrie

Four week old chickens were exposed to limited water, or HgCl₂ in the drinking water or by gavage for 0-3, 0-6, 0-9, 0-12 and 0-15 days. Each group was given a 14-day recovery period. Hg available ad libitum in drinking water caused a decrease in feed and water consumption, weight loss, and an increase in mortality. Red blood cell (RBC), hematocrit (HCT), mean corpuscular volume (MCV), and hemoglobin (Hgb) were increased within 3 days in Hg-treated groups, while the mean corpuscular hemoglobin concentration was decreased. Since the effects of Hg and dehydration were not separated by ad libitum exposure to Hg in drinking water, factorial experiments were performed to separate the effects caused by dehydration, Hg and Hg - dehydration interactions as follows: ad libitum water and Hg (ALW + Hg), ad libitum water without Hg (ALW), limited water and Hg(LW + Hg), and limited water without Hg (LW). Limited water caused reduced feed consumption, body weight loss, increased RBC numbers, HCT, MCV, and Hgb and decreased MCH within 3 days of treatment, while Hg caused reduced feed consumption after 9 days and reduced body weight gain after 12 days. Hg produced variable hematological results. Hg interacted with water limitation to cause a greater reduction in feed intake and weight loss than either caused alone.

659 EFFECTS OF PHENYLALANINE ON THE TERATOGENICITY OF OCHRATOXIN A IN RATS. K. Mayura, W.O. Berndt and T.D. Phillips. Department of Veterinary Public Health, Texas A&M University, College Station, TX 77843.

Ochratoxin A (OA), an important food-borne mycotoxin, is a potent teratogenic and nephrotoxic agent produced by several species of Aspergillus and Penicillium. OA is a known inhibitor of protein synthesis via competitive antagonism of phenylalanine (Phe) in phenylalanyl-tRNA synthetase. Moreover, it has been recently reported that Phe protects mice from lethal poisoning by OA (0.8 mg OA/mouse). This study was designed to determine if Phe could prevent the teratogenic effects of OA in rats. Pregnant Sprague-Dawley rats were injected subcutaneously with a single teratogenic dose of 1.75 mg/kg OA concurrently with 15, 20 or 25 mg/kg Phe on gestation day 7. The incidences of OA-induced fetal resorptions, decreased fetal body weights and fetal malformations were not diminished in the presence of Phe at all dose levels administered. Major types of external malformations (omphalocole, ectopia cordis, anophthalmia and hydrocephaly), visceral malformation (internal hydrocephaly), and skeletal defects involving ribs, vertebrae, sternebrae and skull were observed in rats treated simultaneously with OA and Phe. These results indicate that excess co-administered phenylalanine does not provide fetal protection from low levels of ochratoxin A. (Supported by TAES 58215 and 58620-4)


We have investigated the relationship between the teratogenicity and maternotoxicity caused in rabbits by diflunisal, a new analgesic antiinflammatory drug. Diflunisal treatment during organogenesis at 40 and 60 mg/kg/day induced axial skeletal defects in rabbit fetuses but similar or higher dosage levels were not teratogenic in rats or mice. These dosage levels also caused a severe hemolytic anemia in rabbits but not rats, dogs, or cynomolgus monkeys. The hemolysis was associated with dramatic decreases in erythrocyte ATP levels which occurred in rabbits but not rats, cynomolgus monkeys, or humans. To examine the effects of diflunisal-induced anemia in the absence of the drug, a single dose of 180 mg/kg diflunisal was given to rabbits on Day 5 of gestation. This treatment produced the characteristic axial skeletal defects and anemia which persisted through Day 15 of gestation. Since the diflunisal was cleared from the maternal blood before Day 9 of gestation, the critical day for the induction of similar axial skeletal defects by hypoxia, the skeletal malformations probably resulted from the maternal hypoxia and not from a direct effect of the drug on the embryo. These studies demonstrate that species which exhibit severe maternotoxicity may be unsuitable for studies designed to predict the teratogenic potential of drugs in humans.

659 TERA TOLOGY STUDIES ON ETHYLENEDIAMINE DIHYDROCHLORIDE (EDA) IN FISCHER 344 RATS. L.R. DePaes, M.D. Woodsie, and S.S.H. Yang. Bushy Run Research Center, Export, PA

EDA was fed to the rats on gestation days 6 to 15 at 1.0, 0.25, or 0.05 g/kg/day. At 1.0 and 0.25 g/kg/day, reductions in maternal diet consumption and body weight gain were observed. At 1.0 g/kg, fetal weights and lengths were significantly reduced and there were increases in the percentage of litters with resorptions, in the incidence of skeletal variants and in missing and shortened innominate arteries. To determine the possible effect of reduced food intake on the arterial defects a pair-feeding study was performed in which EDA was fed on gestation days 6 to 15 at 1.0 g/kg/day. A pair-fed control group received the same amount of diet as the EDA-treated rats. An untreated control group was fed ad libitum. Maternal weight gain, fetal weights and lengths and the lengths of the innominate arteries were reduced in the EDA-treated group compared to both control groups. Two fetuses each in the EDA-treated and pair-fed control groups had missing innominate arteries versus none in the untreated controls. Thus, ingestion of EDA resulted in reduced maternal weight gain, fetal size, and length of the innominate artery, but missing innominate artery was not a result of EDA treatment. There was no evidence for teratogenicity of EDA in these studies.

The interaction between ethanol (E) and acetaminophen (APAP) was investigated in pregnant ICR strain mice in reference to effects on progeny. Groups of pregnant ICR strain mice were given E (4.0 ml/kg), APAP (250 or 140 mg/kg), or combinations of E and APAP (4.0 ml and 250 mg/kg or 0.4 ml and 25 mg/kg) by gavage during days 6-15 of gestation. E (4.0 ml/kg) caused resorptions, lower litter weights and pups had hydrenephrosis, micrognathia and a variety of skeletal variations. Offspring mice given 250 mg APAP/kg were smaller and malformed; cleft palate, microcrania, hydrenephrosis and skeletal malformations and variations. The lower (140 mg/kg) dose of APAP caused similar anomalies at lower frequencies. The combination of 4.0 ml E/kg and 250 mg APAP/kg caused reduced litter weights and smaller pups without embryolethality. Malformations were minor and of low frequency: hydrocephalus, micrognathia, hydrenephrosis and skeletal anomalies. These results suggest a need for further interaction studies between embryopathogens. Supported by B5/M5 Fellowship, Union College, Schenectady, NY.

662 TERATOGENICITY OF NITROFEN (2,4-DICHLORO-4'-NITRO DIPHENYL ETHYER) AND THYROID FUNCTION IN THE RAT. J.M. Manson, T.J. Brown, and D.M. Baldwin. University of Cincinnati, Cincinnati, OH.

Nitrofen is an herbicide with potent teratogenic activity in rodent species. Studies have been carried out to determine if nitrofen or any of its metabolites have thyroid hormone activity, whether teratogenic exposure to nitrofen resulted in alteration of maternal or fetal thyroid function, and if this alteration could be related to induction of birth defects. The parent compound and metabolites failed to compete with 125I-T4 for binding to T4 antibodies, while a single metabolite (4-hydroxy-2,5-dichloro-4'-amino diphenyl ether) competed with 125I-T3 for binding with a potency 0.8% of T3. In adult thyroidectomized rats, nitrofen exposure for 2 weeks resulted in a suppression of TSH levels. When a single dose was administered to euthyroid rats, a reduction in the release of TSH after a TRH challenge occurred. Euthyroid rats given a single dose of nitrofen on day 11 of pregnancy had depressed TSH and T4 levels 24 hrs after exposure, and fetal T4 values were markedly depressed at term. Administration of T4 on days 2-22 of pregnancy plus nitrofen on days 9-11 to thyroidectomized dams resulted in a 70% reduction in the frequency of malformed fetuses compared to nitrofen exposure alone. These results have been interpreted to indicate that nitrofen teratogenicity is mediated via alteration in maternal and fetal thyroid hormone status, and may be due to pre-mature and pharmacologic exposure of the embryo to a T3-active metabolite.


CR is a diazo dye similar in structure to more than 80 other dyes which are of concern because they are synthesized from benzidine, a human carcinogen. While the adsorption, distribution and metabolism of these dyes have been studied, their teratogenic potential has not been investigated. The present study is a description of the effects of prenatal administration of CR on the reproductive development of the mouse. CR (92%) was administered orally at 1 g/kg/day on days 8-12 of gestation. The dams were allowed to deliver and the postnatal growth, viability, ages at vaginal opening and breeding, litter sizes, testes, seminal vesicle weights, sperm conc. in the vas were measured and the testes and ovaries were examined histologically. The treated breeding pairs produced fewer litters and had smaller litters, an effect that was subsequently demonstrated to be due to the effect of CR on the females. Ovarian histology revealed that the CR females had accelerated ovarian aging as indicated by follicular cysts and ovarian atrophy. The CR males had smaller testes, reduced sperm conc. in the vas and tubular atrophy. Prenatal exposure to CR reduces the gametogenic potential of mice.


Oral administration of 2, 4-Dichlorophenoxyacetic Acid to pregnant Fischer 344 rats during the period of organogenesis was neither teratogenic nor embryotoxic at doses of 75 mg/kg/day and less. The test material was admixed in Mazola® Corn Oil and administered orally as a single daily dose from day 6 through 15 of gestation. Dosage levels of 8, 25, and 75 mg/kg/day were used. A concurrent control group was dosed with the corn oil vehicle. Throughout gestation all females were observed twice daily for toxicity, weighted at appropriate intervals and sacrificed on gestation day 20 for the scheduled Cesarean section. All fetuses were weighed, sexed and examined for external malformations. One-half of the fetuses were examined skeletalily after staining with Alizarin Red S. The remaining fetuses were fixed in Boulin's solution, weighed and examined for visceral anomalies and developmental variations. No maternal or embryotoxic effects were observed at dosages of 25 mg/kg/day and less. There was a slight inhibition of maternal body weight gain at the highest tested dosage during the initial four days of test maternal administration. There were no remarkable differences in the number or percentage of fetuses and litters with malformations or in the types of anomalies observed at all levels tested when compared with the control group. (Sponsored by the Industrial Task Force on 2, 4-D Research Data).

Oral administration of 2, 4-Dichlorophenol to pregnant Fischer 344 rats during the period of organogenesis was not teratogenic at dosages of 750 mg/kg/day and less. The test material was admixed in Mazola® Corn Oil and administered orally to bred female rats as a single daily dose from day 6 through 15 of gestation. Dosage levels of 200, 375, and 750 mg/kg/day were used. A concurrent control group was dosed with the corn oil vehicle. Throughout gestation all females were observed twice daily for toxicity, weighed at appropriate intervals and sacrificed on gestation day 20 for the scheduled Cesarean section. All fetuses were weighed, sexed and examined for external malformations. One-half of the fetuses were examined skeletally after staining with Alizarin Red S. The remaining fetuses were fixed in Bouin’s solution and examined for visceral anomalies and developmental variations. Maternal body weight gain inhibition occurred in all treated groups; the effects were both dose-related and statistically significant. A slight increase in early embryonic death occurred in the high dose group only. Fetal weights were lower in the high dose group than the control group. The reduced fetal weights and intrauterine survival may represent a slight degree of embryotoxicity or fetotoxicity in this group but was more likely a secondary effect caused by excessive maternal toxicity. (Sponsored by the Industrial Task Force on 2, 4-D Research Data).


Yellow phosphorus in corn oil was administered by gavage to three groups of 25 mated Sprague Dawley rats at dosages of 0.1, 0.3 or 0.75 mg/kg/day on gestation days 6 through 19. A control group of 25 mated females received corn oil. In a second study, two groups of 25 mated female rats per group received either 0.6 mg/kg/day phosphorus in corn oil or corn oil on a comparable regimen. On gestation day 20, all animals were sacrificed. Fetuses were examined for external malformations, one half each for visceral or skeletal malformations.

Eighty-four percent of the animals receiving the 0.75 mg/kg/day phosphorus died between gestation days 14 and 18. In the 0.6 mg/kg/day dosage group, six of 25 females died during gestation days 17 to 20. Animals given 0.3 or 0.5 mg/kg/day phosphorus gained significantly less weight when compared to the control animals. Because of high maternal mortality in the 0.75 mg/kg/day dosage group, only survival body weights were recorded for this group. No compound related effects on reproductive parameters were observed. Yellow phosphorus did not produce a teratogenic response at or below 0.6 mg/kg/day.

TRANSPLACENTAL KINETICS OF METALS FROM THE ACTINIDE SERIES. B.J. Kelman and M.R. Sikov, Pacific Northwest Laboratory, Richland, WA.

We previously reported that monomeric $^{239}$Pu at a maternal plasma concentration of $<100$ ng/g ($<$50 Ci/g) is associated with a decrease in maternal blood flow to the placenta. Further experiments have confirmed that the effect is reproducible, independent of the dosing vehicle, and has an apparent threshold between maternal doses of 5 and 30 Ci/kg (82 and 490 µg/kg) body weight. This unusual effect at low chemical concentrations indicated a need for similar measurements using other actinides. We therefore employed the same technique to measure the clearance of $^{241}$Am from mother to fetus (Am-F). Indirect measurements of maternal blood flow to the placenta (using the clearance of tritiated water) indicated that, unlike Pu, $^{241}$Am had no effect on maternal blood flow to the placenta at maternal doses of 30 Ci/kg (9 µg/kg). Am-F measured 3.4±0.7 µl/min (mean±SE) which was not significantly different from similar measurements of Pu (2.3±0.6 µl/min) uncorrected for blood flow. When corrections are made for the disrupted maternal blood flow observed following Pu administration, Pu-F was 5 times greater than Am-F on a radiological basis. Am accumulation in fetuses was significantly less than that of Pu; differences in transplacental movements of Pu and Am are the most likely explanation for reported differences in the fetal accumulation of the two actinides. (Work supported by the U.S. Dept. of Energy Contract No. DE-AC06-76RL0-1830).


Previous study indicated that the neonatal hippocampal formation, particularly between postnatal days (PND) 9-13, was extremely vulnerable to the toxicity of trimethyltin (TMT) compounds. The present report presents in more detail the neuropathology induced by this compound. Neonatal rats (Sprague-Dawley) were injected (i.p.) with TMT chloride at a dose of 6.0 mg/kg b.w. on PND 11. Animals were sacrificed on PND 15 and PND 24. Extensive destruction of the hippocampal formation was observed 4 days after TMT exposure (PND 15). The Ammon's horn was much more affected than the fascia dentata, displaying pyknotic nuclei and eosinophilic cytoplasm. Many of the surviving neurons acquired a swollen appearance with eccentric and distended cytoplasm. By PND 24, the fascia dentata returned to a normal appearance while the entire Ammon's horn had disappeared. By means of electron microscopy, lysosomal accumulation and multifoci of electron-dense materials were observed. These deposits had a filamentous, crystalloid appearance and were believed to be dystrophic calcification changes of the necrotic tissues. This study confirms that the neonatal nervous system is very vulnerable. The TMT toxicity leading to extensive destruction of the Ammon's horn in a relatively short period of time. (Supported by EPA CR809360-02.)
CORRELATION OF LESION INDUCTION BY TRIMETHYLTHIN WITH VARIOUS DEVELOPMENTAL STAGE AND FUNCTIONAL MATURITY OF THE HIPPOCAMPAL FORMATION. L.W. Chang, Dept. of Pathology, Univ. of Ark. for Medical Sciences, Little Rock, AR

While the pathological effects of trimethylthion (TMT) compounds on the adult limbic system are well recognized, our present investigation was designed to study the pathologic impact of TMT on the developing hippocampus as a result of exposure at various ages of postnatal life. Sprague-Dawley rats were injected between postnatal day 1-30 with a single administration of TMT at a dose of 6.0 mg/kg b.w. Animals were sacrificed between 4 to 30 days after injection. While there was no significant pathological damage in the hippocampal formation of rats injected before PND 3, increased destruction of the Ammon's horn was observed in animals injected between PND 5-15. The progression of neuronal involvement was CAG, CAG, CAG, CAG, CAG, CAG, and CAG, and CAG - entire Ammon's horn (CAG). This pattern of pathological lesion was in good concert with morphological and functional development of the hippocampal formation in various stages of postnatal life. A gradual reduction of toxic sensitivity was observed after PND 20, and the pathological pattern assumed that of adult animals as reported in previous studies. Our present findings suggest that the maturational and functional conditions of the mossy fibers may play an important role in the induction of pathological lesions in the Ammon's horn as a result of TMT intoxication. (Supported by EPA CR809360-02.)


A program has been established to investigate endocrinologic and exogenous factors related to perinatal pregnancy performance in non-human primates. One investigation has concentrated upon examining the effects of chronic methylmercury exposure upon the growth and behavior of primate offspring. Procedures evaluate changes in menstrual cycle length, gross and fine motor coordination and visual functioning in adult females. Timed matings produce gestation known offspring and pre-partum examinations evaluate fetal viability. Pregnant females are monitored 24 hrs/day and labors and deliveries are recorded. Infants are separated from their mothers at delivery and hand raised in a 24 hour nursery. Assessments of offspring begin at birth and continue until 1 year of age. Evaluations include tests of early reflex behaviors, neonatal activity patterns, gross and fine motor coordination and caloric intake and physical growth. Plagelatian procedures are used to monitor the development of object permanence and standard WGSTA tasks evaluate discrimination capabilities, response set formation and breaking and learning set. Results using these procedures indicate that reproductive dysfunction and retarded cognitive development may be the initial signs of MeHg toxicity. NIEHS ES-00677 and ES 07032.


Cynomolgus rabbits were dosed from birth with 0, 50, or 100 µg/kg/day of lead. This resulted in blood lead levels of 2, 16, and 20 µg/dl at 30 days of age and stable levels of 3, 12, and 50 µg/dl thereafter. In order to examine central nervous system impairment, monkeys were tested at about 3 years of age on a series of three discrimination reversal tasks: non-spatial form discrimination, non-spatial color discrimination with irrelevant form cues, and non-spatial form discrimination with irrelevant color cues. Lead-treated monkeys made more errors than controls on the first several reversals of the form discrimination, as well as on the total reversal series for the color discrimination task with irrelevant form cues. In addition, treated monkeys exhibited greater attendance to irrelevant cues on the color discrimination task. These results indicate that low-level lead exposure results in impaired ability to adapt behaviorally to changes in the environment.


The acute and subacute toxicologic profile of a novel anxiolytic agent, (4-(2-chlorophenyl)-1,6-dihydro-1,3,9-trimethylimidazo-(1,2-a)pyrazolo-[4,3,f][1,4]diazepine (CI-918), was evaluated in mice, rats and dogs. Following single oral dosing, LD50 values were 508 and 605 mg/kg in female and male GSH mice, and 394 and 324 mg/kg in female and male Wistar rats, respectively. Clinical signs of toxicity evoked by treatment were depression, prostration, hyper-reactivity, ataxia and hypothermia; congestion and hemorrhage of urinary bladder were found at autopsy. Repeated dose studies revealed no drug-related pathologic changes in rats given 25 to 400 mg/kg/day po for 2 weeks. Liver and brain weights were increased in females at 400 mg/kg and in males at 50-400 mg/kg. In dogs, the drug was tolerated on an incremental dosing regimen from 2.5 to 80 mg/kg. At 100 mg/kg, ataxia, tremors and mydriasis were observed, whereas in 2 week studies, dogs given 40 and 80 mg/kg developed transient ataxia, somnolence, and weight loss without associated pathologic findings.

Pentolopam mesylate (SK&F 82526) was infused iv at 0, 5, 25, 50 or 100 𝜇g/kg/min for 24 hrs or administered by bolus injections at 0, 2, 8 or 16 + 20 mg/kg/day for 16-15 days to rats to assess drug toxicity. In the 2-week study, muscular hypotonia and/or increased adrenal weights were observed in the mid- and high-dose groups; no drug-related clinicopathologic or histologic change occurred. Drug infusion, however, produced smooth muscle mediar necrosis and intramedial hemorrhage in the arteries of the pancreas, stomach, intestine, ovary and kidney of some rats in all drug-treated groups. To investigate the possible influence of the experimental conditions on lesion formation and the sequential development of the lesions, additional studies were conducted. Arterial lesions were not seen after 1 hr of infusion but occurred with low incidence after 4 and 8 hrs of infusion. Lesions developed in unrestrained rats, those killed 24 hrs after cessation of infusion, and in those infused with drug dissolved in saline. Thus the experimental methodology did not contribute to the development of the lesions. Since the arterial lesions were observed in tissues believed rich in dopamine receptors, the genesis of the lesion may be related to a specific receptor interaction.

INTERACTION BETWEEN TOLBUTAMIDE AND VERAPAMIL: EFFECT ON BLOOD Glucose LEVELS IN MICE. M. Greening and A.M. Deery. Arnold and Marie Schwartz College of Pharmacy and Health Sciences, Brooklyn, NY. Sponsor: E. Raje

Verapamil is a slow channel calcium blocker used in the treatment of arrhythmias and angina. Tolbutamide is an oral hypoglycemic agent which stimulates insulin release from pancreatic islet beta cells. Intracellular calcium plays a role in this stimulated insulin release. Verapamil (50 mg/kg, po) alone did not significantly alter glucose levels when compared to saline controls. Tolbutamide (200 mg/kg, ip) for three days, resulted in a 39% reduction in blood glucose. When Tolbutamide was followed by Verapamil, the glucose level did not differ significantly from controls. This antagonism of Tolbutamide-induced hypoglycemia by Verapamil suggests an interaction which may have clinical significance.

BCNU ENHANCED DOXORUBICIN TOXICITY. T. Paradisathu, A.B. Combs, and J.P. Kehrer. Department of Pharmacology, College of Pharmacy, Univ. of Texas at Austin, Austin, TX.

The anticancer drugs doxorubicin (DOX) and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) are being used in combination chemotherapy to improve the response. DOX cardiotoxicity is dose-limiting and has been attributed to free radical metabolism and lipid peroxidation. BCNU, which specifically inhibits glutathione reductase activity, might be expected to enhance DOX toxicity by disrupting the glutathione antioxidant system. The 30 day ip LD50 for DOX in female BALB/c mice was 15.7 mg/kg (95% conf. limits, 14.6-16.8 mg/kg). A 13 mg/kg dose was not lethal to any mice. BCNU, 35 mg/kg ip, significantly inhibited heart and liver glutathione reductase activity within 4 hours, and was not lethal to any mice for up to 30 days. The 30 day ip LD50 for DOX given 4 hours after 35 mg/kg BCNU was 12.9 mg/kg (95% conf. limits, 11.8-14.1 mg/kg). Fifty percent of the deaths with combined DOX-BCNU treatments occurred within 14 days, as compared to none with DOX alone. Four hours after a DOX dose of 26 mg/kg, cardiac reduced glutathione content (GSH) was decreased 10%. BCNU, 100 mg/kg decreased cardiac GSH by 11% after 4 hours. Further studies on the cardiac GSH content following the administration of a combination of DOX and BCNU are underway. (Supported by Grant CH-263 from the American Cancer Society.)

SUBACUTE TOXICITY OF 9 RETINOIC ACID AMIDES, R.G. Meeks and B.P. Sani, Southern Research Institute, Birmingham, AL.

The major limitation for continuous administration of natural retinoids for chemoprevention of cancer is their toxicity; however, synthetic retinamides have the desirable quality of reduced toxicity while retaining biological activity. We have evaluated the comparative toxicity of the following all-trans-retinoids: retinoic acid, N-ethyl retinamide(ER), N-2-hydroxyethyl retinamide (2HBR), N-propyl retinamide(Prol), N-2-hydroxypropyl retinamide(2HPro), N-3-hydroxypropyl retinamide(3HPro), N-2,3-dihydroxypropyl retinamide (2,3-HPro), N-buty1 retinamide(BR), N-4-hydroxysteri-6-butyl retinamide(4-HBR), N-4-hydroxyphenyl retinamide(4-HPR). The estimated LD50s following administration of 27 daily doses of each retinoid in mice were determined. Evaluation of the LD50s and slopes of the lethality curves indicate that retinoic acid was the most toxic followed by 2-HPro>3-HPro>4-HPro>3-HPro>4-HBR>Prol>ER. In toxicity studies, changes in clinical chemistry and hematologic parameters following administration of each retinamide included dose-dependent nonregenerative anemia evidenced by erythrocytopenia, hemoglobinemia and decreased pack cell volume. Retinamide treatment also caused increased plasma alkaline phosphatase activity and decreased serum albumin levels. Histopathological lesions included degeneration and enlargement of hepatocytes and arrest of spermatogenesis and degeneration of spermatocytes within their ducts. Supported by NIH Contract No. CP-85616.

Ac-Di-Sol® croscarmellose sodium, Type A, National Formulary XV (ADS) is marketed by FMC Corporation for use as a disintegrant in pharmaceutical tablets/capsules and may be viewed as an insoluble form of sodium carboxymethylcellulose. Two groups of 20 male and 20 female rats received ADS at dietary concentrations of 10,000 and 50,000 ppm. A control group of 20 males and 20 females received untreated diet for 13 weeks. A fourth group received sodium carboxymethylcellulose (NaCMB) at 50,000 ppm. There were no deaths attributable to treatment. Slightly lower body weight gain, probably associated with the lower nutritional value of the diets, was noted for both ADS treated groups. Higher moisture content in feces was noted for animals in the 50,000 ppm group. Hematology, blood chemistry, urinalysis and organ weight analysis did not reveal any treatment related effects. Similar findings were noted in treated and control groups during gross and microscopic exam. All results with NaCMB were similar.

679 CHANGES IN MONOAMINE OXIDASE ACTIVITY FOLLOWING CHLORPHERTERNINE ADMINISTRATION TO RATS. L. Zychniski and M.R. Montgomery, Department of Pharmacology, University of South Florida and VA Hospital, Tampa, FL.

Examination of the effects of chlorphentermine (CP) on monoamine oxidase activity in rat was conducted in vivo. Oxidation of seven amine substrates by rat liver, lung or brain mitochondrial monoamine oxidase was investigated at 2 hours after single ip injection of 60 mg/kg. Deamination of norepinephrine, serotonin and octopamine (type A substrates) was decreased in liver 54%, 55% and 59%; in lung 40%, 41% and 43%; and in brain 49%, 40% and 48%, respectively. Oxidative deamination of tyramine and dopamine (type A and B substrates) was lowered in liver 31% and 31%; in lung 20% and 19%; and in brain 23% and 22%, respectively. Oxidation of benzylamine and tryptamine (type B substrates) was unaffected by CP intoxication in comparison to the control, in liver 95% and 96%; in lung 104% and 101%; and in brain 102% and 97%, respectively. Kinetic study of dopamine oxidation by purified, crystal monoamine oxidase in the absence and presence of CP (0.5-2.0μM) indicated that only Km was affected. These combined data indicate that chlorphentermine is a specific inhibitor of mitochondrial monoamine oxidase, type A with competitive-type inhibition. This study supported by NIH grant ES02846 and VA Medical Research Funds.

678 ACETAMINOPHEN-INDUCED EXTRAHEPATIC DEGENERATION AND NECROSIS IN THE MOUSE. M.E. Placke, D.S. Wyand, and S.D. Cohen. Dept. Pathobiology and School of Pharmacy, Univ. of Connecticut, Storrs, CT 06268

Acetaminophen (APAP) is recognized as essentially an exclusive hepatotoxin, with descriptions of toxic damage to other organ systems (primarily kidney and heart). Other tissues are believed spared toxic effects. This study was designed to assess possible damage in organs other than liver. Standard and germfree, fasted, adult, male, CD-1 mice were given APAP (600 mg/kg) per os and sampled at varying times, from 30 min to 48 h after treatment. Dose- and time-dependent degenerative and necrotic changes were found in 4 non-hepatic tissues. Renal nephrosis developed 2-4 h after treatment and paralleled the course of hepatic damage. Necrosis of bronchial epithelium, in the absence of any inflammation, was evident 4-6 h after APAP exposure in the lung. Onset of testicular changes was delayed until 6-8 h following APAP. Spermatogenic degeneration, tubular necrosis with development of spermatidic multinucleated giant cells were characteristic features. Small areas of lymphoid necrosis were visible in splenic follicles 18-24 h after treatment. Other studies have revealed that each of these organ tissues have mixed function oxidative and glutathione enzyme systems, which are the reported activation and deactivation pathways for APAP. However, the pathogenesis of each of these apparently drug-induced lesions remains to be determined.

680 RETINOIC ACID INDUCES CALCIUM RELEASE FROM FETAL BONES IN CULTURE. C.J. Vial and S.D. Harrison, Jr. Grad. Ctr. for Toxicology, U. of Kentucky, Lexington, KY.

A fetal mouse bone culture assay has been evaluated as a model for retinoid-induced bone thinning, a subchronic toxicologic response that has been observed in animals. Pregnant C57 mice were injected with Ca-45 (50 μCi) on day 17 of gestation. On day 18, mice were euthanized, and fetal radii and ulnas were dissected aseptically and cultured in a defined medium (BGM) at 37°C in a humidified atmosphere of 5% CO2 in air. Following a 24 hr preincubation in the presence of 3H-arachidonate (AA), radii and ulnas were transferred to fresh medium containing parathyroid hormone (PTH, 0.1 to 1.25 U.S.P. units/ml), all-trans-retinoic acid (RA, 3.0 to 300 ng/ml), or diluent (water or 0.1% aqueous EtOH, respectively) and incubated an additional 24 or 48 hrs until harvest. Radioactivity in bones and media aliquots was measured by liquid scintillation spectrometry to determine the amount of Ca-45 released from bones. PTH and RA both induced significant (p < 0.01) dose-dependent increases in Ca-45 released, up to 200% and 63%, respectively, compared to paired controls. Studies with cyclooxygenase inhibitors and analysis of 3H-RA metabolites released into the media are currently in progress. This approach will be valuable for examining the suspected involvement of AA metabolites in the mechanism of RA-induced bone thinning.
Lack of in vivo covalent binding of nafenopin, a carcinogenic peroxisome proliferator, to liver DNA of normal and partially hepatectomized rats. S.K. Goel, N.D. Laiwani, and J.K. Reddy. Department of Pathology, Northwestern University Medical School, Chicago, IL.

Nafenopin ([2-methyl-2-(1,2,3,4-tetrahydro-1-naphthyl) phenoxypyproionic acid]), a potent hepatic peroxisome proliferator, produces hepatocellular carcinomas in rats and mice. Since this compound, like other carcinogenic peroxisome proliferators, has been found to be negative in short-term tests for mutagenicity, we examined the in vivo covalent binding of[^1]nafenopin to rat liver macromolecules. Intact and 20-hour postpartially hepatectomized male rats were given[^1]nafenopin by gavage and sacrificed 16 hours later. Liver DNA, RNA and protein were isolated and analyzed for radioactivity. Although no measurable radioactivity was found in DNA and RNA, binding to protein was detectable. The results suggest that it is unlikely that covalent binding to DNA is the mechanism by which nafenopin induces hepatocarcinogenesis in rodents.

Interation between diisopropylfluorophosphate (DFP) and the opiate system in mice. S.H. Zorn, L.G. Costa*, S.D. Murphy*. Division Toxicology, Dept. Pharmacology, Univ. Texas Med. School, Houston, TX, and *Dept. Environ. Health, Univ. Washington, Seattle, WA.

DFP causes an antinociceptive effect in mice which is blocked by scopolamine, mecamylamine, and naloxone suggesting that besides stimulation of muscarinic and nicotinic cholinergic receptors, the endogenous opiate system may be involved as well (Zorn et al. Toxicologist 1983). In order to study the interaction between DFP and the opiate system we investigated whether DFP could act directly on the opiate receptors and/or alter brain levels of met-enkephalin in mice. To investigate the former, we determined if DFP was able to displace specific [3H]-dihydromorpholine (DMM) binding in mouse forebrain homogenates. Specific DMM binding was defined as the difference in binding in the presence and absence of 10μM levorphanol. DFP did not alter the specific binding of DMM to opiate receptors in vitro. We also determined whether an antinociceptive dose of DFP could alter met-enkephalin content in mouse striatum. We found that the administration of DFP (5 mg/kg, i.p.) to mice resulted in an 80.6% increase in met-enkephalin immunoreactivity in the striatum compared to controls. This data provides further evidence to suggest that DFP-induced antinociception is due in part to the accumulation or release of endogenous opioid peptides in the CNS. (Supported by research grant 1 ES01831 from NIH, and a gift from the Shell Company Foundation).


Two weeks of oral treatment with SC-37211 [1-(phenyl-2-(1H-imidazole-1-yl)-1-butanone,2,4,6 trichlorophenyl hydrazine hydrochloride], an antimicrobial agent, caused histologic changes in the thyroid, including slighty smaller and/or misshapen follicles lined by epithelium which was occasionally taller, more vacuolated and/or more basophilic than controls. At doses of 20, 60, and 200 mg/kg (10 rats/group) there were significant decreases (20-30%) in serum triiodothyronine (T3) and thyroxine (T4). Treatment did not affect in vivo thyroidal iodide uptake nor iodide organification. However, there were dose related significant increases in hepatic UDP-glucuronosyl transferase (UDPGT) activity on both a mg protein (6-24%) and a gram liver (14-52%) basis as well as increased liver weights (12-40%). The above changes were absent following a two week reversal period. These data suggest that increased hepatic UDPGT activity decreased serum T3 and T4, thus increasing thyrotropin (TSH) release and causing the observed reversible thyroid changes.

Effect of organic acid drugs on methotrexate toxicity in the mouse intestine. M.Z. Badr and T.S. Chen. Dept. of Pharm. and Tox., Univ. of Louisville, Louisville, KY 40292.

Methotrexate (MTX), a folate antagonist, has been shown to inhibit intestinal nutrient absorption. The effect of probenecid (prob) and other organic acid drugs on MTX gastrointestinal toxicity was investigated. Male Swiss-Webster mice were given MTX, 25 mg/kg intraperitoneally (ip), once daily for 4 successive days. Using the everted sac technique, the rate (μmole/gm/hr) of L-tyrosine transport was decreased to 5.4 ± 0.8 as compared to 9.2 ± 1.0 for control animals. When intestinal sacs from untreated animals were exposed to MTX (10^-7 M) on the serosal side, there was no effect on the rate of L-tyrosine transport. Administration of probenecid or sodium salicylate (sal), 100 mg/kg, ip 30 min prior and after MTX, decreased the rate of transport to 3.8 ± 1.2 and 3.7 ± 1.8 respectively. In addition, the body weight loss induced by MTX treatment (14.3 ± 2.1%) was potentiated by the concurrent administration of prob (20.1 ± 2.8%), or sal (21.5 ± 2.2%). The results demonstrate that probenecid and salicylate potentiate the inhibitory effect of MTX on intestinal nutrient absorption. Thus caution should be taken in giving such drugs to patients receiving MTX in cancer chemotherapy. (Supported by the Graduate School, Univ. of Louisville).
A series of imidazo[1,2-a]pyridine analogs was synthesized for possible development as anti-ulcer agents. Preliminary studies were conducted to evaluate the pharmacological activity of 35 compounds. Based on this data, 15 analogs were selected for toxicological screening. Groups of 20 male mice were offered via dietary admixture one of the analogs at an estimated mean daily dose of 400 mg/kg for two weeks. None of the animals died during the study. At necropsy, the stomach and liver were examined and weighed, and representative sections collected for microscopic evaluation. Increases in relative stomach and liver weights were found in animals dosed with seven of the analogs and increases in relative liver weight only were found for eight analogs. Microscopically, stomach changes included parietal cell vacuolization and degeneration and liver changes included centrilobular or periportal cell hypertrophy. Structure activity relationships, organ weight data, and microscopic changes for the series will be presented.

Methemoglobin formation induced by an 8-aminoquinoline WR 6026-2HCl (N,N-diethyl-N'-[6-methoxy-4-methyl-8-quinolinyl]-1,5 hexanediamine dihydrochloride) and its reversibility was examined in beagle dogs and was compared with that of Primaquine, a known 8-aminoquinoline antimalarial. The drugs were administered orally once a day for four consecutive days at 0.0116 mmoles/kg/day or a molar equivalent of 3.0 mg (base) of primaquine/kg/day. Peak % methemoglobin (Mb) occurred approximately 4 days after the first dose for both treatment groups. Peak % Mb was significantly different between the WR 6026-2HCl treatment group (21% Mb) and the primaquine treatment group (5.6% Mb). The Mb disappearance half time was approximately 7 days for both groups. Based on area under the concentration versus time curve (AUC), the total Mb production of WR 6026-2HCl was greater than primaquine but failed to be significantly different at equimolar doses.

Tulobuterol, a beta adrenergic agonist, was given intravenously to rats at dosages of 1, 5 or 25 mg/kg/day and to dogs at dosages of 0.6, 2 or 6 mg/kg/day for 1 month. Control groups received saline. After 1 month of treatment, rats or dogs from each respective group were allowed to recover for 2 weeks without treatment. Two rats died at 25 mg/kg/day. Convulsions, jerking movements, hyperactivity, tremors, hypoactivity and ptosis were observed in rats at 25 mg/kg/day. Restlessness, ptosis and hypoactivity were also observed in dogs at 2 and 6 mg/kg/day. Cutaneous and/or mucosal erythema were observed in rats and dogs at all dosages. Increased body weight gain occurred in drug-treated rats and in female dogs given 2 and 6 mg/kg/day. Slight increases in serum creatinine and BUN were seen in rats and dogs at the highest dosages. Absolute and relative heart weights were increased in rats at all dosages. There were no drug-related gross or histopathological changes in either rats or dogs. After 2 weeks of recovery, absolute heart weight was still increased in rats given 25 mg/kg/day. The no-toxic-effect dosages were considered to be 5 mg/kg/day in rats and 6 mg/kg/day in dogs.

Due to clinical interest, the antineoplastic drug SHM was given intravenously to rats as a single dose (x1) and to rats, mice and dogs as 5 consecutive daily doses (x5). The LD50, LD90 and LD90 were 45.6, 64.8 and 91.2 mg/m2 in x1 rats, 11.4, 16.2 and 23.4 mg/m2/day (d) in x5 rats and 24.9, 33.0 and 43.5 mg/m2/day in x5 mice. Clinical signs were decreases in body weight (BW) and activity, increased respiratory rate and piloerection in mice; decreased BW, rough hair coat, hunched back, changes in respiratory rate and activity level, convulsion and hyperextension of forelimbs in rats; and decreases in BW, food and water, drowsiness, dilated pupil, emesis, diarrhea, lethargy, incoordination, labored breathing, convulsion and spasm in dogs. Major hematoletic and pathologic findings were changes in the hematopoetic and lymphatic systems in all three species, lesions in the testes and ovaries in mice and rats, and lesions in the intestines in rats and dogs. In dogs, 1.8 mg/m2/day was defined as the highest non-toxic dose. Reversibility was seen in long-term animals except for reproductive lesions, which had a late occurrence.

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Due to clinical interest, the antineoplastic drug BIS was given intravenously to CD2F1 mice. Fischer 344 rats and Beagle dogs as a single dose (xl) or 5 consecutive daily doses (x5). In mice, the LD10, LD50 and LD90 were 111, 132 and 158 mg/m² for xl and 98, 111 and 125 mg/m²/day for x5. Lethality doses in rats were about 2 times the lethality doses in mice. In the toxicity studies, mouse equivalent LD10 (MELD10), LD50 and 1/2 LD50 were used in rats and 4xMELD10, 2xMELD10, MELD10 and 1/10MELD10 were used in dogs. Clinical signs in mice and rats were erypthalmas, loss of locomotor ability, body jerking and labored breathing. Immediate reactions in dogs were excessive salivation and lacrimation, vomiting, labored breathing, prostration, flushing, convulsion, yelping, excitement and coma. This was followed by decreases in food, water and body weight; soft stool and diarrhea. Clinical pathologic changes were more severe in dogs than in rats and included changes in cellular elements of the blood, serum electrolyte imbalance and possible liver and kidney damage. Very few indications of toxicity were observed in dogs at the 1/10MELD10. Toxicity was dose-related and reversibility was seen in long-term animals.

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PSORALENS AND UVA LIGHT
J. K. Dunnick1, P. D. Forbes2, R. E. Davies2, and W. O. Iverson3. 1National Toxicology Program; 2NIEHS, Research Triangle Park, NC; 3The Center for Photobiology, Temple University Health Sciences Center, Philadelphia, PA 19140; and 3Experimental Pathology Laboratories, Inc., Herndon, VA 22070. Supported: J.B. Mennen

Psoralens and UVA radiation (PUVA therapy) have been used in the treatment of psoriasis and vitiligo, and this therapy has been implicated in increasing the incidence of squamous cell carcinoma. To evaluate the toxicity of psoralens and to establish dose levels for a chronic study, a 13 week subchronic study of four psoralens, with and without UVA light (320-400nm with a measurable contaminant at 313nm), was conducted in hairless (HHA/Skh) mice. 8-methoxypsoralen (8-MOP) and 5-methoxypsoralen (5-MOP) given in the feed at 50, 100, 625, or 1250 ppm three times a week, without UVA, had no toxicity, and UVA light alone at 2J/cm² had no toxicity. 8-MOP or 5-MOP at 1250 ppm followed 1/2 hr. later with 2J/cm² UVA, caused skin toxicity including hyperplasia, inflammation, ulceration and dysplasia (8-MOP) or atypical nuclei of the female mouse had more severe skin toxicity than the male. Corresponding levels of 5-methylpsoralen (5-MPP) or 3-carbethoxypsoralen (3-CPS) with UVA produced no skin toxicity. UVA light alone at 48J/cm² produced hyperkeratosis and mild epidermal hyperplasia of the skin. Skin toxicity was lessened when drug levels were decreased and UVA levels increased proportionately.

REFERENCES


693 EFFECTS OF PERFLUORO-N-DECANOIC ACID (PFDA) ON LIVER ENZYMES AND ULTRASTRUCTURE IN THE RAT. M. J. Van Ralghem and H. E. Andersen, AFAMRL/THB, Wright-Patterson AFB, OH; S. Lane, S. Luking, and E. N. Harrison, Dept. of Biological Chemistry, Wright State University, Dayton, OH.

A single ip dose of PFDA (50 mg/kg) causes hepatic peroxisome proliferation in male F-344 rats, 16 days after dosing. The effects of this dose on liver morphology and a number of hepatic enzymes localized in peroxisomes and other organelles in male Sprague-Dawley rats were assessed at various times up to 30 days after dosing. PFDA caused cessation of growth persisting 10-12 days, after which the rats gained body weight in parallel to controls. After 10 days the liver body weight ratio of treated rats was twice that of controls. Livers remained enlarged for at least 30 days. There was no effect on liver total protein. Peroxosomal fatty acyl-CoA oxidase activity in the liver of treated rats increased 25-50 fold over controls and was maximal at 3-4 weeks after dosing. Plasma membrane alkaline phosphodiesterase activity dropped rapidly to 50% of control value and remained at this level for 30 days. Catalase (peroxisomes), N-acetyl-L-glucosaminidase (lysosomes), and NADPH-cytochrome c reductase (endoplasmic reticulum) were not affected by PFDA treatment. Cytochrome oxidase (mitochondrial) was decreased by 50%. Changes in liver ultrastructure included disruption of mitochondria and rough endoplasmic reticulum, and an increase in peroxisomes.

695 THE ETIOLOGY OF YUSHO POISONING: S. Bandiera, K. Farrell, M. Kelly, R. Bannister, G. Mason, L. Safe and S. Safe, Dept. of Physiology and Pharmacology, College of Veterinary Medicine, Texas A&M University, College Station, TX, 77843

The accidental leakage of PCBs into edible oils resulted in two mass poisonings in Japan and Taiwan. The oils contained PCBs and highly toxic polychlorinated dibenzo-p-dioxins (PCDFs). High resolution GC analyses have identified the following PCBs and PCDFs which persist in the liver of the "Yusoh" poisoning victims: 2,3',4,4',5-penta-, 2',2',5',6'-, 2,2',3',4',5',6-hexa-, 2,2',3,4,4',5,5'- and 2,3',3',4,4',5,5',6-heptachlorobiphenyls and the 2,3,7,8-tetra-, 2,2',4,7,8-, 2,3',3,7,8 and 2,3,4,7,8-penta- and 1,2,3,4,7,8-hexachlorodibenzo-p-dioxins. All of these PCBs and PCDFs have been synthesized and reconstituted to approximate their composition in human liver. A comparison of the dose-response effects of the reconstituted PCB and PCDF mixtures in causing weight loss, thymic atrophy and the induction of cytochrome P-450-dependent monoxygenases indicated that the PCDF mixture was at least 700 times more active than the PCBs. Since the ratio of PCBs/PCDFs persisting in Yusoh patients' blood and liver was less than 600:1 and 5:1 respectively, the results suggest that the PCDFs are the major etiologic agent in Yusoh poisoning. (Supported by N.I.H. Grants No. ES02937 and ES02798.)

694 TOXIC EFFECTS OF PERFLUORO-N-DECANOIC ACID (PFDA) IN RATS. M. E. George and M. E. Andersen, Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB, OH

Perfluoro-n-decanoic acid (PFDA) is extremely toxic with a single dose ip LD50 of 50 mg/kg. It causes a wasting syndrome with delayed lethality. Although PFDA affects lipid metabolism, the metabolic fate and mechanism of action are unknown. Control rats were pair fed with rats given 50 mg PFDA/kg. Body and organ weights and hematological and serum clinical chemistry parameters were determined at 4, 8, 12, 16, and 30 days post-dosing. Liver samples were also taken for determining cholesterol, cholesterol esters, phospholipids, total lipid, fatty acids, protein, and PFDA. The rats became anorectic within 24 hours and did not resume feeding for 16-18 days, losing 40% body weight. There were no significant changes in the hematological or clinical chemistry except for a decrease in serum protein at all times. The liver body weight ratios of exposed rats was twice that of control rats. The absolute liver weights remained constant during the weight loss period (2-16 days) and increased dramatically during refeeding (16-30 days). Kidneys but not heart weights followed this pattern. Total liver lipid content was increased and proteins content decreased. PFDA was found in liver samples with peak levels at 4 days although significant amounts were found at all times.

696 EFFECTS OF ORAL DISOPYRAMIDE PHOSPHATE ON SERUM GLUCOSE AND GLUCOSE COUNTERREGULATION IN THE DOG. J. R. Meenan, J. J. Hjelle, and F. N. Kotsenis, Product Safety Assessment Dept., Searle Research and Development, Skokie, IL.

The hypoglycemic effect of the antiarrhythmic disopyramide phosphate (DPP) was investigated following oral single doses in Beagle dogs. DPP produced statistically significant decreases in the change in serum glucose concentration from baseline values. Maximum decreases in glucose were 9% at 10 mg/kg, 14% at 30 mg/kg, 27% at 100 mg/kg given as single doses, and 30% at 100 mg/kg given as three 33.3 mg/kg doses separated by 4 hr intervals. In each case, glucose concentrations returned to control values within 24-30 hr. The magnitude of the glucose decreases was related to the disopyramide serum concentration. The hypoglycemic effects of DPP and its optical isomers were compared by examining the ratio of the areas under the curve of serum glucose concentration to drug concentration. The ratio for the (S)-(-) isomer was greater than the ratio for DPP and the (R)-(+) isomer, indicating that the hypoglycemic effect of DPP is largely due to its (S)-(+) isomer. No differences of any practical significance were observed between the insulin tolerance curves of control and 50 and 100 mg/kg DPP groups, suggesting that the overall glucose counterregulatory response following insulin was unaffected by DPP.
HAZARDOUS WASTE MANAGEMENT: AN INVESTIGATION OF POTENTIAL GENERATION OF AIR POLLUTANTS.  
M.S. Bilesi, Department of Health and Safety, Indiana State University, Terre Haute, IN.  
Sponsor: F.R. Oehme

The hazardous waste management process involves various waste handling phases, including: waste generation, storage, collection, transportation, treatment, recovery, disposal, site investigation, and clean-up. The purpose of this paper is to incorporate and apply principles of industrial hygiene and toxicology, in order to investigate the potential generation of air pollutants associated with the various waste handling phases. An outlined and schematic presentation depicts the phases of the hazardous waste management process, and (1) identifies the areas or factors recognized as potential generation sources of air pollutants; (2) presents various air sampling strategies along with monitoring and analytical equipment employed for evaluation of air pollutants; and (3) recommends possible methods for employee exposure control, including major elements of a proposed occupational health program.

SODIUM SACCHARIN ASSOCIATED CHANGES IN BLADDER TISSUE MINERAL CONCENTRATIONS.  
R.L. Anderson.  
The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, OH.  
Sponsor: E.V. Buehler

Ingestion of sodium saccharin (NaS) is associated with a dose-dependent, male-specific increase in bladder tissue concentration of K, Mg, and Zn but with no change in tissue Na or Ca. Since NaS ingestion increases the urinary excretion of Ca, Mg and Na with no change in K or Zn it seems unlikely that the bladder mineral increases simply reflected the changes in urinary excretion.

To resolve this issue, the effect of intravenous (i.v.) NaS on bladder mineral concentrations was determined. i.v. NaS did not alter plasma or urinary mineral excretion but did induce bladder tissue concentration increases in Mg, Zn, K and P without altering tissue Na or Ca.

This work shows that NaS in the urine can result in a bladder tissue mineral accumulation that is independent of plasma or urinary mineral concentrations.

EFFECTS OF 6-MERCAPTOPURINE ON DEVELOPING MUSCLE CELLS IN VITRO.  
G. Yander and H. Kaji.  
Thomas Jefferson University, Philadelphia, PA.  
Sponsor: J.J. Keolis.

6-Mercaptopurine (6-MP) is an adenine antagonist that is used in the treatment of acute leukemia. Other investigators have shown that the administration of 6-MP to neonatal rats resulted in a delayed onset degeneration of the thigh and sublumbar muscles. To determine whether or not developing muscles were particularly sensitive to 6-MP we isolated pure populations of embryonic muscle cells from chick embryos and grew them in vitro. We compared the effects of 6-MP on replicating myoblasts and postreplicative myotubes. Treatment with 10-50 µg/ml of 6-MP did not affect the number of viable myoblasts; however, the number of myotubes was decreased. The incorporation of [3H]-leucine into protein and [3H]-thymidine into DNA was inhibited by 6-MP in the myoblasts but not in the myotubes. Because 6-MP is incorporated into DNA as thioquinine and myoblasts undergo a terminal mitosis, we analyzed DNA on agarose gels after restriction endonuclease digestion. No new fragments were observed in the DNA from 6-MP treated cells after digestion with Bam H1 or Eco R1. Further analysis of restriction enzyme digested DNA may elucidate the mechanism of 6-MP induced myotoxicity. (Supported by ESO 7084-05).

LONG-TERM PERITONEAL TISSUE RESPONSE TO PARTICULATE MED-RELEASE AGENTS USED IN SURGEONS’ GLOVE MANUFACTURE.  
British Industrial Biological Research Association, Carshalton, Surrey, SN5 4DS, U.K.  
Sponsor: S.D. Gangoli.

Post-surgical complications have been attributed to wound contamination by modified starch used as glove lubricant, but only recently has the involvement of particulate mold-release agents been suspected. Tobin & Brown (1980, Arch. Surg. 115, 729) identified residues of talc on most available brands. The present study compared peritoneal tissue reactions to talc, calcium carbonate and modified starch (Bio-Sorb® glove powder) in rats. Samples (50 mg) were introduced into the peritoneal cavity and at intervals of 2, 4, 6, 13, 26 and 52 weeks groups of 10 animals were killed and the peritoneum examined. The severity of the lesion in each instance diminished with time, but talc produced a more severe and extensive inflammatory reaction formed largely of macrophages and multi-nucleate giant cells containing talc granules. In addition talc resulted in significantly more peritoneal adhesions than the other two materials. On this basis calcium carbonate would appear the safer mold releasent. (Supported by Surgikos Inc.)
701 PRECHRONIC TOXICITY EVALUATION OF O-BENZYL-P-
CeIlorophenol: COMPARISON BETWEEN F344 RATS AND B6C3F1 MICE Randy Deskin, Sandra Crumbein, Perry Kurtz, Arthur Peters, and Linda Birnbaum* Battelle, Columbus, Ohio, and National Toxicology Program, RTP, N.C.

O-Benzy1-p-chlorophenol (obPC), an aryl halide biocide, is currently used as a disinfectant. The toxicity of obPC was of interest because it is structurally similar to hexachlorophene, a known neurotoxin. A two-week repeated dose toxicity study was conducted in both sexes of F344 rats and B6C3F1 mice. obPC was administered by gavage in corn oil at doses ranging from 1000 to 62.5 mg/kg. Renal lesions were observed in both sexes of rats and mice. Rats were more sensitive than mice to the nephrotoxic effects of obPC. Subsequently, a 13-week subchronic toxicity study was conducted in both sexes of B6C3F1 mice and F344 rats. Animals received obPC at doses of 480, 240, 120, 60, 30, or 0 mg/kg. Renal changes were present in rats in the 480 mg/kg dose group and decreased in frequency and severity with decreasing dose. Male rats appeared to be more sensitive than female rats. Kidneys of mice treated with obPC appeared normal at the end of the subchronic study. Neobehavioral and neurobehavioral effects failed to detect any neurotoxicity associated with obPC. A second subchronic study was performed in mice and kidney lesions were observed at higher doses. (Supported by Contract N01-ES-95653-03 from the NTP).

703 PHTHALATE ESTER MIGRATION FROM POLYVINYL CHLORIDE CONSUMER PRODUCTS. R. L. Hanson and C. H. Hobbs. Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM; B. Bhoochan, U.S. Consumer Product Safety Commission, Bethesda, MD.

Di(2-ethylhexyl)phthalate (DEHP), a widely used plasticizer, has been reported carcinogenic in rats and mice. We determined the migration of phthalate ester plasticizers from polyvinyl chloride consumer products under simulated use conditions to provide an initial estimate of potential human exposure from use of these products. The concentrations of DEHP in 16 products studied had a range of 0.7-40%, with most products containing 10-35% DEHP by weight. Dermal contact was simulated by scrubbing with a cotton cloth containing 330 mg lanolin/cm². Ingestion route was simulated by leaching submerged products with saliva simulant and shaking or squeezing, using a mechanical plunger. Scrubbing a 900 cm² area of products for two min resulted in migration of 22-77 µg DEHP, whereas 30 min of scrubbing resulted in migration of 100-560 µg DEHP. The migration of DEHP from baby pacifiers was greater by leaching with squeezing of the nipple than leaching in a shaker bath. Two pacifiers had 814 and 1070 µg DEHP leached in 2 hr of squeezing, compared to 151 and 174 µg DEHP leached by 22-hr shaking. DEHP migration remained fairly constant for up to 30 days. (Research conducted for Consumer Product Safety Commission under Interagency Agreement CPSC-IGA-1387 under U.S. DOE Contract DE-AC04-76EV01013 and does not necessarily reflect the position or views of CPSC.)


Eight ionophores (12-Crown-4, 15-Crown-5, 18-Crown-6, Dibenzo-18-Crown-6, Dicyclohexano-18-Crown-6, Hexacyclen trisulfate, Lasalocid, and Valinomycin) were evaluated for toxicologic and pharmacologic properties, including lethality and neurobehavioral effects in multiple species by multiple routes, skin and eye irritation, dermal absorption and effects on membrane permeability. Attention was focused on determining minimum neurobehavioral effect levels and in characterizing the spectrum of these effects at different dose levels. A subacute study was performed to identify target organ systems and to evaluate the effects of repeated dosing.

While LD50's followed the pattern valinomycin < lasalocid < dicyclohexano < 12-C-6 hexacyclen trisulfate < 18-C-6 < 15-C-5 < dibenzo-18-C-6 < 12-C-4, this was not the case with other effects. Cluster and factor analysis supported that the primary mechanisms of action are alteration of membrane permeability to specific cations, the ions which are given increased passage vary with ring size and charge dispersion. In the intact animal, this is modified primarily by absorption. Membrane absorption and specific blocker studies support the derived classifications of cationic mechanisms (ie, sodium, potassium, calcium or multiple ion permeability enhancers).

704 POTENTIAL TOXICITY OF INTRAVENOUSLY ADMINISTERED 01-(2-ETHYLHEXYL)PHTHALATE (DEHP) AND MONO-(2-
ETHYLHEXYL)PHTHALATE (MEHP) IN THE NEONATAL RAT. D.B. Wiencekowski, B.A. Gillies, J.A. Thomas, and E.J. Youkilis. Travenol Laboratories, Morton Grove, IL

This study assessed the potential acute toxicity of DEHP and MEHP. Two to four-day-old Sprague Dawley rats received DEHP at dosages of 15.4, 30.3, or 62.4 mg/kg, or MEHP at 8.8, 17.7, or 40.9 mg/kg. These dosages represent the probable exposure for a pediatric patient during an exchange transfusion of 0.5, 1.0, or 2.0 units of blood stored for 35 days in a polyvinyl chloride blood bag. Test articles were administered as suspensions in 4% (w/v) solution of bovine serum albumin (BSA) in saline. Twenty-four litters, with 6 rats in each, were used. In each litter, 3 rats received either DEHP or MEHP at low, intermediate, or high dosages, respectively; one received 4% BSA; one 0.9% saline; and the last no treatment. No toxic signs appeared after treatment or during the 18-day observation period. No alterations in hematologic or clinical chemistry parameters were observed. No treatment-related histopathologic changes were evident. Subsequently, 3-day-old neonatal rats received DEHP iv at approximately 62 mg/kg for 18 days with no apparent toxic effects. Results of both studies indicate that no toxic manifestations resulted following acute and subchronic intravenous administration of DEHP to the neonatal rat.
Toxic Responses in F344 Rats and B6CF1 Mice Given Roxarsone in Their Diets For Up To 13-Weeks. K.M. Abdo, C.A. Montgomery1, R.B. Thompson2, J.B. Prejean2, 1NIH-S-NTP, Research Triangle Park, N.C. 27709, 2Southern Research Institute, Birmingham, AL 35223.

Subchronic (13-week) toxicity studies with roxarsone (an organic arsenical) at 0, 50, 100, 200, 400, or 800 ppm in the diet were conducted in groups (10 animals each) of F344 rats and B6CF1 mice of each sex. Arsenic levels in blood and urine of rats and kidneys and liver of rats and mice were measured in extra animals (30/sex/species) at 0, 100, and 400 ppm. Compounds-related mortalities occurred in both sexes of rats at 800 ppm and mice at 800 and 400 ppm. Significant body weight gain depression occurred in both sexes of rats at 200, 400, and 800 ppm and mice at 800 ppm. Clinical signs of toxicity (trembling, ataxia, and pale skin) were seen primarily in rats and mice at 800 ppm. Lesions associated with roxarsone administration were noted in only the kidneys of rats and were characterized by tubular necrosis and mineralization at the cortico-medullary junction. Arsenic levels in urine, blood, liver, and kidneys increased with time on study and were directly proportional to the level of roxarsone in feed. These levels were 5-6 times higher in rats than mice and were about two times higher in males vs females, in blood vs urine, and in kidneys vs livers. The no-observable-effect level for roxarsone was estimated at 100 ppm for rats and 200 ppm for mice. No histology or clinical chemistry effects were found in rats or mice of either sex.


The chemical properties of polycetylenes makes these substances particularly attractive to the chemical industry. Our studies were conducted to assess the mammalian toxicity of a series of diacetylenes varying in both chain length and terminal functional groups. The compounds evaluated, in order of ascending mol. wt. were: 2,4-hexadiyn-1,6-diol; 5,7-dodecadiyn-1,12-diol; 5,7-dodecadyn-1,12-bisethylurethane; 2,4-hexadiyn-1,6-bis-p-toluenesulfonate; and 5,7-dodecadyn-1,12-bis-(butylcarbomethylene)urethane. Only the six carbon diol was appreciably soluble in aqueous solution. The five diacetylenes were evaluated for acute systemic oral (rat) and dermal (rabbit) toxicity, ocular and dermal irritation (rabbit) and dermal sensitization (guinea pig) using standard methodologies. Systemic oral toxicity was greatest with the two diols; the five carbon diol being the most toxic. Oral toxicity of the other three diacetylenes was minimal. No appreciable dermal toxicity was associated with any of the compounds tested. The two diols and the sulfonate were ocular irritants while only the six carbon diol was a dermal irritant. Both of the diols and the sulfonate were dermal sensitizers. Our results suggest that molecular weight was the most important determinant of both systemic oral toxicity and dermal irritation while terminal functional groups were an additional factor in determining ocular irritation and dermal sensitization potential.


Fyrol 6 [Diethyl N,N-bis[2-hydroxyethyl] aminomethyl phosphonate], a fire retardant used primarily in rigid urethane foam, was administered in corn oil to rats (22/group) by gavage at levels of 20, 100 or 500 mg/kg/day for 3 months. Controls received the corn oil vehicle. Mean body weights and food consumption of all test groups were comparable to control values throughout the study. No treatment related clinical signs were observed. Relative (organ/body) liver and kidney weights were increased in high dose males and in mid and high dose females. No biologically significant changes were found with respect to clinical signs, urinalysis, hematology or clinical chemistry. Histopathologic examination revealed very slight hepatocellular hypertrophy in mid and high dose males and females. Increased eosinophilia of centrolobular hepatocytes was observed in mid and high dose males. No evidence of toxicity was seen in males or females given Fyrol 6 at the 20 mg/kg/day level.
A significant enhancement in the biliary excretion of lanthanum sulfate (LASS) and phenol-3,6-dihromophthalein glucuronide (DBP) was observed in rats maintained on diets containing 0.5% BHT for periods of 10 days. This effect was not due to the increased metabolism of these drugs by DBP and ouabain are not metabolized in rats prior to biliary excretion and in case of PABA biliary excretion of unconjugated free from rather than conjugated form was increased by BHT. The enhanced biliary excretion of these drugs in BHT treated rats appears to correlate with the increase in bile flow produced by BHT. A marked increase in biliary salt concentration suggested that the bile salt independent flow is stimulated. The increased bile flow was due to an increase in canaliculare bile production rather than a change in ductular secretion or reabsorption of fluid since bile to plasma concentration ratios of erythritol were unchanged and no permeability change in the biliary tree was observed when mannitol was administered by rectangular intrabiliary injection. It appears that the increase in bile flow produced by BHT is due to osmotic cholestasis related to the secretion of B HT metabolites rather than true bile acid independent cholestasis.

Chlorine dioxide (ClO2) and monochloramine (NH2Cl) represent alternatives to chlorine for disinfecting drinking water. Previously we demonstrated that subchronic exposure to ClO2 (9 mg/kg/day in drinking water) depressed serum thyroxine (T4) in monkeys, whereas Cl2 and NH2Cl were without effect on thyroid function at comparable doses. Subsequently we have shown that a single oral dose of ClO2 (25 ppm) in rats induced covalent binding of dietary iodide (I") to the G.I. contents and to mucusal surfaces, a finding consistent with the strong oxidizing properties of ClO2. Since Cl2 and NH2Cl are also oxidizing substrates, one may anticipate that their effects on I" absorption would be similar to that of ClO2. In this study we examined the gastrointestinal fate of Cl2 and NH2Cl solution at molar and at normal concentrations equivalent to 25 mg/l of ClO2. Our data indicate that neither Cl2 or NH2Cl induce significant covalent binding of radiiodide comparable to ClO2. This may infer that the gastrointestinal interaction between trace elements (such as I") and ClO2 may have mechanistic features unique to this disinfectant. (This abstract does not necessarily reflect EPA policy.)

Chlorine dioxide (ClO2) in drinking water has been shown by this laboratory to depress thyroxine (T4) levels in monkeys. One proposed mechanism is that ClO2 oxidizes dietary iodide (I") to a reactive species which binds to macromolecules in the G.I. tract. This could inhibit absorption of I", leading to iodine deficiency, and a decrease in T4 synthesis. This study examined the binding of iodide to laboratory animal chow and the G.I. mucosa. In vitro, ClO2 greatly enhanced the binding of I" to laboratory animal chow in a dose-dependent manner. Furthermore, binding of I" to the gastric lining in an isolated rat stomach increased severalfold in the presence of 15 ppm ClO2. When ClO2 and I" were concurrently administered to rats orally, I" retention by the G.I. tract increased. The portion of the I" retained by the G.I. tract would not be available for T4 synthesis. However, ClO2 in single dose produced no significant changes in blood I" levels or in the amount taken up by the thyroid gland during the first 24 hours. Therefore, it appears that a single dose of ClO2 would not affect T4 synthesis. Adverse effects of chronic exposure on the thyroid gland cannot be excluded. (This abstract does not necessarily reflect EPA policy.)

Production of free radicals and lipid peroxidation have been associated with gastrointestinal (GI) injury caused by ulcerogenic compounds. To examine the possibility that antioxidants may protect against this type of GI damage, the influence of the antioxidants alpha-tocopherol, propyl gallate, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), ascorbic acid, and glutathione (GSH) on GI bleeding induced by the nonsteroidal antiinflammatory drug indomethacin was investigated in 5-10 week old male rats. A single dose of indomethacin (30 mg/kg) was administered orally to rats treated 24 and 2 hr previously with oral doses of an antioxidant (2.4 mmole/kg). GI bleeding was determined by measuring the amount of hemoglobin present in feces collected for 3 days after the indomethacin dose. Ascorbic acid, GSH, and BHA had no effect on the amount of blood present in the feces. Alpha-tocopherol caused a significant reduction in fecal blood during the first 24 hr, whereas BHT, which had no effect on fecal blood levels at 24 hr, reduced significantly the amounts of fecal blood at 48 and 72 hr. Propyl gallate increased the amount of blood present in feces at 24 hr. BHT was the only antioxidant which prevented the reduction in body weight that occurred over the 72-hr period following the indomethacin dose.

Influence of Antioxidants on Indomethacin-Induced Gastrointestinal Bleeding in Rats. D. J. Murphy, E. B. Taylor, and J. J. Northing, Dept. of Toxicology, Squibb Institute for Medical Research, New Brunswick, NJ. Sponsor: J. S. Kulesza.

α-Dichlorobenzene (DCB) is a relatively unknown liver and kidney toxin in animals. Our objective was to assess acute toxicity of the compound using clinical chemistry and to correlate these indices with the generation of urinary DCB metabolites, namely the 2,3- and 3,4-dichlorophenols and thio-
ethers in rats pretreated with phenobarbital (80 mg/kg/day 1.p., 3 days) or SKF 525-A (50 mg/kg 1.p., 1 hr prior to treatment). DCB solubilized in vegetable oil was injected i.p. at 1, 2 or 4 mmol/kg and the rats were housed in metabolism cages for 24 hr. Plasma creatinine increased and urinary creatinine decreased jointly in the pheno-
obarbital-treated rats only, suggesting impairment of renal function. Plasma alanine and aspar-
tate aminotransferase (ALT, AST) activities in-
creased significantly above that observed in control animals with both phenobarbital and SKF 525-A pretreatments. At 1 and 2 mmol DCB/kg, 20-fold and 1.5-fold increases in ALT were obtained with these treatments, respectively. Thioether and dichlorophenol production was not influenced by either pretreatment. Since generation of the 2,3- and 3,4-dichlorophenols of the hypothetical inter-
mediate epoxide was not enhanced in phenobar-
bital pretreated rats, we suggest that the greatly increased hepatic toxicity of DCB upon induc-
tion of the hepatic microsomal mixed function oxid-
dase system must parallel an alternate metabolic pathway more closely. (Supported by IRSSS, Québec)

ACUTE TOXICITY OF ETHYLENE GLYCOL, MONOALKYL ETHERS ON THE HEMOPOEITIC SYSTEM OF RATS. D. Grant, S. Salih, W.H. Butler, British Industrial Biological Research Association, Woodhouse Road, Surrey, SM5 9DS, U.K.

Glycol ethers are widely used as solvents in industry. They have a variety of toxic effects on experimental animals, with the haemopoietic system being particularly sensitive. The sites of action and severity of toxicity depend upon the type of glycol ether used. Accordingly we conducted a comparative study on ethylene glycol monomethyl (EGM) and monobutyl (EGB) ethers to examine acute toxicity and recovery. Young (60-70g) male F344 rats were given daily oral doses of EGM (100 or 500 mg/kg) or EGB (500 or 1000 mg/kg) for 4 consecutive days. Six animals from each group were killed on days 1, 4, 8 and 22 after the final treatment. There was a profound drop in the RBC count, PTC, Hb and WBC, and an increase in MCV, % reticulocytes and MCH after treatment with EGB. In contrast EGM had no discernable effect on RBC parameters immediately after treatment, although the WBC count was depressed. There was a slight but significant drop in RBC count at days 4 and 8 with EGM. By day 22, RBC parameters were normal with both glycol ethers, whereas the WBC count remained slightly depressed. There were profound differences between EGM and EGB in the severity of toxicity to the bone marrow and kinetics of extramedullary haemopoiesis in the spleen. Both compounds caused lymphocyte depletion in the thymus. These changes were all reversible. This forms part of a programme to assess acute/chronic toxicity relationships in the haemopoietic system. (Supported by Food and Drink Industries Council U.K.)

PRENATAL AND POSTNATAL EXPOSURE TO TRICHLOROETHY-

Rat pups were exposed to trichloroethylene (TCE) via their dams' drinking water at concentrations of 625 mg/l, 1250 mg/l, and 2500 mg/l throughout gestation until weaning at 21 days post parturition. Activity of the rats was measured from 50 to 60 days of age using a residential cage apparatus. The animals were maintained on a 12:12 light:dark photo period. TCE exposed rats exhibited hyperactivity as measured by increased use of running wheels during the dark portion of the photo period and decreased time spent in nest-
boxes during the light portion. These differ-
ences were observed at all doses and were dose
related. These data support the hypothesis that pre- and postnatal exposure of rats to TCE can affect the behavior of adult rats. (This abstract does not necessarily reflect EPA policy).

TOXICITY OF BENZENE METABOLITES TOWARD MOUSE BONE MARROW STROMAL CELLS IN A CO-CULTURE SYSTEM. R.W. Gado and D. Wierda. Dept. of Pharmacology and Toxicology, West Virginia University Medical Center, Morgantown, WV 26506.

Bone marrow myelotoxicity that results from benzene exposure may be caused by damage to the hemopoietic microenvironment. In the present study, we examined the effect of benzene metabolites on the ability of stromal cells, which comprise the hemopoietic microenvironment, to influence the growth of granulocyte/monocyte colony forming units (G/M-CFU-G) in an in vitro co-culture system. Adherent stromal cells (4 X 10^6 cells/ml) from BALB/c mice were plated in 2 ml of RPMI media in 35 mm tissue culture dishes. The stromal cells were cultured for 14 days to allow for adherent colony formation and then exposed to doses of hydroquinone, a benzene metabolite. After 3 days, the media and metabolite were removed and an agar:RPMI layer containing 10^6 fresh bone marrow cells was plated over the stromal cell layer. Seven days later, the cultures were scored for G/M colony formation. Hydroquinone exerted a dose related toxic effect on adherent cell number and G/M colony formation. A 50% decrease in G/M colony formation occurred with a dose of 4.5 X 10^-9M. These results demonstrate that a benzene metabolite can affect the hemopoietic microenvi-
ronment, ultimately altering normal hemopoiesis. (Supported in part by a grant from Travenol Labs, Inc.)
2-Ethoxyethanol (ethylene glycol monochloroether, EGE), a widely used water-miscible solvent, is volatile and easily absorbed through the skin. Recent evidence indicates that EGE is both embryotoxic and teratogenic in the rat. The present study was designed to determine appropriate doses for a subchronic dermal toxicity study in rabbits. The systemic toxicity and dermal irritancy of EGE were evaluated following acute topical application to the shaved backs of female New Zealand white rabbits; dermal irritancy was scored by the Draize method over a 14-day post-treatment observation period. EGE was applied under occlusive dressings at 5 dose levels ranging from 0.9 to 6.0 g EGE/kg bw. Blood samples, obtained from all animals at hourly intervals from 1 to 6 hours during treatment, were analyzed for EGE. The mean peak concentrations of EGE in blood were dependent upon both dose and sampling interval after administration; higher doses were associated with higher and more delayed maximum blood levels. The estimated LD₅₀ was 3.9 g/kg, which corresponded to a maximum blood concentration of approximately 900 µg/mL 4 hours after beginning dose application. EGE produced only minimal irritation in most animals. Based on these findings, the dose range from 0.5 to 2.0 g/kg/day was chosen for further study in a modified subchronic dermal study.

A rapid and noninvasive method for determining CO₂ production in mice is described. Metabolic and pharmacological challenges were performed to demonstrate the ability of the method to detect changes in CO₂ production and spontaneous locomotor activity under controlled conditions. CO₂ production and locomotor activity were then measured in mice having inhaled toluene or n-hexane at 0, 100, 1000, or 3000 ppm, 5 hr/day for 90 days. Mice tested after 3000 ppm toluene showed decreased CO₂ production in the first week of toluene, with a return to normal during week 2. Hexane produced no significant changes in CO₂ production in this protocol. Followup studies at 3000 ppm toluene and n-hexane confirmed the reduction in metabolic rate following inhalation of 3000 ppm toluene. Exposure to 3000 ppm n-hexane caused a transient elevation in CO₂ production. The effect of toluene persisted for two weeks of daily exposure, while those of n-hexane were absent by the third day of exposure, indicating that tolerance to these solvents developed at different rates. These metabolic effects demonstrate that the present method can detect changes in metabolic rate, without change in locomotor activity, resulting from inhalation of these solvents, and suggest that different mechanisms of tolerance may be involved. (Supported by Grant No. OH-00093 from NIOSH)

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Methyl chloride (MeCl) is a solvent widely used in the chemical industry, which has been found to induce neurotoxicity, teratogenicity and renal cancer in mice. Female C57Bl/6 and male C3H mice were exposed for 6 hr/day, 5 days/wk for 2 wks, to 1,500 ppm MeCl. Focal and diffuse maldes, involving the inner granular layer of the cerebel- lum, was more severe in the C57Bl/6 strain while renal tubular necrosis and basophilic tubules in the renal cortex were prominent in the C3H strain. Autoradiographic studies using pulse labelling with [³H]-thymidine demonstrated a high rate of cell turnover in the epithelial cells of basophilic foci in the renal cortex of MeCl exposed mice. The brain lesions were mostly found in the ventrolateral cerebellum. The earliest ultrastructural and electron microscopic changes in the cerebellum were observed in the nuclei of granule cells, with progression from slight confluence of heterochromatin to complete nuclear condensation or karyorrhexis. It was concluded that the cerebellar granular cell and the epithelium of the renal proximal tubules represent important target sites for acute MeCl toxicity in mice. The presence of a proliferative response in the form of basophilic foci in the kidney requires further investigation in relation to the renal carcinogenicity of MeCl.
721 PERFORMANCE ON A ROTA-ROD AS A PREDICTOR OF CNS DEPRESSION: EVALUATION WITH ETHANOL. P.E. Kish, I. Rosenblum, and R. Abraham. Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, NY.

There is a remarkable and well known correlation of the oil/water partition coefficient with concentration of volatile solvents required to produce anesthesia. The predictive value of the oil/water partition coefficients for CNS depression with solvents at non-anesthetic concentrations is not known. Ethanol's effect on motor performance was measured with an accelerating rotarod. Male Sprague-Dawley albino rats weighing 200-225 g were pre-trained to remain on a 3 in diameter rod which was accelerated 3 to 40 rpm in three min. The trained animals were divided into 3 experimental groups and injected with 0.75, 1.25, or 1.75 g/kg of ethanol (15% in saline). After injection, 3 animals from each group were tested for decrement of motor performance at 15 min intervals. Following testing on the accelerating rotarod, rectal temperatures were recorded to determine the degree of hypothermia caused by ethanol. Blood and brain samples were obtained after sacrificing, and concentrations of ethanol determined. Linear regression modeling of performance vs. time and blood or brain concentration vs. time showed positive correlations. Correlation of performance vs. tissue concentration may show potential to predict CNS depression for other solvents based solely upon a physical constant. Supported by NIEHS Grant No. ST32ES07058.

722 A 90-DAY INHALATION STUDY OF NITROBENZENE IN F-344 RATS, CD RATS AND B6C3F1 MICE. T.E. Hamm, Jr., M. Phelps, T.H. Raynor and R.D. Icons. Chemical Industry Institute of Toxicology, Research Triangle Park, NC

Nitrobenzene (NB) is an important commodity chemical whose TLV is currently 4 ppm. 10 Male and 10 female F-344 rats, CD rats, and B6C3F1 mice were exposed to 0.5, 1.6, or 5 ppm NB, 6 hr/day, 5 d/wk, for 90 days. There was no effect on body weight gain or mortality. Mean methemoglobin concentrations were: F-344: 1.4 ± 0.6%; 3.1 ± 1.0, 4.2 ± 1.3, and 10.3 ± 1.4; CD: 1.4 ± 0.7, 1.6 ± 0.6; 3.5 ± 0.5, and 9.9 ± 2.3; and B6C3F1: 1.0 ± 0.8, 1.2 ± 0.5, 2.1 ± 1.0, and 5.5 ± 1.3. For the control, 5 ppm, 16 ppm and 50 ppm groups respectively. Changes secondary to methemoglobinemia were seen in the spleens of all treated groups. Interestingly, F-344 rats also had splenic capsular lesions similar to those described in F-344 rats exposed to aniline while CD rats had only minimal splenic capsular lesions, and B6C3F1 mice had none. Both the F-344 and CD 50 ppm group rats had bilateral degeneration of seminiferous epithelium and a reduction or absence of sperm in the epididymis. Mice had no testicular lesions. A variety of other lesions were found in the liver, adrenal glands and kidneys of exposed animals. The rat strain difference in splenic capsular lesion development and the testicular lesions seen in both rat strains require further study.

723 EFFECTS OF XYLENE ISOMERS ON OPERANT RESPONDING IN MICE. V.C. Moser, E.M. Coggeshall, and R.L. Balster. Department of Pharmacology, Medical College of Virginia, Richmond, VA. Sponsor: L.W. Reiter.

Xylene, a widely used industrial solvent, is a mixture of the ortho-, meta-, and para- isomers. In this study we examined the effects of each individual isomer, as well as a commercial-grade mixture of xylenes, on the performance of 15 mice trained to lever-press on a DRL (differential-reinforcement-of-low-rates) 10-sec schedule. The 15-min operant sessions immediately followed the 30-min exposures to solvent vapors (5000-7000 ppm), or air, in static inhalation chambers. All mice were exposed to each isomer in a counterbalanced order, and the mixture was given last in all cases. Ortho-, meta-, para-, and mixed xylenes produced similar biphasic effects on response rates, and concentration-dependent decreases in reinforcement rates. The lowest significantly effective concentration for each isomer on any variable was 1400 ppm. Half-maximal response rate decreases were produced by 5200-6200 ppm of each isomer. Only slight recovery from the solvent effects was observed during test sessions. The temporal distribution of responses during the 10-sec intervals was not disrupted, even at high concentrations. Xylene, therefore, produced pronounced behavioral actions following acute exposure, and no differences were obtained in the overall effects of the individual isomers or the commercial mixture.

724 NEUROTOXICITY OF METHYL CHLORIDE (MeCl) IN CONTINUOUSLY VERSUS INTERMITTENTLY EXPOSED FEMALE C57BL/6 MICE. Landry, T.D., Quast, J.F., Goshow, T.S. andMattsson, J.L. Toxicrology Research Lab., Dow Chemical USA, Midland, MI 48640. Sponsor: M.J. McKenna

This study evaluated the relationship between MeCl exposure duration and neurotoxicity in a very sensitive animal. Female C57BL/6 mice were exposed to MeCl for 11 days, either continuously (22 hr/day) or intermittently (5.5 hr/day). Cerebellar granule cell layer degeneration was observed in mice exposed continuously to 100 ppm MeCl and in mice exposed intermittently to 400 ppm. This histopathologic effect was observed at lower concentrations than a decrement in rotarod running performance. No effects were observed in mice exposed to 50 ppm continuously, or to 150 ppm intermittently. The no observable effect levels for continuous and intermittent MeCl exposures were nearly proportionate to exposure concentration multiplied by duration, although the dose response curve was much steeper for continuously exposed mice. Continuous exposure to MeCl produced the cerebellar lesion with little effect on other tissues than did intermittent exposure. Mice exposed to 2400 ppm intermittently also had renal and hematopoietic effects. Since the effect of exposure duration on MeCl toxicity is complex, careful judgment is necessary when extrapolating intermittent exposure data to a continuous exposure situation.
725 EFFECT OF CIMETIDINE ON THE ACUTE HEPATOTOXICITY OF STYRENE IN RATS. S. A. Kerim, S. Chakrabarti b and G. Sirois a. Fac. pharmacie a et Dép. méd. trav. hyg. mil. b, Univ. Montréal, Montréal, Québec, Canada.

Hepatotoxicity of styrene (S) is due to its metabolic activation to intermediate styrene oxide(s) mediated by a liver microsomal monooxygenase containing cytochrome P-450. Cimetidine (C) inhibits hepatic drug metabolism. It is expected to reduce the toxicity of S by inhibiting the formation of styrene oxide. So, we studied the effect of co-administration of C on S-induced acute hepatotoxicity in adult male Sprague-Dawley rats. Groups of pre-fasted (16 h) rats were treated i.p. with 0, 80, 160 and 240 mg/kg C simultaneously with either 0, or 600 or 900 or 1200 mg/kg S in corn oil. After collecting urine for 24 h, the rats were sacrificed. The hepatotoxicity of S (measured by serum transaminase activities at 24 h) at 900 and 1200 mg/kg was significantly potentiated by co-administration of either 160 or 240 mg/kg C but not by 80 mg/kg C. Thus, increased activity of SGOT at 900 mg/kg S was further significantly enhanced by 52 or 60% and that of SGPT, by 56 or 60% due to 160 or 240 mg/kg C. C alone had no effect on such activities. No hepatotoxic interaction was noticed between 600 mg/kg S and C at any dose level. So failure of C to reduce the hepatotoxicity of S strongly suggests that the mechanism of activation/deactivation process involving S is complex and that alternative pathways not dependent on cytochrome P-450 alone might also be involved. (Supported by CAFIR, Université de Montréal).

726 INFLUENCE OF STYRENE AND TRICHLOROETHYLENE ON THE ACUTE HEPATOTOXICITY OF ACETAMINOPHEN IN RATS. P. Colin a, J. Henri a, S. Chakrabarti b and G. Sirois a Fac. pharmacie a et Dép. méd. trav. et hyg. mil. b, Univ. Montréal, Montréal, Québec, Canada.

Acetaminophen (AAP), when taken in large doses, can cause hepatorenal damage. Such necrosis is thought to involve metabolic activation of AAP to its toxic reactive intermediate followed by its covalent binding to cellular macromolecules subsequent to depletion of hepatic glutathione. Exposure to industrial solvents such as styrene (S) and trichloroethylene (TCE) is known to produce hepatorenal dysfunction due to a mechanism similar to that of AAP. So, to determine whether hepatotoxic response to AAP could be modified due to simultaneous exposure to S or TCE, we have studied the effect of an acute threshold toxic dose of S or TCE on the dose-response curve of AAP in adult male Sprague-Dawley rats. Groups of control and S (800 mg/kg, i.p. in corn oil)-treated rats were simultaneously given i.p. 0, 600, 800 and 1000 mg/kg AAP in 1% carboxymethyl cellulose suspension. After collecting the urine for 24 h, the animals were sacrificed. Hepatotoxic response (as measured by the activities of serum transaminase, SGOT and SGPT) induced by AAP at different doses was further increased significantly in an additive manner in rats treated with S. Similar additive effect on such toxicity due to AAP was again noticed when 0.5 ml/kg of TCE was co-administered i.p. These initial studies suggest the ability of S or TCE to potentiate the hepatotoxicity of AAP. (Supported by CAFIR, Univ. Montréal).


Groups of adult male Sprague-Dawley rats, pretreated with phenobarbital (50 mg/kg, i.p., 7 days) were given i.p. injection of 0, 0.25, 0.50, 0.75, 1.0, 1.50 and 2.0 ml/kg TCE in corn oil 16 h after fasting. After collection of urines for 24 h following each dose, the animals were sacrificed. The hepatotoxic response, as measured by the Activities of serum transaminase, was significantly enhanced with increasing dose of TCE. Decrease of liver microsomal enzyme activities including cytochrome P-450 was observed and reached an apparent saturation at 1 ml/kg TCE. Urinary excretion of both total chloro-compounds as well as of trichloroethanol (TCE) was increased non-proportionally with increasing dose of TCE. However, the percent of dose excreted as TCE and trichloroacetic acid was reduced with increasing dose of TCE. Urinary thiocarbamate was always decreased from 47.9 μmol/kg (control) to 10 μmol/kg in 2 ml/kg TCE-treated rats, suggesting a non significant role of GST/GSH-transferases detoxification pathway in the metabolism and acute toxicity of TCE. Treatment of rats (one dose of 0.5 ml/kg per day or 2 doses of 0.25 ml/kg at interval of 4 h per day for 3 weeks) showed no changes in the transaminase activities, whereas similar acute doses produced a significant increase in such activities. This suggests a development of an apparent tolerance to TCE toxicity due to such subchronic treatment with TCE. (Supported by IRSST, Québec).

728 TOXIC INTERACTIONS BETWEEN LEAD AND TOLUENE IN FISHER 344 RATS. B. Tuchweber a, b, S. Chakrabarti, R. Goyal b and F. Gratton a. Dép. nutrition a et Dép. méd. trav. et hyg. mil. b, Fac. méd., Univ. Montréal, Montréal, Québec, Canada.

Hepatorenal damage following exposure to lead acetate (LA) or toluene (T) is well studied, but little is known about their possible toxic interactions. Combined exposure to lead and T occurs during industrial spray painting, newspaper printing and operation of leaded petrochemicals. So we have examined the effects of lead acetate (2.5 g/kg) or T (30 or 40 mmole/kg) given orally alone or combined T and LA (T 2 h before LA) on functional and structural alterations in the liver and kidneys of Fisher 344 rats (100-130 g). Animals were sacrificed 24 or 48 h after collection of urines. Increase in SGPT activity, though not significant, and increase in relative kidney weight were observed due to combined treatment. Urinary phosphate was increased due to combined exposure of LA and T. Urinary proteins, glucose and serum creatinine were increased, but not significantly, and lead in blood and kidney was decreased due to combined treatment. Light microscopy showed moderate to severe accumulation of lipids in the hepatocytic cytoplasm due to combined exposure to T and LA. Such accumulation was localized into the centrilobular areas of the liver parenchyma. Renal lesions, due to such combined exposure, consisted of cytoplasmic vacuolization in epithelium of proximal and distal tubules of the renal cortex. (Supported by IRSST, Québec and CAFIR, Université de Montréal).

Five chlorinated hydrocarbon solvents--1,1,2 and 1,2-dichloroethane (DCE), 1,1,1 and 1,1,2-trichloroethene (TCE), and trichloroethylene (TCE)--were tested for carcinogenic promotion and initiation activity in a rat liver focus assay using glutamyl transpeptidase (GGT) as a putative preneoplastic marker. In the promotion protocol, rats were given partial hepatectomies and, 24 hr later, a single ip dose of diethyl nitrosamine (DEN) (30mg/kg body wt). The rats were gavaged 5 times weekly with the test solvents in corn oil at the MTD (or 3/4 MTD for 1,1,1-TCE) for 7 weeks and sacrificed one week later. In the initiation protocol, a single oral dose (MTD) of the chlorinated hydrocarbon was substituted for DEN, and phenobarbital (0.05% in the diet) was used as the promoter. When tested for promotion, 1,1,2-TCE (with or without DEN initiation) and 1,1-DCE (with DEN initiation) produced an average increase in GGT+ foci of 2-fold. In the initiation protocol with phenobarbital promotion, 1,2-DCE produced a significant increase in the average number of GGT+ foci per tissue; slight increases in the number of GGT+ foci were obtained with 1,1,1-TCE as a promoter. TCE was without effect in these assays. (This work was supported by EPA Contract 68-02-3703.)

ACETONE POTENTIATION OF CHRONIC LIVER INJURY INDUCED BY REPETITIVE ADMINISTRATION OF CCI4. M. Chabonneau, B. Tuchweber and C.L. Pian, Université de Montréal, Montréal, Québec.

The ability of ketonic compounds to modify the chronic liver injury (cirrhosis) induced by halogenated ketones is unknown. To investigate this problem, male Sprague-Dawley rats were treated twice weekly (3:00 pm, Tues. & Thurs.) for 12 weeks with acetone (A, 2mM/kg in corn oil (CO) or CO alone (10ml/kg). 18 hr after each pretreatment, the rats were treated with CCI4 (500mg/kg in CO, po); controls received CO (10ml/kg). The rats were killed after 4, 8, 10 or 12 weeks of treatment. Liver-kidney/body weight ratios (LR, KR) were computed; biochemical analyses were performed on plasma (ALT, bilirubin, BUN) and liver (collagen content) samples. Body weight gain was slower in A-pre-treated rats given CCl4; 35% die. Compared to CO-pre-treated rats, A+CCI4-treated animals showed significantly lower LR and higher KR values at all four times. Significantly higher bilirubin levels and collagen contents occurred at 8, 10, 12 and 4, 8, 10 weeks, respectively. No significant differences were observed in ALT and BUN values between these two groups. After 10 weeks, light microscopy revealed a slight to moderate cirrhosis in CO+CCI4-treated rats, whereas much more severe lesions was observed in A+CCI4-treated rats. We conclude that acetone accelerates the appearance of the cirrhosis induced by CCl4, without modifying the pattern of the lesion. (Supported by IRSS Québec and Health/Welfare Canada.)

SUBCHRONIC TOXICITY OF CARBON TETRACHLORIDE (CT) ADMINISTERED BY ORAL GAVAGE TO CD-1 MICE. J.R. Hayes, L.W. Condie*, and J.F. Borzelleca, Dept. of Pharmacology and Toxicology, Medical College of VA, Richmond, VA 23298; *U.S.E.P.A., 26 W. St. Clair St., Cincinnati, OH 45268.

Although CT has been extensively utilized as a model hepatotoxin, its subchronic toxicity has not been adequately evaluated in mice. To alleviate this, male and female mice were divided into five treatment groups containing 20 mice of each sex. CT was orally administered for 90 days in corn oil at doses of 0, 12, 120, 540 and 1200 mg/kg/day. Final body weight was unaltered while liver and spleen weight increased at the third highest doses in females and all doses in males. Kidney weight increased at the high dose in females, but remained unchanged in males. The remaining organ weights were not altered. Hemoglobin decreased at the highest doses in females and at the high dose in males, with hematocrits being decreased in the high dose females. Serum LDH, SGPT, SGOT, ALP and cholesterol were increased at the two highest doses in females and at the highest dose in males. Serum creatinine levels were increased at the two highest doses in females only and BUN was not changed in either sex. This study indicates that CT at doses as low as 12-120 mg/kg/day produces alterations of possible toxicologic significance in mice. (Supported by E.P.A. Contract No. 808861010. This report does not necessarily reflect E.P.A. views)


We have investigated the subchronic toxicity of two materials produced by the EDS direct coal liquefaction process using adult New Zealand white rabbits as the test species. Recycle Solvent (RS; 204-427°C) and fuel oil (PO; 204-539°C) were applied to the intact dorsal surface of 5 (20) or 10 (RS) rabbits/sec/dose group, 5 days per week for 4 weeks. Materials were applied as 2.5 and 10% (w/v) suspensions in mineral oil; mineral oil alone was administered to concurrent control groups. Both materials elicited gross signs of toxicity including severe dermal irritation, loss of body weight (16-25%) and mortality (4/20 in the high dose group treated with RS). Systemic effects included liver enlargement as evidenced by histologic findings of diffuse hepatocytomegaly, cytoplasmic degeneration and hepatocellular vacuolation as well as elevated serum cholesterol. There was also evidence of testicular, seminal vesicle and thymic atrophy; more pronounced effects were apparent in the high dose groups. The liver enlargement was probably a physiologic response to elevated systemic levels of aromatic hydrocarbons. Thymic and testicular atrophy may have been a secondary response to dermal irritation, stress or body weight loss.

Sulfolane produces convulsions and increases sensitivity to pentylentetrazol (PTZ) seizures. Because sulfolane also produces hypothermia, a condition that alters seizure susceptibility, three types of seizure activity were tested under isothermic conditions. Audogenic (1 min ultrasound stimulus) and PTZ (100 mg/kg ip) seizures were assessed in rats (N=160) exposed to 0, 200, 400 or 800 mg/kg sulfolane. Seizure latency, duration and severity were scored. Primary afterdischarge (PAD) thresholds were determined in 30 rats implanted with chronic bipolar electrodes in the dorsal hippocampus who were treated with 0 or 800 mg/kg. All animals were dosed by ip injections one hour before testing and were maintained in warm (30°C) environments. Audogenic seizures were observed in 45% and 5% of the 400 and 800 mg/kg groups, respectively, with no seizures evidenced by the 2 low dose groups. PTZ seizure duration, severity and incidence of death were increased significantly at 400 and 800 mg/kg, while control and 200 mg/kg values did not differ. Although PAD thresholds were unaffected, behavioral seizures were evoked in 20% of treated rats during testing, an event not seen in controls. These results indicate that sulfolane increases general seizure susceptibility in the absence of hypothermia. The unusual incidence of audogenic seizures in these animals suggests brainstem involvement.

735 A COMPARATIVE 90-DAY DERMAL EXPOSURE STUDY OF DIETHYLENE GLYCOL MONOMETHYL ETHER (DEGME) AND ETHYLENE GLYCOL MONOMETHYL ETHER (EGME) IN THE GUINEA PIG. D. W. Hobson, A. P. D’Addario and D. E. Uddin; Naval Medical Research Institute/Toxicology Detachment, Wright-Patterson AFB, Dayton, OH 45433 Sponsor: R. W. Gardier.

The addition of EGME or DEGME to Navy jet fuel inhibits formation of ice crystals which can clog fuel lines during flight operations. This experiment was performed to determine whether DEGME produced similar toxicity to EGME following dermal exposure. Male guinea pigs were dermally exposed to 1.00, 0.20, 0.04 or 0 (control) ml/kg/day DEGME for 13 weeks, 5 days/week, 6 hrs/day. EGME animals were similarly exposed to 1.00 ml/kg/day. Parameters monitored included clinical signs, body weight, clinical chemistries, hematological and pathological changes. No mortalities were observed during the experiment. A greater than 6% decrease in growth rate was observed in animals exposed to 1.00 ml/kg/day DEGME and EGME. At necropsy, severe testicular atrophy (27% of control mean) was seen in all EGME treated animals. No evidence of testicular atrophy was observed in other groups. EGME treated animals exhibited a significant increase (2.68 X control) in serum lactate dehydrogenase activity and an apparent lymphocytic leucopenia which was not observed in other treatment groups. In summary, DEGME produced only minimal toxicological changes following dermal exposure; whereas, EGME produced more significant changes.


The subcutaneous dermal toxicity of DOPMA was evaluated in young adult New Zealand White rabbits (Rb), and its potential to produce delayed contact sensitization (DCC) was evaluated by a modified Buehler’s closed patch technique in Hartley guinea pigs (Gp). In the dermal toxicity study, 4 groups of Rb (5/treatment group) received 18 daily doses (uncooked, over 4 weeks) of DOPMA in acetone at 0 (acetone only), 10, 107, and 1067 (undiluted) mg/kg/day at 1 ml/kg. No DOPMA-related effects on mortality, systemic toxic signs, body weights, hematology, clinical chemistry, urinalysis, or histopathology of tissues (except skin) were observed. Slightly increased feed consumption in females, and slightly increased kidney weights and kidney/body weight ratios in males occurred at the high dose. In the absence of histopathologic changes indicative of DOPMA-related systemic effect, these slight increases were not toxicologically significant. The only DOPMA-related toxic response was slight or moderate skin irritation at the mid and high doses. Its severity was dependent on the number of doses and the concentration of DOPMA. Maximal skin irritation occurred after 1 week. In the DCC study, Gp received 6 hr induction doses (2 doses/wk for 3 wk) of 0.5 ml 100% DOPMA and then were challenged with 0.5 ml of 50% (W/V) DOPMA 2 weeks later. No erythema or edema was observed in any challenged Gp in either the treated and control groups. The no-observed-effect level for systemic toxicity of DOPMA in male Rb was at least 1067 mg/kg/day; no irritation, slight, or moderate irritation occurred in Rb exposed to 10 and 100X DOPMA, respectively. DOPMA was not a skin sensitizer in Gp.


Previous studies have produced histopathological evidence that a renal lesion can be induced in male rats after oral doses of pure branched-chain hydrocarbons and petroleum distillate fractions. It was proposed that branched-chain chemical components were the nephrotoxic species in complex distillates. The purpose of this study was: (I) to monitor biochemical markers of nephrotoxicity in male rats after dosing with 2,4,4-trimethylpentane (TMP) and decane; (II) to determine if TMP could produce a renal lesion in male guinea pigs and mice. Rats were dosed 2 weekly for 4 weeks (0.1, 0.3, 1.0 ml/kg, p.o.); 24-hour urine collections were obtained in high dose and controls in order to evaluate NAG, urea, creatinine, gamma-GTP, alk. phos., GGT and pH. Guinea pigs were treated with TMP at 2 dose levels (0.4, 1.0 ml/kg/week, p.o. 4 weeks). Serum chemistries and hematozoology were analyzed at necropsy (day 30) for rats and guinea pigs. Mice were dosed at 0.3 and 1.0 ml/kg (2 weeks, 2 weeks, 4 weeks). The only consistent biochemical response was an elevation of urinary GGT for TMP-treated vs. control rats. Histopathological results showed the expected dilated tubules at the CM junction and hyaline droplet degeneration for TMP rats. There were no distinguishable pathological differences from controls for guinea pigs, mice and decane-treated rats.
After studies with ten different membrane-active toxins, Schanne et al. (1) suggested that the influx of calcium across liver cell plasma membranes represents, or at least initiates, a final common pathway for the toxic death of cells damaged by a range of different mechanisms. New techniques involving cryoultramicroscopy of rapidly frozen tissue, high resolution scanning transmission electron microscopy, X-ray microanalysis and computer assisted image enhancement were used to study liver cellular component calcium concentrations under in vitro conditions. Results confirm earlier conclusions from work using cell homogenates and fractionation (2) that high levels of external calcium enter the cytoplasm and are sequestered in the mitochondria in response to in vivo administration of carbon tetrachloride. (Schanne, F.A.X., Kan, A.B., Young, E.E. and Barber, J.L. Science 206(4):700-702 (1979). Moore, L., Davenport, G.R., Landon, E.J. J. Bio. Chem. 251(4):1197-1201 (1976). (This abstract does not necessarily reflect EPA policy.))

Carnfentanil, a potent opiate analgesic, produces muscular rigidity and loss of righting reflex (LRR) in various animal species which is reversible by naloxone. However, aside from opiate receptor mediation, neural mechanism(s) responsible for carfentanil induced immobility are unknown. In male mice, the s.c. ED50 for immobilization, assessed by LRR, was 56.5 (40.3-79.4) mg/kg. Following administration of 0.4 mg/kg (s.c.), animals appear excited for 1-2 min followed by LRR of 2-4 min duration and recovery. Pretreatment with chlorpheniramine (15 mg/kg; i.p.) or diphenhydramine (10 mg/kg; i.p.) resulted in 35 and 55%, respectively, of mice exhibiting LRR following carfentanil administration (0.4 mg/kg; s.c.), compared to 90% in untreated controls. The protective effect appeared specific since chlorpheniramine or diphenhydramine pretreatment did not decrease the duration or incidence of pentobarbital induced LRR. The possibility that H3-histaminergic antagonists, at the doses used in this study, protected from carfentanil induced LRR by a non-specific blockade of H1-histaminergic or cholinergic receptors was also examined. However, pretreatment with either cimetidine, atropine or mecamylamine did not decrease the duration or incidence of carfentanil induced LRR. These data suggest involvement of H3-histaminergic receptors in carfentanil induced immobilization.

Male Sprague-Dawley rats (275-300g) were administered TCDD orally (50 mg/kg) or by bilateral microinjection (0.25 µg/site) into the lateral hypothalamus (LH). Vehicle-treated rats were either fed ad libitum or were pair-fed to the TCDD-treated rats. Oral TCDD treatment produced decreases in food intake (FI) and body weight. The magnitude and duration of the weight loss were similar for both oral TCDD-treated and pair-fed control rats. Both groups of rats also exhibited similar decreases in spontaneous motor activity, and total and resting oxygen consumption. LH injection of TCDD produced an immediate-onset, but transient hypophagia. This effect was reduced in marked contrast to the gradual-onset but persistent hypophagia caused by oral dosing. However, we do not think that the LH represents a primary site of action for production of hypophagia induced by oral TCDD for two reasons. First, regardless of the route of administration, the hypothalamic concentration of 9H-TCDD equivalents remained constant throughout the experimental period even though daily FI was changing. Second, regardless of the time postinjection and the level of FI the hypothalamic concentration of radioactivity following LH injection was 1000 times higher than that following oral administration (Supported by NIH Grant ES01332).
A longitudinal metabolic profile following 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) treatment in the Sprague-Dawley rat. B.J. Christian, L.A. Menahan, and R.E. Peterson, Univ. of Wisconsin, Madison, WI.

TCDD, in a lethal dose, results in a wasting syndrome in the rat which is characterized by hypophagia and weight loss. Rats pair-fed to TCDD-treated animals had identical patterns of weight loss and lethality (Pharmacologist 25:169, 1983). To further examine the role of hypophagia in the toxicity of TCDD, indices of carbohydrate, lipid, and protein metabolism were evaluated in mature (-450 g) rats treated with a lethal dose (75 μg/kg) of TCDD. When compared to vehicle-treated rats that were pair-fed to the TCDD-treated animals, the plasma concentration of alanine and triacylglycerols was elevated 2 through 8 days after treatment. Also, pyruvate levels in plasma were increased at 4 and 6 days after treatment with TCDD. Yet, the plasma values for glucose, lactate, total ketone bodies, and free fatty acids were similar in TCDD-treated and pair-fed groups. Likewise, the 24-hr urinary excretion of urea, ammonia, and creatinine was alike in both groups during the 8 days of the profile. Thus, selective indices of intermediary metabolism are altered within several days after TCDD administration, and these changes seem to be independent of the hypophagic component of the toxicity. (Supported by NIH Grant ES01332).

Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on food intake, body weight and lethality in guinea pigs, rats and mice: a pair-feeding study. C.K. Kelling, B.J. Christian and R.E. Peterson, Univ. of Wisconsin, Madison, WI.

Our aim was to assess the role of TCDD-induced hypophagia on weight loss and lethality. Male C57BL/6 mice given TCDD (360 μg/kg) displayed a significant reduction in food intake and body weight. Pair-fed control mice exhibited a similar pattern of weight loss but a lower incidence of lethality. Body composition of pair-fed and TCDD-treated mice at death revealed similar carcass protein, fat and ash content. Carcass water content was greater at death in TCDD-treated mice due to edema. Male guinea pigs given TCDD (2 μg/kg) displayed a significant reduction in food intake and body weight. Pair-fed control guinea pigs exhibited less weight loss than TCDD-treated animals yet the time course and magnitude of lethality in the two groups was similar. Pair-fed control guinea pigs were in a more hydrated state than TCDD-treated guinea pigs at the time of death. Carcass protein, fat and ash contents of the two groups were similar. Male Fischer 344 rats given TCDD (100 μg/kg) did not display a significant reduction in food intake and body weight. Pair-fed control rats exhibited an identical pattern of weight loss but a different time course of lethality. We suggest that hypophagia is primarily responsible for weight loss in rats, mice and guinea pigs following TCDD exposure and that its role in lethality is species dependent (NIH Grant ES01332).

Enhancement of bradykinin and histamine-induced paw edema by 2,3,7,8-tetrachlorodibenzo-p-dioxin. H.M. Theobald, R.C. Bookstaff and R.E. Peterson, University of Wisconsin, Madison, WI.

The edemagenic carrageenan and dextran are more potent producers of edema when injected into the hindpaws of rats treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) than in control rats. Since this response to TCDD suggests that it may affect the actions of certain endogenous mediators of vascular permeability, we characterized it by using drugs to inhibit carrageenan and dextran-induced edema formation, and by quantifying the edema produced after injecting the mediators themselves into the paw. At low and moderate doses of carrageenan and dextran TCDD treatment did not affect the abilities of indomethacin, dexamethasone and an equimolar mixture of ciproheptadine and pyrilamine to inhibit edema formation. Since TCDD treatment also did not affect the time-course of edema formation, it appears that the same mediators are responsible for irritant-induced edema in TCDD treated and control rats. TCDD treatment increased the edemagenic potency of histamine and bradykinin, but not prostaglandin F2α and serotonin. Therefore, the increased potency of carrageenan and dextran is at least partly due to the increased edemagenic activities of bradykinin and histamine. We speculate that the mechanism of edema enhancement may involve TCDD treatment potentiating the ability of bradykinin and histamine to stimulate phospholipase activity in endothelial cells. (Supported by USPHS Grant ES01332.)

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745 EFFECTS OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ON THE PITUITARY-TESTIS AXIS IN RATS. R.H. Moore, C.L. Potter, J.A. Robinson, R.E. Peterson. Univ. of Wisconsin, Madison WI 53706.

We have previously reported that 2,3,7,8-tetrachlorodibenzop-dioxin (TCDD) depressed serum androgens and sex organ weights in male rats (Fed. Proc. 42, 355 (1983)). The dose-related nature of the effects of TCDD on circulating androgens and on luteinizing hormone (LH) have now been determined. Dose-dependent decreases in plasma testosterone and dihydrotestosterone (to as little as 10% and 25%, respectively, of ad lib control values) were seen seven days after rats received a single oral dose of TCDD (0, 6.25, 12.5, 25, 50, or 100 μg/kg). Half-maximal decreases were seen at about 15 μg TCDD/kg. The reductions previously seen in accessory sex organ weights could be attributed to the decreased androgen concentrations: no evidence for disproportionate dehydration was found. Androgen assays in plasma from pair-fed control rats indicated that hypophagia could account for up to a 50% decrease in each androgen but not for the full 10- and 4-fold decreases seen in the hypophagic TCDD-treated rats. LH was assayed in order to determine whether insufficient pituitary secretion of this hormone could account for the low androgen concentrations. TCDD, however, had no effect on immunoreactive plasma-LH concentrations. The mechanisms whereby TCDD depresses plasma-androgen concentrations beyond the decreases caused by hypophagia remain to be determined. (Supported by NIH Grant ES01332.)

746 THE EFFECT OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) ON ADRENA L STEROIDGENESIS, IN VIVO. M.J. DiBartolomeis, C.R. Jefcoate, B.J. Christian, R.W. Moore, and R.E. Peterson. University of Wisconsin, Madison, WI.

Rats receiving a lethal oral dose of TCDD (50 μg/kg, 13 days post-treatment) exhibit a severe wasting syndrome. In order to relate these observations to a possible hormonal imbalance, the effect of TCDD on adrenal steroidogenesis was investigated. Plasma cortisol levels were reduced by 50% in TCDD-treated rats as compared to pair-fed controls, 10 min after corticotropin (ACTH) administration or under non-stressed conditions. However, isolated mitochondria from TCDD-treated rats exhibited a 35% increase in side chain cleavage (SCC) activity. This increased activity was the result of a parallel elevation in free cholesterol levels, thereby increasing availability of cholesterol to cytochrome P-450sc. This step is activated within minutes after ACTH administration. The increased free cholesterol levels affected by TCDD is identical to that resulting from a 10-min exposure to aminoglutethimide, a known inhibitor of SCC activity. TCDD did not affect total mitochondrial P-450 levels. These results suggest that, in response to TCDD, glucocorticoid synthesis in ACTH-stressed rats is suppressed, presumably as the result of an indirect inhibition of the rate-limiting step. (NIH grants AM 18585 and ES 01332.)


Exposure to TCDD elevates serum TG in several species including rats and humans. Adult (12 wk) male, Fischer rats are given 0 or 80 μg TCDD in corn oil/kg body wt. by gavage once a week for one. Serum TG increased, 81.6 ± 10.3 and 311 ± 10.4 mg/dl, control and TCDD-exposed, respectively. Control and TCDD-exposed rats were intubated with 1.5 ml of corn oil that contained [14C]-palmitic acid (0.5 mCi/rat). Mesenteric lymph was collected by cannulation and chylomicra (CM) were isolated. [14C]-TG labeled CM from each control and TCDD-exposed rat contained 7.1 (X) or 28.4 (4X) μg TG/ml (1.6 ml/rat). Control or TCDD-treated 14C-CM were injected into control and TCDD-exposed recipient rats via the left jugular vein and aliquots of blood were drawn from the right jugular vein at 2 min intervals for 20 min. Serum was prepared and counted for [14C] content to determine the rate of CM clearance. Control CM clearance rate was independent of CM dose in control recipient rats and was slower only when given at 4X level to TCDD-exposed rats, 7.7 vs. 13.3 min. A more pronounced effect was seen with TCDD-exposed CM, 7.3 and 14.8 min. 4X TCDD-CM injected into control and TCDD-recipient rats, respectively. These results represent a decreased metabolism of CM TG in the TCDD-exposed rat. The possible hybrid effect implies both aberrant CM and removal processes.


After a single oral dose of TCDD, 30-day LD50 values of 182, 2570 and 296 μg TCDD/kg body weight were obtained for the C57Bl/6J, DBA/2J and B6D2F1/J mouse, respectively. The relative lethal toxicity of TCDD in the three strains of mice was consistent with a specific mediated by the Ah locus controlled cytosolic receptor protein. The acute toxicity was characterized by a dose-related loss of body weight in mice exposed to lethal or near lethal doses of TCDD. Decreased body weight was not due to reduced food consumption in the B6D2F1/J mouse. One week after a single oral dose of TCDD, the C57Bl/6J and DBA/2J mouse exhibited significant doserelated decreases in serum total and esterified cholesterol, glucose and triglyceride. Liver triglyceride levels were increased in both the C57Bl/6J and DBA/2J mouse. Concentrations of serum non-esterified fatty acid, glycerol and protein were not affected by TCDD exposure. The C57Bl/6J mouse exhibited an order of magnitude greater sensitivity to the TCDD induced alterations in carbohydrate and lipid metabolism than the DBA/2J mouse. The observed changes in metabolism do not resemble changes observed in the rat. The similarities between TCDD toxicity and fasting in the mouse suggest that the observed effects of TCDD result from an imbalance in energy supply.
ALTERNED APOPROTEIN (apo) COMPOSITION OF CHOLI-
CRA AND VERY LOW DENSITY LIPOPROTEINS (VLDL) FROM
MESENTERIC LYMPH AFTER EXPOSURE OF ADULT MALE
FISHER RATS WITH 2,3,7,8-TETRACHLORODIBENZO-P-
DIOXIN (TCDD). C.R. Shoaf, R. Walden, D.E.
Chapman and C.M. Schiller, Lab. of Pharm., NIHEH/
NIH, Research Triangle Park, N.C.

Twelve-week old, male Fischer rats were given 0
or 80 µg TCDD in corn oil/kg body wt. by gavage
one week before use. Rats were intubated with
1.5 ml corn oil and later there were larger aggrega-
tions of sacchar lipids in the intestinal epithe-
al cells and more total triglyceride in the mesen-
tery lymph of TCDD-exposed rats than that of
the control rats. The protein to triglyceride ratio
in TCDD-exposed lymph was also reduced.

Proteins were labeled by injecting [14C]-leucine
(control) or [3H]-leucine (TCDD-exposed) via tail
vein, and mesenteric lymph was collected after
conulation. Apo from lymph lipoproteins were
obtained by differential centrifugation, agarose
column chromatography and degraditation. Analysis
of the mixture of exposed to control ratio (K/0)
of individual apo bands on SDS-polyacrylamide gels
compared to the ratio of the total sample applied
revealed 32.7% decrease in apo A-I ratio and 100%
increase in apo E ratio of chylomicra. VLDL re-
vealed 42.8% decrease in apo A-I ratio and 89.6%
increase in apo E. This data suggested that al-
tered apo composition on the surface of chylomi-
cra and VLDL does occur after TCDD exposure, and
these are important factors which may explain the
altered transport of intestinal and removal of
serum lipids after TCDD exposure.

ALTERNED REGULATION OF HUMAN EPIDERMAL CELL PROLI-
FERATION AND DIFFERENTIATION BY 2,3,7,8-TETRA-
CHLORODIBENZO-P-DIOXIN (TCDD). R. Osborne
and W.F. Greenlee, Chem. Ind. Inst. of Toxicol.,
Res. Tri. Pk. NC 27709.

In humans TCDD typically produces pathological
changes in skin characterized by thickening of the
epidermis (acanthosis), hyperkeratosis, and squa-
moous metaplasia of the epithelium lining of the
sebaceous glands. We have examined actions of
TCDD in two human epidermal cell culture systems:
sequeous cell carcinoma (SCC) lines derived from
epidermis and tongue, and normal epidermal cells
derived from neonatal foreskin. Cells were cul-
tured in the presence of a feeder layer of murine
3T3 fibroblasts. In sparse cultures of SCC 9
cells, TCDD (10 nM) increased keratinization (in-
dicated by Rhodonite blue staining) and inhibited
colyon expansion. Similar results were obtained
in SCC 12F cells, which have growth requirements
similar to normal epidermal cells. TCDD inhibited
DNA synthesis (3H-thymidine incorporation into
acid-precipitable DNA) by 40 to 70% in confluent
cultures of SCC 9 and 12F cells in the presence or
absence of serum. In confluent and sparse cul-
tures of normal epidermal cells, TCDD (10 nM) pro-
duced a hyperkeratinization response, and a 30 to
40% inhibition of basal and epidermal growth fac-
tor-stimulated DNA synthesis. These results in-
dicate that TCDD stimulates differentiation of
human epidermal cells in culture, and suggest that
enhanced commitment of proliferating basal cells
to terminal differentiation is an important ini-
tial step resulting in hyperkeratinization.

2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) INHIB-
ITS THE INDUCTION BY THYMIC EPITHELIAL (TE) CELLS
OF T-LYMPHOCYTE MITOGEN RESPONSIVENESS. W.F.
Inst. of Toxicol., Res. Tri. Pk., NC 27709.

In young animals TCDD produces severe thymic atro-
phy characterized by a depletion of cortical thymo-
cytes. Several target sites have been proposed
including lymphocyte precursors and thymic epithe-
lium. We have cultivated TE cells from C57Bl/6
mice using conditions which select against thymic
fibroblasts and macrophages. The epithelial ori-
gin of the TE cells was confirmed using an immuno-
fluorescence sandwich technique with anti-prekera-
tin antibody. Thymocytes co-cultivated with TE
monolayers or incubated in TE-conditioned medium
showed a 10- to 20-fold enhanced response to the
mitogens Con A and PHA, suggesting that the cul-
tured TE cells are producing humoral factors which
promote thymocyte differentiation. TE monolayers
were treated with varying concentrations of TCDD
(1 to 10 nM) for 48 hr, washed, and then co-cul-
tivated with thymocytes for 48 hr. The enhanced mi-
togen response was inhibited in thymocytes co-
cultivated with TCDD-treated TE monolayers (30% of
control with 10 nM TCDD). TCDD treatment did not
result in detectable cytotoxicity to TE cells.
The enhanced antigen response of thymocytes was
not inhibited following incubation in the presence
of 10 nM TCDD added to TE-conditioned medium
from control cultures. These findings indicate that
TE cells are a target site for TCDD and suggest that
TCDD alters the capacity of TE cells to support
the intrathymic differentiation of T-lymphocytes.

MIXED FUNCTION OXIDASE INDUCTION AND TOXIC
EFFECTS OF TCDD IN POULTRY: D. Jones, T.W.
Sawyer, K. Rossanford and S. Safe, Dept. of Phy-
siology and Pharmacology, College of Veterinary
Medicine, Texas A&M University, College Station,
TX 77843

Polyhalogenated aromatic hydrocarbons (PHA) have
been implicated as contaminants in commercial
products causing substantial agricultural los-
ses. Poultry seem to be the most sensitive to the
adverse effects of these compounds. In

Recent years several cases involving the loss of
millions of birds and eggs have occurred in
Japan and the United States due to PHA contami-
nation. The sensitivity of White Leghorn cocke-
rels to ip injection of 2,3,7,8-tetrachlorodi-
benzop-dioxin (TCDD) has been studied with
respect to the induction of the cytochrome P-450
dependent mixed function oxidases. Involvement
of the Bursa of Fabricius was also examined as a
measure of toxicity. Ethoxyresorufin-O-deethyl-
ylase, aryl hydrocarbon hydroxylase, 4-amino-
antipyrine N-demethylase and aldrin epoxidase
activities were all found to be dramatically
increased by as little as 0.03 µg/kg of TCDD.
Involvement of the Bursa of Fabricius was found
to be an extremely sensitive index of toxicity
with as little as 0.3 µg/kg of TCDD signifi-
cantly decreasing organ weights as compared to
controls. (Supported by the Texas Agricultural
Research Station No. S-6376.)

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TCDD toxicity is markedly reduced in thyroidectomized (Th) rats (Rozman, K. et al. Toxicol. Appl. Pharmacol., in press). Therefore, it was of interest to investigate whether T₄ and T₃ are equivalent in modulating TCDD toxicity. 30 male Sprague Dawley rats were divided into 3 groups, 2 of which were thyroidectomized (Th) by 3.5°C/kidney rat. Four days later one of the Th groups was made euthyroid by the administration of T₄ (5 mg/kg, daily p.o.) whereas the other one by T₃ (50 mg/kg, twice a week p.o.). After the elapse of 1 month, all rats received TCDD (100 mg/kg i.p.). Body weights, food consumption and lethality were monitored every two days. 33 days after TCDD, T₄ and T₃ levels were slightly reduced in all groups, probably as a result of enzyme induction. Mortality, weight loss and weight of food consumption were similar in all 3 groups. This group was euthyroid by the administration of T₄ (5 mg/kg, daily p.o.) whereas the other one by T₃ (50 mg/kg, twice a week p.o.). After the elapse of 1 month, all rats received TCDD (100 mg/kg i.p.). Body weights, food consumption and lethality were monitored every two days. 33 days after TCDD, T₄ and T₃ levels were slightly reduced in all groups, probably as a result of enzyme induction. Mortality, weight loss and weight of food consumption were similar in all 3 groups.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean time to death at 62 days</th>
<th>Mortality to death at 62 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>nonTh + TCDD</td>
<td>41 ± 4</td>
<td>51 ± 2%</td>
</tr>
<tr>
<td>Th + T₃ + TCDD</td>
<td>34 ± 3</td>
<td>51 ± 2%</td>
</tr>
<tr>
<td>Th + T₄ + TCDD</td>
<td>40 ± 3</td>
<td>48 ± 2%</td>
</tr>
</tbody>
</table>

Results show that T₄ and T₃ are about equivalent in the mediation of TCDD toxicity.


60 male Sprague Dawley rats (300 ± 20 g) were divided into 6 equal groups. Group 3, 4, 5 and 6 rats were thyroidectomized (Th) by 3 mCi [¹³¹I]/kg rat. 5 weeks later group 2, 4 and 6 rats received i.p. 100 μg TCDD/kg body weight in olive oil. Group 1, 3 and 5 rats served as vehicle controls. 3 days after dosing and every 7th day thereafter group 5 and 6 rats were given i.p. 45 μg thyroxine (T₄)/kg rat. Lethality, body weight, food intake and thymin weight were recorded. The course of TCDD toxicity was similar in nonthyroidectomized and euthyroid rats, but was different in athyroid rats. At day 10 after dosing mortality (P < 0.1) as well as the mean time to death (P < 0.01) was lower in athyroid rats than in euthyroid groups. Body weight loss was much slower in athyroid (<1 g/day) than in nonthyroidectomized or T₄-treated rats (<8 g/day). The weight loss of TCDD dosed nonthyroidectomized and T₄-treated rats correlated well with reduced food intake. Food intake of athyroid rats was not affected by TCDD.

736 UPTAKE, DISTRIBUTION, METABOLISM AND ELIMINATION OF 2,3,7,8-TCARTCHLOROBENZO-π-DIOXIN (TCDD) IN FISH. J.M. Kleeman, S.M. Ghent, C.L. Potter, J.R. Olson, and R.E. Peterson. SUNY, Buffalo, NY, and Univ. Wisc., Madison, WI.

Rainbow trout and yellow perch were fed a diet containing [¹⁴C]-TCDD (494 pg/g) for 13 weeks followed by TCDD-free diet for 13 weeks. Both species accumulated the highest concentration of radioactivity in visceral fat. Lower concentrations were observed in gill, kidney, liver, skin, heart and muscle. No significant differences in growth or lethality were seen between TCDD-treated and control fish. Elimination of TCDD-derived radioactivity was slow and species-and tissue-dependent. Metabolism of TCDD was studied in rainbow trout, bluegill, largemouth bass, striped bass and white sucker given 60 μg/kg [¹⁴C]-TCDD (ip). Radioactivity in gall bladder bile and tissue extracts was analyzed by HPLC. Tissue extracts of white suckers contained TCDD but no metabolites. At 2 and 7 days following exposure, the bile from all species was found to contain TCDD and at least 3 polar metabolites. Hydrolysis of rainbow trout bile with S-glucuronidase and aryl sulfatase suggests that the major metabolite fractions may be a glucuronide conjugate(s). Freshwater fish therefore accumulate TCDD from the diet, concentrate it in body fat, and are capable of metabolizing TCDD to a limited extent. Once formed, the metabolites appear to be rapidly eliminated.
To determine effects of TCDD on growth, lethality and histology in juvenile rainbow trout and yellow perch, each species was treated with graded ip doses of TCDD ranging from 1-125 μg/kg. In trout, doses of 25 and 125 μg/kg produced >95% lethality 2-4 weeks after treatment. At a dose of 5 μg/kg, cumulative lethality after 11 weeks was 20%. In TCDD-treated trout, a dose-dependent decrease in body weight was observed. While no gross pathological lesions were observed in any of the TCDD-treated trout, light microscopic examination after 2 weeks revealed thymic atrophy and focal necrosis of cardiac glandular mucosa in the 125 μg/kg group. In perch, doses of 25 and 125 μg/kg produced >95% lethality 1-4 weeks after treatment. Unexpectedly, this lethality was not preceded by a significant loss of body weight. At 5 μg/kg, 85% lethality occurred by week 11. Fin necrosis and cutaneous hemorrhage were seen in the 5, 25 and 125 μg/kg treated perch at times of peak lethality. Histopathological lesions after 2 weeks revealed a dose-related depletion of lymphoid tissue from the spleen and abnormal hepatocyte vacuolation. Thus, trout and perch are as responsive to TCDD as some of the more sensitive mammalian species. Furthermore, neither the loss of body weight nor the histopathologic lesions observed were considered of sufficient magnitude to cause death. (Sea Grant)

CONSIDERATION ON THE MECHANISM FOR 6-METHYLCOUMARIN PHOTOALLERGENICITY. T. Seki, S. Kato, Y. Katsurada, T. Kobayashi and S. Kusuhara, Dept. of Dermatology and Toxicology, Showa University School of Medicine, Tokyo. Sponsor: Y. Miya

Photoallergenic property of 6-methylcoumarin (6-MC), a synthetic fragrance material, has been reported both in man and in guinea pigs. The purpose of our study was to investigate a possible mechanism of the photoallergic reaction of 6-MC.

6-Methylcoumarin in ethanol solution was irradiated with long wavelength ultraviolet light (UVA: 200J/cm²) ranging from 320 to 400 nm. The UVA-irradiated solution was examined for its contact allergic hypersensitivity by means of a modified guinea pig maximization test. Positive responses were observed. The solution was further studied to isolate and identify the contact allergens formed during the UVA irradiation. The gel permeation chromatography, high performance liquid chromatography, mass spectrometry and nuclear magnetic resonance were employed as analytical methods. Ethyl esters of 6-MC dimer were identified and confirmed to be contact sensitizers. The results of this study indicate that the UVA plays a catalytic role to form contact allergens as a possible mechanism in the elicitation of 6-MC photosensitization.

ASSESSMENT OF LEAD TOXICITY IN TRAFFIC CONTROLLERS OF ALEXANDRIA (EGYPT) ROAD INTERSECTIONS


FACULTIES OF AGRICULTURE AND MEDICINE, UNIVERSITY OF ALEXANDRIA, ALEXANDRIA, EGYPT

Blood lead level (BPL) was determined in 45 traffic controllers working on Alexandria road intersections. Central nervous system dysfunction in the studied subjects was investigated by means of performance tests. Biochemical indicators related to lead exposure such as 8-amino-9-levulinic acid dehydratase and haemoglobin in their blood were also determined.

Results indicated that most of the studied subjects have a comparatively high BPLs. They also showed significantly poorer performance scores than that obtained in a previous study with a group of textile workers of the same age and educational levels. The mean of the BPL in the traffic controllers was found to be 68.26 ± 13.22 μg/dl. This is a very high level compared to an acceptable level of 30.00 μg/dl. All neurobehavioral symptoms demonstrated in the traffic controllers could be attributed to high level of lead exposure.

THE TIME COURSE, DOSE RESPONSE, AND HISTOLOGY OF CHEMICALLY INDUCED SKIN IRRITATION IN THE MOUSE, K. Patrick, H. Mathbach and A. Burghalter, University of California, San Francisco, Ca. The mechanism by which chemicals produce skin irritation has not been well defined. In studies investigating the mechanism time course and the dose response of irritant inflammatory responses to ethyl phenylpropionate (EPP), croton oil, methyl salicylate, dinitrochlorobenzene (DNCB), and 4-ethoxyacetyl-2-phenyl-2-oxazolin-5-one (oxazolone) were determined in the mouse. Responses to each chemical following application to one ear of outbred mice were measured as changes in ear thickness. Responses were maximum as follows: methyl salicylate 20 minutes, DNCB 1 hour, croton oil 6 hours, and oxazolone 8 hours. The time course of irritant response to EPP showed two peaks, a small early peak (1 hour) and a larger peak at eight hours. The histology of treated and control ears of animals sacrificed at varied times after application was examined. The peak responses to methyl salicylate and DNCB were primarily vascular with no cellular infiltration. The early response to EPP was also vascular. The response to croton oil and late response to EPP was predominately cellular (PMN and monocytes) infiltration. These findings suggest inflammatory responses to irritants have different underlying mechanisms.
Para-tertiary Butyl Benzoic Acid (ptBBA) is an industrial chemical with a wide variety of applications, including polymer manufacturing. It is a solid particulate, and exposure to ptBBA dust may occur in the workplace. Male and female Fischer 344 rats were exposed to 0.0, 495, 668, 958 or 1802 mg/m³ ptBBA for 4 hours, or to 0.0, 12.5, 106.1 or 515.2 mg/m³ ptBBA for 6 hours per day on 7 days. Major toxicological effects were observed in the central nervous system (CNS), testes, liver, kidney and body weight. The CNS effects were seen following single or repeated exposure and were characterized by forelimb neuropathy and described microscopically as multifocal poliomyelopathy. Testicular lesions were evident as decreased testes size, reduced sperm counts and microscopic changes. The 4-hour LC₅₀ was greater than 1802 mg/m³, however, the lowest level tested, 495 mg/m³, caused CNS, testicular and body weight changes. A no-effect level of 12.5 mg/m³ was found for CNS and body weight effects following repeat inhalation, but that level was seen to induce liver, kidney and testicular changes.

A new NTP testing system entitled "Fertility Assessment by Continuous Breeding" which involves Task 1: Dose Finding, Task 2: Continuous Breeding, and Task 3: Determination of Affected Sex, was employed in this study. In task 2, male and female mice housed as breeding pairs had free access to feed containing 0.00, 0.10, 0.25, and 0.50% theobromine, exposure was only suspended during the task 3 mating period. In task 3, the task 2 high dose males were randomly paired with control males, and control males with control females for 7 days or less if a copulatory plug was detected. The task 2 results showed significant decreases in litters per pair, live pups per litter, and mean pup body weight in 0.25, and 0.50% groups compared to controls. In task 3, high dose females bred to control male mice delivered significantly fewer live pups; pups had lower body weights than the other pairs. Although the above parameters in the high dose male and control female pairs were unaffected compared to controls, there was a significant increase in the incidence of abnormal sperm in the high dose males without significant effects on motility and sperm count.

(Supported by Contract #N01-ES-2-5013 & ES-95615)
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